

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
19 October 2006 (19.10.2006)

PCT

(10) International Publication Number
WO 2006/108679 A2

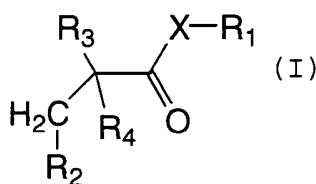
- (51) International Patent Classification: Not classified
- (21) International Application Number: PCT/EP2006/003464
- (22) International Filing Date: 13 April 2006 (13.04.2006)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
10 2005 018 641.6 15 April 2005 (15.04.2005) DE
10 2005 018 642.4 15 April 2005 (15.04.2005) DE
- (71) Applicant (for all designated States except US): **BIOMAC PRIVATINSTITUT FÜR MEDIZINISCHE UND ZAHNMEDIZINISCHE FORSCHUNG, ENTWICKLUNG UND DIAGNOSTIK GMBH** [DE/DE]; Chopinstrasse 15, 04103 Leipzig (DE).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **HUSE, Klaus** [DE/DE]; Hauptstrasse 93, 04416 Markkleeberg (DE). **BIRKENMEIER, Gerd** [DE/DE]; Chopinstrasse 15, 04103 Leipzig (DE). **BIRKENMEIER, Monika** [DE/DE]; Chopinstrasse 15, 04103 Leipzig (DE).
- (74) Agent: **UHLEMANN, Henry**; KAILUWEIT & UHLEMANN, Bamberger Str. 49, 01187 Dresden (DE).
- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: SUBSTANCES AND PHARMACEUTICAL COMPOSITIONS FOR THE INHIBITION OF GLYOXALASES AND THEIR USE TO COMBAT CANCER



(57) Abstract: The present invention pertains to substances of the formula (I) wherein X is O or S; and R1 is a branched or non-branched alkyl, cycloalkyl, branched or non-branched alkenyl, cycloalkenyl, branched or non-branched alkynyl, cycloalkynyl, alkoxyalkyl, alkoxycarbonylalkyl, aryl or a sugar residue; and R2 is H or a branched or non-branched alkyl, cycloalkyl, branched or non-branched alkenyl, cycloalkenyl, branched or non-branched alkynyl, cycloalkynyl, alkoxyalkyl, alkoxycarbonylalkyl, aryl or a sugar 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). 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 cancer.

Substances and pharmaceutical compositions for the inhibition of glyoxalases and their use to combat cancer

FIELD OF THE INVENTION

5

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).

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 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 cancer.

BACKGROUND OF THE INVENTION

25

Cancer is a disease in which cells originating from different organs (e.g. liver, brain, prostate, blood, kidney), are mutated by endogenous processes (errors in different processes during cell division) or exogenous factors (among others chemical mutagens, radiation) such that they are able to elude growth control, leave their tissue of origin and resettle in another organ after spread via the blood flow or lymphatic system and penetrate another organ (metastasis). These cells can proliferate endlessly and form malignant tumors in the organ of origin as well as in an organ colonized secondarily, which can lead to the death of the affected.

Cancer can affect all tissues or organs of higher organisms, and occur at all stages of life, though there is a marked increase in cancer frequency with age. Some forms of cancer
5 are relatively benignant, whereas others progress rapidly, metastasise early and lead to rapid death.

The diversity of their origin and the diversity of their formation renders all tumor cells generated unique. Tumors
10 exist which are dependent on growth factors (e.g. EGF, PDGF, FGF, TGF, IGF, IL, CSF, EPO, NGF, INF, VEGF, HGF, BMP, leptin, and many others) or hormones (e.g. male or female sex hormones) or which produce autocrine stimuli. In many cases the growth stimulating factors are even unknown. Many recent
15 approaches in the treatment of cancer aim at certain surface molecules, such as EGFR. However, the expression of such molecules is highly diverse between different cancers, and even amongst the cancer cells of a single individual.

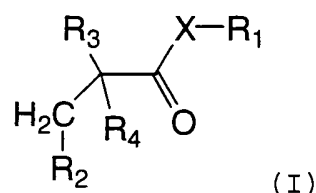
20 As a result, an effective common therapy for the different kinds of cancer is not presently available. Moreover, conventional cancer therapies, such as chemotherapy, surgery or radiation are associated with severe side effects, and limited clinical efficacy. Most therapies are primarily life
25 prolonging, but fall short of curing the disease. Because of these significant shortcomings of existing therapies, cancer remains a leading cause of premature death.

Therefore, there is a general need for the development of
30 novel cancer therapies.

SUMMARY OF THE INVENTION

The problem underlying the invention thus resides in
35 providing substances, compositions, medicaments and methods for the treatment of cancer.

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



5

wherein X is O or S; and

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

10

alkoxycarbonylalkyl, aryl or a sugar residue; and

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

15

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, said substances are for inhibiting glyoxalase I and/or II.

20

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. In a preferred embodiment R₁ comprises 1 to 4 carbon atoms and R₂ is H or comprises 1 to 4

25

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 compounds according to formula (I) wherein X = S, as well as the compounds according to table 1.

30

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
5 the racemic mixture thereof, 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
10 medicament, and methods of treatment comprising the administration of said substances. The treatment with and/or administration of substances of the invention to a mammal, including humans, in need thereof, with a therapeutically effective amount of said substance is also encompassed by the
15 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 increased
20 glycolytic metabolism, comprising diseases associated with one or more of: increased formation of oxoaldehydes, such as methylglyoxal, increased activity of glyoxalase I and/or II activity, and enhanced cell growth/proliferation. In one embodiment, said disease is cancer.

25 In one embodiment, the pharmaceutical composition or medicament comprises one or more additional pharmaceutically active ingredients, comprising ingredients selected from chemotherapeutics, such as one or more selected from
30 cyclophosphamide, doxorubicin, taxol, 5-fluorouracil or cisplatin. 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
35 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, intraarterial, subcutaneous, intramuscular, intradermal, intraperitoneal, rectal, intranasal, epidural, percutaneous, transdermal, pulmonary or intratumoral administration, or for administration as an aerosol, via mini-pumps, as mouth lavage, 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 cancer in a mammal including human, wherein said cancer comprises malignant or benign tumors, solid or non-solid tumors, or the ex-vivo purging of cancer cells. Optionally, the cancer is resistant to chemotherapeutic agents and/or radiation therapy.

More specifically, the cancer is one or more selected from carcinomas including breast, lung, bladder, thyroid gland, prostate, intestine, rectum, pancreas, stomach, liver, uterus, and ovary carcinomas; lymphomas including non-Hodgkin-Lymphoma, Hodgkin-Lymphoma, myeloma; leukemia including acute and chronic lymphoblastic leukemia, acute and chronic myeloblastic leukaemia; brain tumors including astrocytoma, glioma, medulloblastoma, glioblastoma, oligodendroglioma, neuroblastoma; sarcomas including fibrosarcoma, liposarcoma, angiosarcoma, mesothelioma, chondrosarcoma, osteosarcoma; and melanoma.

In one embodiment, the pharmaceutical composition or medicament is for the treatment of a mammal concomitantly suffering from an infectious disease, comprising bacterial, protozoal or fungal infections as well as worms, such as infectious diseases caused by one or more of Candida, Aspergillus, Cryptococcus, Zygomycetes, Dermatophytes,

Blastomyces, Histoplasma, Coccidioides, Sporothrix, Trypanosoma, Leishmania, Plasmodium, Toxoplasma, helminthes, Acrobacter, Actinobacillus, Actinomyces, Bacteroides, Brucella, Chlamydia, Clostridium, Campylobacter, Escherichia, Enterobacter, Enterococcus, Eubacterium, Fusobacterium, Helicobacter, Hemophilus, Legionella, Listeria, Mycobacteria, Mycoplasma, Neisseria, Pasteurella, Peptostreptococcus, Pneumococcus, Pneumocystis, Porphyromonas, Prevotella, Pseudomonas, Salmonella, Shigella, Spirochetes, Staphylococcus, Streptococcus, Treponema, Vibrio, Yersinia, Escherichia coli or Pneumocystis carinii. Some of these infectious diseases may be an opportunistic infection, and/or may be characterized by antibiotic resistance.

15 In one embodiment the pharmaceutical composition or medicament is for use in a mammal having a reduced blood glucose level.

The invention further relates to the use of pharmaceutical compositions or medicaments of the invention in mammals that are going to receive, are currently receiving, or have received conventional cancer therapy, such as one or more of chemotherapy, surgery, radiotherapy or brachytherapy.

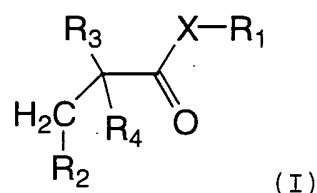
25 It is to be understood that all embodiments described in the context of pharmaceutical compositions or medicaments of the invention equally apply to methods of treatment, and vice versa. Thus, the mentioning of a particular embodiment in the context of one or more of a substance of the invention,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

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



- 10 wherein X is O or S,
 wherein R₁ is a branched or non-branched alkyl, branched or non-branched alkenyl, branched or non-branched alkynyl, alkoxyalkyl, or alkoxycarbonylalkyl, each preferably with a chain length of C1 to C10, more preferably C1 to C8, more preferably C1 to C4, in particular C1, C2, C3 or C4;
 15 or a cycloalkyl, cycloalkenyl, cycloalkynyl, 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
 20 R₂ is H or a branched or non-branched alkyl, branched or non-branched alkenyl, branched or non-branched alkynyl, alkoxyalkyl, or alkoxycarbonylalkyl, each preferably with a chain length of C1 to C10, more preferably C1 to C8, more preferably C1 to C4, in particular C1, C2, C3 or C4;
 25 or a cycloalkyl, cycloalkenyl, cycloalkynyl 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.

30

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

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

5

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	=0	=0	Isopropyl pyruvate
Isobutyl	H	=0	=0	Isobutyl pyruvate
Isopentyl	H	=0	=0	Isopentyl pyruvate
Isohexyl	H	=0	=0	Isohexyl pyruvate
Isoheptyl	H	=0	=0	Isoheptyl pyruvate
Isooctyl	H	=0	=0	Isooctyl pyruvate
Cycloalkyl				
Cyclohexyl	H	=0	=0	Cyclohexyl pyruvate
Cyclohexyl methyl	H	=0	=0	Cyclohexylmethyl pyruvate
Cyclopentyl	H	=0	=0	Cyclopentyl pyruvate
Cyclopentyl methyl	H	=0	=0	Cyclopentylmethyl pyruvate
Alkenyl residues				
Vinyl	H	=0	=0	Ethenyl pyruvate
Allyl	H	=0	=0	Propenyl pyruvate
Butenyl	H	=0	=0	Butenyl pyruvate
Pentenyl	H	=0	=0	Pentenyl pyruvate
Hexenyl	H	=0	=0	Hexenyl pyruvate
Heptenyl	H	=0	=0	Heptenyl pyruvate
Octenyl	H	=0	=0	Octenyl pyruvate
Branched Alkenyl				
Isopropenyl	H	=0	=0	Isopropenyl pyruvate
Isobutenyl	H	=0	=0	Isobutenyl pyruvate
Isopentenyl	H	=0	=0	Isopentenyl pyruvate
Isohexenyl	H	=0	=0	Isohexenyl pyruvate
Isoheptenyl	H	=0	=0	Isoheptenyl pyruvate
Isooctenyl	H	=0	=0	Isooctenyl pyruvate
Cycloalkenyl				
Cyclohexenyl	H	=0	=0	Cyclohexenyl pyruvate
Cyclohexenyl methyl	H	=0	=0	Cyclohexenylmethyl pyruvate
Cyclopentenyl	H	=0	=0	Cyclopentenyl pyruvate

Cyclopentenylmethyl	H	=0	=0	Cyclopentenylmethyl pyruvate
Alkynyl				
Ethynyl	H	=0	=0	Ethynyl pyruvate
Propynyl	H	=0	=0	Propynyl pyruvate
Butynyl	H	=0	=0	Butynyl pyruvate
Pentynyl	H	=0	=0	Pentynyl pyruvate
Hexynyl	H	=0	=0	Hexynyl pyruvate
Heptynyl	H	=0	=0	Heptynyl pyruvate
Octynyl	H	=0	=0	Octynyl pyruvate
Branched Alkynyl				
Isopentynyl	H	=0	=0	Isopentynyl pyruvate
Isohexynyl	H	=0	=0	Isohexynyl pyruvate
Isoheptynyl	H	=0	=0	Isoheptynyl pyruvate
Isooctynyl	H	=0	=0	Isooctynyl pyruvate
Cycloalkynyl				
Cyclooctynyl	H	=0	=0	Cyclooctynyl pyruvate
Alkoxyalkyl				
Methoxymethyl	H	=0	=0	Methoxymethyl pyruvate
Ethoxymethyl	H	=0	=0	Ethoxymethyl pyruvate
Methoxyethyl	H	=0	=0	Methoxyethyl pyruvate
Alkoxyalkylalkyl				
Methoxycarbonylmethyl	H	=0	=0	Methoxycarbonylmethyl pyruvate
Ethoxycarbonylmethyl	H	=0	=0	Ethoxycarbonylmethyl pyruvate
Aryl				
Phenyl	H	=0	=0	Phenyl pyruvate
Naphthyl	H	=0	=0	Naphthyl pyruvate
Sugar	H			
Glucosyl	H	=0	=0	Glucosyl pyruvate
Galactosyl	H	=0	=0	Galactosyl pyruvate
Mannosyl	H	=0	=0	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	=0	=0	Glucosyl 2-oxopentanoate
Alkyl	Alkenyl			
Propyl	Butenyl	=0	=0	Propyl 2-oxoheptenoate
Cycloalkyl	Alkenyl			
Cyclohexyl	Butenyl	=0	=0	Cyclohexyl 2-oxoheptenoate
Alkyl	Alkynyl			
Propyl	Butinyl	=0	=0	Propyl 2-oxoheptinoate
Butyl	Butinyl	=0	=0	Butyl 2-oxoheptinoate
Cycloalkyl	Alkynyl			
Cyclohexyl	Butinyl	=0	=0	Cyclohexyl 2-oxoheptinoate
Alkoxyalkyl	Alkyl			
Methoxymethyl	Ethyl	=0	=0	Methoxymethyl 2-oxopentanoate
Ethoxymethyl	Ethyl	=0	=0	Ethoxymethyl 2-oxopentanoate
Methoxyethyl	Ethyl	=0	=0	Methoxyethyl 2-oxopentanoate
Alkoxyalkyl	Alkyl			
Methoxycarbonylmethyl	Ethyl	=0	=0	Methoxycarbonylmethyl 2-oxopentanoate
Ethoxycarbonylmethyl	Ethyl	=0	=0	Ethoxycarbonylmethyl 2-oxopentanoate
Alkyl	Alkoxyalkyl			
Ethyl	Methoxycarbonylmethyl	=0	=0	Ethyl-4-methoxycarbonyl-2-oxobutanoate
Ethyl	Ethoxycarbonylmethyl	=0	=0	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
5 pyruvate, isobutyl pyruvate, isopentyl pyruvate, isohexyl pyruvate, isoheptyl pyruvate, isooctyl pyruvate, cyclopentyl pyruvate, cyclopentylmethyl pyruvate, cyclohexyl pyruvate, cyclohexylmethyl pyruvate, butenyl pyruvate, hexenyl pyruvate, isobutenyl pyruvate, isohexenyl pyruvate, butinyl
10 pyruvate, hexinyl pyruvate, methoxymethyl pyruvate, ethoxymethyl pyruvate, ethoxycarbonylmethyl pyruvate, methyl-2-oxobutanoate, ethyl 2-oxobutanoate, butyl-2-oxobutanoate, methyl-2-oxopentanoate, ethyl-2-oxoheptanoate, butyl-2-oxopentanoate, methyl-2-oxohexanoate, ethyl-2-oxohexanoate, butyl-2-oxohexanoate, methyl-2-oxoheptanoate,
15 ethyl-2-oxoheptanoate, butyl-2-oxoheptanoate, isobutyl-2-oxobutanoate, isobutyl-2-oxohexanoate, cyclohexyl-2-oxopentanoate, cyclohexylmethyl-2-oxopentanoate, propyl-2-oxoheptanoate, cyclohexyl-2-oxoheptanoate, butyl-2-oxoheptanoate, methoxymethyl-2-oxopentanoate,
20 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
25 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
30 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.

5

More preferred compounds of the invention comprise methyl pyruvate, ethyl pyruvate, propyl pyruvate, butyl pyruvate, isobutyl pyruvate, ethyl-2-oxobutanoate, ethyl-2-oxopentanoate, cyclohexylmethyl pyruvate,

10 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-buturate

15

or the said compounds wherein X = S, in particular S-ethyl pyruvate, and/or the said compounds wherein R3 or R4 is -OH.

a) Substances according to formula (I) inhibit glyoxalases

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

All living 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

25

30

35

combination with oxidative phosphorylation, aerobic energy generation.

Glycolysis, however, is always accompanied by the formation
5 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
10 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
15 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
20 (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.

25 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
30 mechanisms leads to the inhibition of cell proliferation.

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
35 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
5 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
10 and/or II, as well as to the respective glyoxalases of non-mammalian eukaryotic and prokaryotic organisms, such as glyoxalase I and II of yeast or other microorganisms.

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

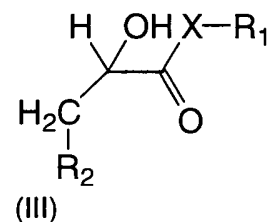
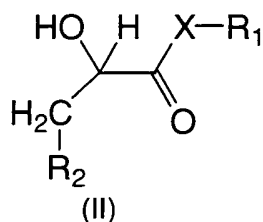
According to the invention, the substances according to formula (I) are for direct inhibition of glyoxalase I and/or
20 II when R3 and R4 together are =O.

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
25 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
30 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
35 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
 5 (III),



10 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, ethyl lactate, propyl lactate, butyl lactate, and ethyl-2-hydroxybutanoate, which
 15 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
 20 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
 25 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)
 30 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
 5 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				
Ethynyl	H	H / OH	=O	Ethynyl lactate
Propynyl	H	H / OH	=O	Propynyl lactate
Butynyl	H	H / OH	=O	Butynyl lactate
Pentynyl	H	H / OH	=O	Pentynyl lactate
Hexynyl	H	H / OH	=O	Hexynyl lactate
Heptynyl	H	H / OH	=O	Heptynyl lactate
Octynyl	H	H / OH	=O	Octynyl lactate
Branched Alkinyl				

Isopentynyl	H	H / OH	=O	Isopentynyl lactate
Isohexynyl	H	H / OH	=O	Isohexynyl lactate
Isoheptynyl	H	H / OH	=O	Isoheptynyl lactate
Isooctynyl	H	H / OH	=O	Isooctynyl lactate
Cycloalkynyl				
Cyclooctynyl	H	H / OH	=O	Cyclooctynyl 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	Glucosyl lactate
Galactosyl	H	H / OH	=O	Galactosyl lactate
Mannosyl	H	H / OH	=O	Mannosyl lactate
Alkyl	Alkyl			
Methyl	Methyl	H / OH	=O	Methyl 2-hydroxybutanoate
Ethyl	Methyl	H / OH	=O	Ethyl 2-hydroxybutanoate
Propyl	Methyl	H / OH	=O	Propyl 2-hydroxybutanoate
Butyl	Methyl	H / OH	=O	Butyl 2-hydroxybutanoate
Methyl	Ethyl	H / OH	=O	Methyl 2-hydroxypentanoate
Ethyl	Ethyl	H / OH	=O	Ethyl 2-hydroxypentanoate

Ethyl	Ethyl	H / OH	=S	S-Ethyl 2-hydroxypentanethionate
Propyl	Ethyl	H / OH	=O	Propyl 2-hydroxypentanoate
Butyl	Ethyl	H / OH	=O	Butyl 2-hydroxypentanoate
Methyl	Propyl	H / OH	=O	Methyl 2-hydroxyhexanoate
Ethyl	Propyl	H / OH	=O	Ethyl 2-hydroxyhexanoate
Propyl	Propyl	H / OH	=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	Alkynyl			
Propyl	Butinyl	H / OH	=O	Propyl 2-hydroxyheptinoate
Butyl	Butinyl	H / OH	=O	Butyl 2-hydroxyheptinoate
Cycloalkyl	Alkynyl			
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	Methoxyc	H / OH	=O	Ethyl-4-

	arboylm ethyl			methoxycarbonyl-2- hydroxybutanoate
Ethyl	Ethoxyca rbonylme thyl	H / OH	=O	Ethyl-4- ethoxycarbonyl-2- hydroxybutanoate
Alkyl	Alkoxyal kyl			
Ethyl	Methoxym ethyl	H / OH	=O	Ethyl 4-methoxy-2- hydroxybutanoate
Ethyl	Ethoxyme thyl	H / OH	=O	Ethyl 4-ethoxy-2- hydroxybutanoate
Ethyl	Methoxye thyl	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, 5 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, 10 isobutenyl lactate, isohexenyl lactate, butinyl lactate, hexinyl lactate, methoxymethyl lactate, ethoxymethyl lactate, ethoxycarbonylmethyl lactate, methyl-2-hydroxybutanoate, ethyl 2-hydroxybutanoate, butyl-2-hydroxybutanoate, methyl-2-hydroxypentanoate, ethyl-2-hydroxyheptanoate, butyl-2- 15 hydroxypentanoate, methyl-2-hydroxyhexanoate, ethyl-2-hydroxyhexanoate, butyl-2-hydroxyhexanoate, methyl-2-hydroxyheptanoate, ethyl-2-hydroxyheptanoate, butyl-2-hydroxyheptanoate, isobutyl-2-hydroxybutanoate, isobutyl-2-hydroxyhexanoate, cyclohexyl-2-hydroxypentanoate, 20 cyclohexylmethyl-2-hydroxypentanoate, propyl-2-hydroxyheptanoate, cyclohexyl-2-hydroxyheptanoate, butyl-2-hydroxyheptanoate, methoxymethyl-2-hydroxypentanoate, ethoxycarbonylmethyl-2-hydroxypentanoate, ethyl-4-

methoxycarbonyl-2-hydroxybutanoate, ethyl-4-methoxy-2-hydroxybutanoate,
or the said compounds, wherein X=S.

5 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.
10 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
15 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
20 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.

25 Lactate and alkyl lactate, respectively, are transported over the cell membrane by a lactate shuttle (monocarboxylate transporters (MCT's)) (Garcia et al., 1994; von Grumbkow et al., 1999) in combination with a proton transporter. For the
30 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
35 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.

5 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.

10 In addition, it is a typical feature of cancer cells to have increased activity of lactate dehydrogenase, achieved mainly by specific induction of lactate dehydrogenase A in tumors (Lewis et al., 2000). Therefore, the use of "prodrugs" of the invention, i.e. compounds according to formula (I) wherein R3
15 or R4 is -OH, such as butyl lactate leads to an increased selectivity of the proliferation inhibiting effect for cancer cells.

Therefore, it is advantageous to use compounds wherein R3 or
20 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
25 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".

30 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
35 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.

5

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

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

10

From the known effect of methyl pyruvate its influence on glyoxalases was not predictable. For years methyl pyruvate has been intensely investigated as an insulinotropic compound (Düfer et al., 2002; Valverde et al., 2001; Lember et al.,
15 2001). This effect is mediated by influencing potassium channels and mitochondrial effects. Inhibitory effects on LDH have also been proposed (Lluis, 1976).

Furthermore, it has been described that the administration of
20 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
25 attributable to abolishing the effect of NF- κ B (Han et al., 2005; Yang et al., 2004; Fink et al., 2004; Miyaji et al., 2003; Ulloa et al., 2002). However, opposite observations also exist in this respect (Mulier et al., 2005).

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

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

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

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

The present invention relates to the medical use of compounds 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.

5

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

10 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
15 the presence of more than one substance of the present invention. This does also not exclude the presence of non-pharmaceutically active additives, i.e. substances which contribute to preparing a galenic formulation, such as fillers, flavouring agents, stabilizers, etc.

20

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
25 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
30 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.

35 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.

- 5 For example, a combination of a compound of the present invention, such as ethyl pyruvate with common 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
- 10 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.
- 15 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
- 20 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

25 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

30 acetate, and/or the lactate dehydrogenase inhibitor oxamate.

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,

35 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.

5

The pharmaceutical composition can also be used for the treatment of cancer in combination with an agent stimulating tumor growth. Such provocation of fast tumor cell proliferation increases the rate of glycolysis of the tumor and thus, its sensitivity towards compounds of the present invention. Such stimulating agents are for example growth factors (EGF, PDGF, FGF, TGF, IGF, IL, CSF, EPO, NGF, INF, VEGF, HGF, BMP, leptin) and hormones (insulin, estrogens, androgens, thyroid hormones, adrenocorticoids, hypothalamic hormones, pituitary hormones), nicotine and others. The composition can advantageously be used in combination with the stimulant (growth factors, hormones, etc.) required by the different tumors. Thus, therapy with compounds of the present invention can be individualized to the respective tumor characteristics of an affected patient.

In case a tumor has been removed by surgery, cancer cells from the explanted tumor tissue can for example be cultured and it can be tested which agents (hormones, cytokines like TGF- β , medicaments and other chemicals) are able to stimulate the cancer cells. This can for example also be performed in combination with molecular biology methods wherein the expression of receptors is determined. Proliferation factors identified in this way can then be used for therapy in combination with the compounds of the present invention, e.g. ethyl pyruvate.

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 antibiotic agents.

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.

5

The pharmaceutical composition of the invention comprises at least one compound of the invention in a therapeutically effective amount. The skilled person can readily determine the therapeutically effective amount in standard in vitro or
10 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
15 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,
20 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
25 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
30 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
35 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 in vivo upon
5 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.
10 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
15 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
20 can be circumvented by local application.

An example of local administration is intratumoral administration, preferably under stereotactic or ultrasound control, or e.g. via locally positioned probes in combination
25 with pumps, or in case of superficially located tumors via creams or other means of local administration.

Pharmaceutical compositions comprising the compounds of the invention can be administered according to generally known
30 methods - including but not limited to oral, intravenous, subcutaneous, intramuscular, intradermal, intraperitoneal, rectal, intranasal, epidural, percutaneous, or transdermal administration, or administration as an aerosol, via mini-pumps, as mouth lavage, gel, oil, ointment, cream, spray,
35 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
5 respective manipulation. An example thereof is, amongst others, so called microbubbles as described in Bekeredjian et al. (2005) and Bekeredjian et al. (2003).

The pharmaceutical composition can also be a food supplement
10 and/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
15 that is consumed by subjects without medical supervision to achieve a prophylactic or therapeutic effect.

C. Medical indications

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

The invention encompasses the administration or use in a vertebrate animal, more particularly a mammal including
25 humans, in need thereof.

In the context of this application, the term "animal" is meant to encompass vertebrate animals, comprising non-mammalian vertebrates and mammals, which mammals comprise
30 man. Thus, the term animal encompasses humans.

In particular, the pharmaceutical composition is for a vertebrate animal, including man, suffering from a disease associated with increased glycolytic metabolism. Such
35 diseases may further be associated with one or more selected from increased formation 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 cancer, including the various specific types
5 of cancer discussed below.

a) Cancer

It was surprisingly found that the compounds of the formula (I) can inhibit growth and/or proliferation of cancer cells.
10 The substances can in addition kill cancer cells and/or induce senescence.

In the context of this application, "inducing senescence" means that cells acquire a more differentiated phenotype and
15 loose their property to divide, and possibly undergo cell death.

Anti-cancer effects of substances of the invention have previously been unknown.

20

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.

25 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,
30 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.

35 Moreover, ethyl lactate can be added to shampoos used in the treatment of canine superficial bacterial pyoderma (de Jaham, 2003). Fungicidal effects have also been suggested for this

compound (US 2005/0020678). Methyl pyruvate has been suggested to treat fish parasites (WO 02/102366).

Ethyl pyruvate has been used to improve cataracts

5 (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.

10 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 the treatment of cachexia, a clinical symptom that is the sequel of cancer. However, these
15 documents do not relate to the treatment of cancer per se. Marx et al, 1988 suggests the inhibition of cancer cells by lactate. The document fails, however, to disclose ester compounds of lactate. Similarly, Stanko et al, 1994, discusses a role of pyruvate in the treatment of cancer, but
20 fails to disclose any pyruvate containing esters. Moreover, the data of Stanko et al, 1994 are difficult to interpret, as the authors themselves admit. Comparative tests on the effects of pyruvate on cancer cells performed by the present inventors clearly demonstrate that pyruvate does not inhibit,
25 but even promotes cancer cell growth (see experiment 16).

Though not intending to be bound by this hypothesis, it is suggested that the cancer inhibiting activity of the compounds of the invention is due to their inhibition of
30 glyoxalase I and/or II.

Cancer cells require significant amounts of energy for cell division. In addition, cell division in many tumors occurs at reduced oxygen supply, because tumors are oftentimes poorly
35 vascularized and solid tumors always show large areas of poor blood supply, which are hypoxic.

A general feature of tumors is a high rate of glycolysis in accordance with the hypoxic conditions, which proceeds even in areas of better vascularization as "aerobic glycolysis". This characteristic of cancer cells, which is also called

5 "missing Pasteur effect" has already been described by Warburg more than 70 years ago and has been confirmed many times since. It is only controversial if an increased rate of glycolysis has to be seen as a cause or consequence of transformation in tumorigenesis (Garber K., 2004).

10

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

15

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

20

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

25 and thus is highly dependent on glycolysis.

A more recent approach to therapeutic intervention aiming at influencing the modified and increased glycolysis as a tumor-cell specific target is therefore no longer related to the inhibition of glycolysis, but to the inhibition of

30 glyoxalases (Thornalley et al., 1994; Vander Jagt et al., 1990; Pemberton and Barrett, 1989; Creighton et al., 2003; Hamilton & Batist, 2004).

35

Cancer cells generally have a very high concentration of glyoxalases to cope with the increased amount of methylglyoxal resulting from increased glycolysis. Under the

effect of glyoxalases, 2-oxo-aldehydes like methylglyoxal are transformed into the corresponding 2-hydroxy acids like D-lactate via the intermediate S-D-lactoyl-glutathione.

- 5 If degradation of methylglyoxal is prevented by glyoxalase inhibitors, cells, which due to increased glycolysis produce increased methylglyoxal, such as cancer cells, accumulate this cytotoxin. This leads to inhibition of their growth, senescence and apoptosis of the cells.

10

Thus, the inhibition of glyoxalases can serve as a "universal" therapy for a plurality of cancers.

15

The term "cancer" encompasses malignant and/or benign tumors, and relates to solid and non-solid tumors. The terms "cancer" and "tumor" are understood to be interchangeable. Moreover, the terms "proliferation" and "growth" are used interchangeably.

20

The term "treatment" encompasses subjects suffering from any of the various disease stages, and encompasses after-treatment as well as prophylaxis.

25

"After-treatment" means a treatment following conventional therapy, such as radiation therapy, chemotherapy, surgery, etc. 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
30 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.
35 throughout the further courses of chemotherapy).

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
5 disease has not fully evolved or has not been diagnosed.

The term "treating cancer" in the stricter sense relates to the treatment of clinically manifest disease. It is meant to encompass both cytotoxic and cytostatic effects. Thus,
10 "inhibition of cancer cells" encompasses the inhibition of cell proliferation as well the killing of the cells. The killing of cells by necrosis or apoptosis or induction of senescence is encompassed by the invention.

15 Examples for cancer which can be treated by the present invention are carcinomas (breast, lung, bladder, thyroid gland, prostate, intestine, rectum, pancreas, stomach, liver, uterus, ovary), lymphomas (non-Hodgkin-Lymphoma, Hodgkin-Lymphoma, myeloma), leukemia (acute and chronic lymphoblastic
20 leukemia, acute and chronic myeloblastic leukemia), brain tumors (e.g. astrocytoma, glioma, medulloblastoma, glioblastoma, oligodendroglioma, neuroblastoma), sarcomas (fibrosarcoma, liposarcoma, angiosarcoma, mesothelioma, chondrosarcoma, osteosarcoma), plasmacytoma and melanoma.
25 Moreover, the ex-vivo purging of cancer cells, e.g. in the context of autologous or heterologous stem cell transplantation, is within the scope of the invention.

Further examples for tumors which can preferably be treated
30 by the present invention are hormone-dependent tumors such as prostate carcinoma, breast cancer, carcinoma of the thyroid gland and/or preferably leukaemia. Moreover, to the extent substances of the invention, in particular substances of the formula (I) wherein R3 or R4 are -OH, can easily cross the
35 blood-brain-barrier, tumors of the CNS are preferably treated according to the present invention.

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.

In this context it is important that inhibitors of glyoxalase I can compensate the effect of resistances against chemotherapeutics (Kamiya et al., 2005). Thus, an originally successful therapy that would have to be discontinued after tumor resistance evolved, can be continued or restarted with the simultaneous application of compounds of the present invention. In a second round of chemotherapy also the first chemotherapeutic may thus be used and other chemotherapeutics exhibiting more severe side effects may be avoided. Advantageously, compounds of the present 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.

Therefore, in one embodiment, the cancer is resistant to 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.

According to the invention cell proliferation is inhibited
5 mainly in cancer cells of vertebrate animals, in particular mammals including humans.

Advantageously therapy is performed in combination with positron-emission-tomography (PET) using for example 2-[18F]
10 fluoro-2-deoxyglucose or similar diagnostic methods observing the influx of glucose. Tumors with a high rate of glycolysis can be displayed via these therapeutic methods (Gatenby and Gillies, 2004) limited only by the resolution of PET. Each tumor that can be identified and localized by PET can
15 therefore be treated by the method according to the present invention.

A combination of the compounds of the present invention with supporting physical therapies, e.g. hyperthermy for the
20 treatment of cancer is also within the scope of the invention.

A further aspect is the use before, during or after application of physical treatment methods like e.g. surgery,
25 radiation therapy or radio therapy (brachytherapy). The kind of physical therapy is not limited for the present invention. Examples encompass radiation therapy using cesium, iridium, iodine or cobalt as radiation source.

30 In one embodiment the treatment of actinic keratoses with methyl- or ethylpyruvate is excluded. In a further embodiment, the treatment of epidermal carcinoma is excluded.

b) Treatment of concomitant infectious disease

35 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 ubiquitous, typically non-pathogenic organisms because of this weakened immune defence, also known as
5 opportunistic infections.

Thus, in one embodiment, the substance, pharmaceutical composition, medicament or method of treatment of the invention is for the treatment of a mammal concomitantly
10 suffering from an infectious disease, comprising bacterial, protozoal or fungal infections, also comprising opportunistic infections. Specific examples of infectious organisms that can be treated according to the present invention comprise species belonging to the genus *Candida*, *Aspergillus*,
15 *Cryptococcus*, *Zygomycetes*, *Dermatophytes*, *Blastomyces*, *Histoplasma*, *Coccidioides*, *Sporothrix*, *Trypanosoma*, *Leishmania*, *Plasmodium*, *Toxoplasma*, helminthes, *Acrobacter*, *Actinobacillus*, *Actinomyces*, *Bacteroides*, *Brucella*, *Chlamydia*, *Clostridium*, *Campylobacter*, *Escherichia*, *Enterobacter*,
20 *Enterococcus*, *Eubacterium*, *Fusobacterium*, *Helicobacter*, *Hemophilus*, *Legionella*, *Listeria*, *Mycobacteria*, *Mycoplasma*, *Neisseria*, *Pasteurella*, *Peptostreptococcus*, *Pneumococcus*, *Pneumocystis*, *Porphyromonas*, *Prevotella*, *Pseudomonas*, *Salmonella*, *Shigella*, *Spirochetes*, *Staphylococcus*,
25 *Streptococcus*, *Treponema*, *Vibrio*, *Yersinia*, *Escherichia coli* or *Pneumocystis carinii*.

Degradation of glucose by glycolysis and the formation of glyoxal compounds are ubiquitous metabolic pathways, which
30 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. They turn off other energy producing processes by glucose and use
35 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.

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.

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.

Pathogenic fungi of humans or animals, including man, which cover their energy consumption mainly by glycolysis when glucose is available are for example *Candida* spp., *Aspergillus* spp., *Cryptococcus* spp., *Zygomycetes* spp., *Dermatophytes*, *Blastomyces* spp., *Histoplasma* spp., *Coccidioides* spp., *Sporothrix* spp.. These organisms switch off other energy producing processes by glucose and use glycolysis (catabolite repression) or grow under hypoxic conditions. It is therefore not surprising that such a multitude of cells and organisms can be inhibited in their growth and killed by compounds of the present invention.

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).

In addition, bacterial infections represent a significant problem in cancer therapy. The weakening of the patient by cancer per se, as well as by cancer therapy, such as chemotherapy, which weakens the immune system, favors

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. 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).

The simultaneous treatment of cancer and infectious organisms by the compounds according to the present invention is highly beneficial to the subject receiving such therapy, who otherwise would have to be treated with different agents at the same time. Thus, the compounds of the present invention result in synergistic effects in cancer. Moreover, the treatment of cancer with compounds of the present invention results in a prophylactic effect for the prevention of bacterial or fungal infections, at the same time as treating the tumor. These effects represent a significant therapeutic improvement, and actually lead to the reversal of side effects commonly associated with conventional chemotherapies, i.e. an increased susceptibility to infections.

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 of cancer in postprandial states 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 tumors represent the primary consumers of glucose, under conditions of reduced blood glucose levels (Sugden and Holness, 2002).

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

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

BRIEF DESCRIPTION OF THE FIGURES

15 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).

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

25 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).

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

35 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 androgen stimulated growth of androgen dependent prostate-carcinoma cells (LNCaP) by ethyl D-lactate (DEL).

5 Fig. 9: Concentration dependent inhibition of androgen stimulated growth of LNCaP cells by ethyl D-lactate (DEL).

Fig. 10: Inhibition of androgen stimulated growth of LNCaP cells by ethyl L-lactate (LEL).

10

Fig. 11: Inhibition of androgen stimulated growth of androgen dependent prostate-carcinoma cells (LNCaP) by ethyl pyruvate (EP).

15 Fig. 12: Inhibition of TGF- β 1 stimulated growth of LNCaP cells by ethyl D-lactate (DEL).

Fig. 13: Inhibition of leptin stimulated growth of LNCaP cells by ethyl D-lactate (DEL).

20

Fig. 14: Effect of pyruvate on proliferation of prostate-carcinoma cells (LNCaP).

Fig. 15: Influence of ethyl D-lactate (DEL) and ethyl L-lactate (LEL) on growth and morphology of human astrocytoma cells

25

Fig. 16: Inhibition of growth of human brain tumor cells by ethyl pyruvate (EP).

30

Fig. 17: Inhibition of growth of human leukemia cells by ethyl pyruvate (EP).

35 EXPERIMENTS

A. General experimental procedures

a) Determining enzymatic activity of yeast glyoxalase I

Measuring glyoxalase I (E.C.4.4.1.5, lactoyl-glutathione
5 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
10 formation of the product is observed using the extinction
coefficient $\epsilon = 2,86 \text{ mM}^{-1} \text{ cm}^{-1}$ at 240 nm. The measurement
was performed in 50 mM sodium phosphate buffer, pH 7,0. For
that purpose 2 mM methylglyoxal and 2 mM reduced glutathione
were incubated for 2 minutes at 30°C for the formation of the
15 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
20 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
25 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.
30 (1982).

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

c) Determining enzymatic activity of human erythrocyte glyoxalase II

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

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

An identical number of LNCaP cells (androgen dependent prostate carcinoma cells; DSMZ No ACC 256) were routinely 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% CO_2 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.

5

e) Protocol for experiments using human astrocytoma cells (1321 N1; ECACC no. 86030102)

100000 human astrocytoma cells (1321 N1; ECACC no. 86030102) were incubated in DMEM (Gibco, no. 41966-092),
10 penicillin/streptomycin (100 units of penicillin/ml; 100 µg streptomycin/ml, Gibco; no. 15140/122) in the presence of 10% calf serum (Biochrom, S0113/5), 2 mM L-glutamine, (Gibco, no. 25030-024) at 37°C, 5% CO₂ in 6 well plates (Greiner, no. 657160).

15

B. Data

Example 1

Inhibition of the enzymatic activity of glyoxalase I of yeast
20 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
25 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
30 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

35

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
 5 inhibition curves of each compound.

The data (Table 3) show that alkyl 2-oxo-derivatives inhibit the enzymatic activity of yeast glyoxalase I with different IC₅₀s, while the alkyl 2-hydroxy-derivatives revealed no
 10 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.

15 Table 3

Alkyl 2-Oxo-Derivatives		IC ₅₀ GLO1*
Ethyl 2-oxobutyrate	CH ₃ -CH ₂ -O-CO-CO-CH ₂ -CH ₃	1,8 mM
Butyl pyruvate	CH ₃ -CH ₂ -CH ₂ -CH ₂ -O-CO-CO-CH ₃	7,5 mM
Ethyl pyruvate	CH ₃ -CH ₂ -O-CO-CO-CH ₃	10 mM
Methyl pyruvate	CH ₃ -O-CO-CO-CH ₃	15 mM
Diethyl oxalate	CH ₃ -CH ₂ -O-CO-CO-OCH ₂ -CH ₃	>50mM
Alkyl-2-Hydroxy Derivatives		
(-)-Butyl L-Lactate	CH ₃ -CH ₂ -CH ₂ -CH ₂ -O-CO-CHOH-CH ₃	no inhibition
(-)-Ethyl L-Lactate	CH ₃ -CH ₂ -O-CO-CHOH-CH ₃	no inhibition
(+)-Ethyl D-Lactate	CH ₃ -CH ₂ -O-CO-CHOH-CH ₃	no inhibition
Methyl L-Lactate	CH ₃ -O-CO-CHOH-CH ₃	no inhibition
(-)-Isopropyl L-Lactate	CH ₃ -CH(CH ₃)-O-CO-CHOH-CH ₃	no inhibition

(+)-Isobutyl L-Lactate	CH ₃ -CH(CH ₃)-CH ₂ -O-CO-CHOH-CH ₃	no inhibition
------------------------	--	---------------

*GLO 1 = glyoxalase I

Example 3

Effect of prodrugs

5

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

10

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

15

Table 4

	Inhibition of enzyme	Inhibition of cell proliferation	
	yeast GLO 1	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.

20

25

Example 4

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

5 Synthesis of 2-Oxopropanethioic acid S-ethyl ester

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; 10 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 15 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 20 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 25 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, n_D 1.4750 (21 °C), purity > 95 % (NMR).

30

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.

5 Determination of glyoxalase I activity

Determination of glyoxalase I activity was performed according to the general protocol as described above. The influence of 2-oxopropanethioic acid S-ethyl ester on the activity of the enzyme was investigated by addition of
10 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
15 manner.

Example 5

Influence of ethyl pyruvate (EP), butyl pyruvate (BP) and butyl L-lactate (BL) on the enzymatic activity of yeast
20 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
25 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).

30

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.

5 Example 6

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

A colony of strain HD65-5a (*Saccharomyces cerevisiae*) was
10 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
15 in the stationary growth phase (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 (Schwöck et al., 2004).
Subsequently the disrupted cells were centrifuged at 23000 x
20 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
25 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
30 incubation period of 15 min at 25°C the formation of
glutathione was measured at 412 nm ($\epsilon = 13.6 \text{ mM}^{-1} \text{ cm}^{-1}$). The

activity of the glyoxalase II (IU) correlates with the amount of enzyme hydrolysing 1 μ mol 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.

- 5 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
10 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, 15 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
20 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
25 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
30 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
35 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

10 Inhibition of androgen stimulated growth of androgen dependent prostate-carcinoma cells (LNCaP) by ethyl D-lactate (DEL).

LNCaP cells were cultured according to the general protocol described above, and were incubated with: (i) 0,09% DMSO, (ii) 10 nM DHT (Sigma; A-8380) in DMSO, (iii) 10 nM DHT + 10 mM Ethyl D-lactate (DEL) and (iv) 10 mM DEL.

The experiment (Fig. 8) shows that DHT stimulated the growth of LNCaP cells ($p = 0,006$ [blank vs. DHT]). In the presence of DEL androgen stimulated growth is suppressed ($p = 0,01$ [DHT vs. DHT+DEL]). DEL does not show an effect on the growth of unstimulated tumor cells ($p = 0,88$ [blank vs. DEL]).

Example 11

Concentration dependent inhibition of androgen stimulated growth of LNCaP cells by ethyl D-lactate (DEL).

LNCaP cells were cultured according to the general protocol described above, and were incubated with: preparation 1 (0,09 % DMSO); preparation 2 (10 nM DHT); preparation 3 (10 nM DHT + 1 mM DEL); preparation 4 (10 nM DHT + 5 mM DEL); preparation 5 (10 nM DHT + 10 mM DEL); preparation 6 (10 nM DHT + 20 mM DEL); preparation 7 (10 nM DHT + 50 mM DEL).

The experiment (Fig. 9) shows that the proliferation of DHT-stimulated LNCaP cells depends on the concentration of DEL.

The IC₅₀ of proliferation is already reached at 1 mM DEL. Inhibition of proliferation by increasing DEL concentrations is significant in all cases ($p < 0,05$).

5 Example 12

Inhibition of androgen stimulated growth of LNCaP cells by ethyl L-lactate (LEL).

10 LNCaP cells were cultured according to the general protocol described above, and were incubated with: (i) 0,09 % DMSO, (ii) 10 nM DHT in DMSO, (iii) 10 nM DHT + 10 mM ethyl L-lactate (LEL) and (iv) 10 mM LEL.

15 The experiment (Fig. 10) shows that DHT stimulates the growth of LNCaP cells ($p = 0,006$ [blank vs. DHT]). In the presence of LEL, the androgen stimulated growth is suppressed ($p = 0,045$ [DHT vs. DHT + LEL]). LEL does not show an effect on the growth of un stimulated tumor cells ($p = 0,49$ [blank vs. LEL]).

20

Example 13

Inhibition of androgen stimulated growth of androgen dependent prostate-carcinoma cells (LNCaP) by ethyl pyruvate (EP).

25

LNCaP cells were cultured according to the general protocol described above, and were incubated with: (i) 0,09% DMSO, (ii) 10 nM DHT (Sigma; A-8380) in DMSO, (iii) 10 nM DHT + 1 mM ethyl pyruvate (EP), (iv) 10 nM DHT + 5 mM ethyl pyruvate (EP) (v) 10 nM DHT + 10mM ethyl pyruvate (EP).

30

The experiment (Fig. 11) shows that DHT stimulated the growth of the LNCaP cells ($p = 0,007$ [blank vs. DHT]). In the presence of EP the androgen stimulated growth is suppressed ($p = 0,043$ [1 mM EP], $p = 0,02$ [5mM], and $p < 0.001$ [10mM EP], DHT versus DHT + EP).

35

Example 14

Inhibition of TGF- β 1 stimulated growth of LNCaP cells by ethyl D-lactate (DEL).

5 LNCaP cells were cultured according to the general protocol described above, and were incubated with: preparation 1 (equivalent volume of RPMI-SF); preparation 2 (10 ng/ml TGF- β 1 (Stratman, No. 9515500); preparation 3 (10 ng/ml TGF- β 1 + 10 mM DEL); preparation 4 (10 mM DEL).

10

The experiment (Fig. 12) shows that DEL significantly inhibits the TGF- β 1-caused stimulation of tumor cell proliferation ($p = < 0,001$ [blank vs. TGF- β 1]; $p = 0,003$ [TGF- β 1 vs. TGF- β 1 + DEL]).

15

Example 15

Inhibition of leptin stimulated growth of LNCaP cells by ethyl D-lactate (DEL).

20 LNCaP cells were cultured according to the general protocol described above, and were incubated with: preparation 1 (equivalent volume of RPMI-SF); preparation 2 (100 ng/ml leptin); preparation 3 (100 ng/ml leptin + 10 mM DEL); preparation 4 (10 mM DEL).

25

The experiment (Fig. 13) shows that DEL significantly inhibits leptin-caused stimulation of tumor cell proliferation ($p = 0,001$ [blank vs. leptin]; $p = 0,001$ [leptin vs leptin + DEL]).

30

Example 16

Effect of pyruvate on proliferation of prostate-carcinoma cells (LNCaP).

35 LNCaP cells were cultured according to the general protocol described above, and were incubated with: preparation (1) 0,09 % DMSO; (2) 10 nM DHT; (3) 10 nM DHT + 1 mM pyruvate;

(4) 10 nM DHT + 5 mM pyruvate; (5); 10 nM DHT + 10 mM pyruvate; (6) 1 mM pyruvate; (7) 5 mM pyruvate; (8) 10 mM pyruvate.

5 The experiment (Fig. 14) shows that pyruvate did not diminish the DHT-induced proliferation of LNCaP cells ($p < 0,001$ [blank vs. DHT]; $p > 0,05$ [DHT vs. DHT+1 mM pyruvate]; $p > 0.05$ [DHT vs. DHT+5 mM pyruvate]; $p > 0.05$ [DHT vs. DHT+10 mM pyruvate] as has been demonstrated for the compounds of the invention.

10 These data clearly contradict earlier speculations on pyruvate (Stanko et al, 1994). Rather, pyruvate itself exhibits stimulatory effects on proliferation of tumor cells in the absence of DHT ($p < 0,001$ [1 mM pyruvate]; $p < 0,001$ [5 mM pyruvate]; $p < 0.001$ [10 mM pyruvate]).

15

Example 17

Influence of ethyl D-lactate (DEL) and ethyl L-lactate (LEL) on growth and morphology of human astrocytoma cells

20 Human astrocytoma cells (1321N1; ECACC no. 86030102) were cultured as described above. After addition of 20 mM DEL and LEL, respectively, the tumor cells were for another 24 hours. Thereafter the cells were inspected using a light optical microscope (Axioplan 2, Carl Zeiss) and documented (Fig. 15).

25

Fig. 15A shows non treated tumor cells (normal cell configuration; adherent growth; broad cytoplasm; strong cell-cell contacts), Fig. 15B shows DEL treated tumor cells (needle shaped structure; almost no cytoplasm; loss of cell-cell contacts; low cell adhesion, reduced cell number), Fig. 15C shows LEL treated tumor cells (needle shaped; missing cytoplasm; loss of cell-cell contacts, low cell adhesion, reduced cell number).

35 The experiments show that ethyl lactate dramatically changes the morphology and functional characteristics of tumor cells and inhibits their growth by starvation.

Example 18

Inhibition of growth of human brain tumor cells by ethyl pyruvate (EP).

5

Human astrocytoma cells (1321N1; ECACC no. 86030102) were cultured as described above. After addition of increasing concentrations of ethyl pyruvate the tumor cells were incubated for another 24 hours. Thereafter 20 µl WST-1 reagent (Roche, no. 1644807) were added to each well and absorption at 450/620 nm was measured after 3 hours. The staining is proportional to cell number.

At all concentrations marked with an asterisk (5 mM, 10 mM and 20 mM) significant differences in cell number can be observed in comparison to untreated cells ($p < 0,05$).

The experiment (Fig. 16) shows that proliferation of human brain tumor cells is significantly inhibited by EP.

20

Example 19

Inhibition of growth of human leukemia cells by ethyl pyruvate (EP).

Human acute monocytic leukaemia cells (THP-1; DSMZ no. ACC 16) were cultured in RPMI-1640 medium (Gibco no. 21875-034) in the presence of 10% calf serum (Biochrom, S0113 / 5), penicillin/streptomycin (100 units penicillin/ml; 100 µg streptomycin/ml, Gibco; no. 15140/122), 20 mM Hepes (Gibco, no. 15630-056), 2 mM L-glutamine (Gibco; no. 25030-024) at 37°C and 5% CO₂ for 24 hours. 10000 cells each were pipetted into the wells of 96 well culture plates (Greiner; no. 655180) and were mixed with ethyl pyruvate until the indicated concentrations were obtained. Thereafter the cells were cultured for another 24 hours. Subsequently, 20 µl WST-1 reagent (Roche, no. 1644807) was added to each well and absorption at 450/620 nm was measured after 3 hours.

35

According to manufacturer's instructions the staining is proportional to the number of cells.

At all concentrations marked by an asterisk significant
5 differences in cell numbers can be observed in comparison to untreated cells ($p < 0,05$).

The experiment (Fig. 17) shows that proliferation of leukaemia cells is inhibited by ethyl pyruvate.

10

Example 20:

Pharmaceutical composition for infusion

A solution for infusion comprising the substances of the
15 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
20 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
25 as follows:

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

35 Example 21:

Pharmaceutical composition for bolus injection

A solution for bolus injection can be prepared according to Example 22, wherein the concentration of the substance of the invention is adapted accordingly.

5 Example 22:

Cream

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

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

Example 23:

25 Ointment

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

30	A	product of the invention	10-20%
		butyleneglycol	5%
		glycerol	4%
		sodium dihydroxy cetylphosphate,	
		isopropyl hydroxy cetylether	2%
35		water	to 100%
	B	glycolstearate SE	15%

65

	octylcocoate	11%
5	C butyleneglycol, metylparabene ethylparabene, propylparabene, pH: adjusted to 5,5	2%

Literature

Andersen PH, Jensen NJ.

5 Mutagenic investigation of flavourings: dimethyl succinate,
ethyl pyruvate and aconitic acid are negative in the
Salmonella/mammalian-microsome test.
Food Addit Contam. 1984 Jul-Sep;1(3):283-8

Ayoub F, Zaman M, Thornalley P, Masters J.

10 Glyoxalase activities in human tumour cell lines in vitro.
Anticancer Res. 1993 Jan-Feb;13(1):151-5.

Bekeredjian R, Grayburn PA, Shohet RV.

15 Use of ultrasound contrast agents for gene or drug delivery
in cardiovascular medicine.
J Am Coll Cardiol. 2005 Feb 1;45(3):329-35.

Bekeredjian R, Chen S, Frenkel PA, Grayburn PA, Shohet RV.

20 Ultrasound-targeted microbubble destruction can repeatedly
direct highly specific plasmid expression to the heart.
Circulation. 2003 Aug 26;108(8):1022-6. Epub 2003 Aug 11.

Bennett JE, Izumikawa K, Marr KA.

25 Mechanism of increased fluconazole resistance in Candida
glabrata during prophylaxis.
Antimicrob Agents Chemother. 2004 May;48(5):1773-7.

Birkenmeier G, Heidrich K, Glaser C, Handschug K, Fabricius
EM, Frank R, Reissig D.

30 Different expression of the alpha2-macroglobulin
receptor/low-density lipoprotein receptor-related protein in
human keratinocytes and fibroblasts.
Arch Dermatol Res. 1998 Oct;290(10):561-8.

35 Bowmer CT, Hooftman RN, Hanstveit AO, Venderbosch PW, van der
Hoeven N.

The ecotoxicity and the biodegradability of lactic acid, alkyl lactate esters and lactate salts.
Chemosphere. 1998 Sep;37(7):1317-33.

5 Bradford M. M.

A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding.

Anal. Biochem. 1976, 72:248-254.

10

Clary JJ, Feron VJ, van Velthuijsen JA.

Safety assessment of lactate esters.

Regul Toxicol Pharmacol. 1998 Apr;27(2):88-97.

15 Clugston SL, Daub E, Kinach R, Miedema D, Barnard JF, Honek JF.

Isolation and sequencing of a gene coding for glyoxalase I activity from *Salmonella typhimurium* and comparison with other glyoxalase I sequences.

20 Gene. 1997 Feb 20;186(1):103-11.

Creighton DJ, Zheng ZB, Holewinski R, Hamilton DS, Eiseman JL.

Glyoxalase I inhibitors in cancer chemotherapy.

25 Biochem Soc Trans. 2003 Dec;31(Pt 6):1378-82.

Cunha BA.

Methicillin-resistant *Staphylococcus aureus*: clinical manifestations and

30 antimicrobial therapy.

Clin Microbiol Infect. 2005 Jul;11 Suppl 4:33-42.

Devamanoharan PS, Henein M, Ali AH, Varma SD.

Attenuation of sugar cataract by ethyl pyruvate.

35 Mol Cell Biochem. 1999 Oct;200(1-2):103-9.

Dong YQ, Yao YM, Wei P, Liu H, Dong N, Yu Y, Sheng ZY.

Effects of ethyl pyruvate on cell-mediated immune function in rats with delayed resuscitation after burn injury.

Zhongguo Wei Zhong Bing Ji Jiu Yi Xue. 2005 Jan;17(1):12-5.

- 5 Douglas KT, Gohel DI, Nadvi IN, Quilter AJ, Seddon AP.
Partial transition-state inhibitors of glyoxalase I from human erythrocytes, yeast and rat liver. Biochim Biophys Acta. 1985 May 20;829(1):109-18.

- 10 Dufer M, Krippeit-Drews P, Buntinas L, Siemen D, Drews G.
Methyl pyruvate stimulates pancreatic beta-cells by a direct effect on KATP channels, and not as a mitochondrial substrate.
Biochem J. 2002 Dec 15;368(Pt 3):817-25.

15

Fan X, Subramaniam R, Weiss MF, Monnier VM.
Methylglyoxal-bovine serum albumin stimulates tumor necrosis factor alpha secretion in RAW 264.7 cells through activation of mitogen-activating protein kinase, nuclear factor kappaB
20 and intracellular reactive oxygen species formation. Arch Biochem Biophys. 2003 Jan 15;409(2):274-86.

Fink MP.

Ethyl pyruvate: a novel treatment for sepsis and shock.

- 25 Minerva Anesthesiol. 2004 May;70(5):365-71.

Garber K.

Energy boost: the Warburg effect returns in a new theory of cancer.

- 30 J Natl Cancer Inst. 2004 Dec 15;96(24):1805-6

Garcia CK, Goldstein JL, Pathak RK, Anderson RG, Brown MS.
Molecular characterization of a membrane transporter for lactate, pyruvate, and other monocarboxylates: implications
35 for the Cori cycle.

Cell. 1994 Mar 11;76(5):865-73

Gatenby RA, Gillies RJ.

Why do cancers have high aerobic glycolysis?

Nat Rev Cancer. 2004 Nov;4(11):891-9.

- 5 von Grumbkow L, Elsner P, Hellsten Y, Quistorff B, Juel C.
Kinetics of lactate and pyruvate transport in cultured rat
myotubes.

Biochim Biophys Acta. 1999 Mar 4;1417(2):267-75.

- 10 Hamilton D, Batist G.

Glutathione analogues in cancer treatment.

Curr Oncol Rep. 2004 Mar;6(2):116-22.

Hamilton DS, Creighton DJ.

- 15 Inhibition of glyoxalase I by the enediol mimic
S-(N-hydroxy-N-methylcarbamoyl)glutathione. The possible
basis of a tumor-selective anticancer strategy.

J Biol Chem. 1992 Dec 15;267(35):24933-6.

- 20 Han Y, Englert JA, Yang R, Delude RL, Fink MP.

Ethyl Pyruvate Inhibits Nuclear Factor- κ B-Dependent
Signaling by Directly Targeting p65.

J Pharmacol Exp Ther. 2005 Mar;312(3):1097-105. Epub 2004 Nov
03.

25

Heymans M, Singh AK.

Deriving phylogenetic trees from the similarity analysis of
metabolic pathways.

Bioinformatics. 2003;19 Suppl 1:i138-46.

30

Iwami Y, Schachtele CF, Yamada T.

Mechanism of inhibition of Glycolysis in Streptococcus mutans
NCIB 11723 by chlorhexidine. Oral Microbiol. Immunol. 1995
Dec; 10(6):360-4).

35

de Jaham C.

Effects of an ethyl lactate shampoo in conjunction with a systemic antibiotic in the treatment of canine superficial bacterial pyoderma in an open-label, nonplacebo-controlled study.

5 Vet Ther. 2003 Spring;4(1):94-100.

Johansson AS, Ridderstrom M, Mannervik B.

The human glutathione transferase P1-1 specific inhibitor TER 117 designed for overcoming cytostatic-drug resistance is

10 also a strong inhibitor of glyoxalase I.

Mol Pharmacol. 2000 Mar;57(3):619-24.

Kalsi A, Kavarana MJ, Lu T, Whalen DL, Hamilton DS, Creighton DJ.

15 Role of hydrophobic interactions in binding S-(N-aryl/alkyl-N hydroxycarbamoyl) glutathiones to the active site of the antitumor target enzyme glyoxalase I.

J Med Chem. 2000 Oct 19;43(21):3981-6.

20 Kamiya D, Uchihata Y, Ichikawa E, Kato K, Umezawa K.

Reversal of anticancer drug resistance by COTC based on intracellular glutathione and glyoxalase I.

Bioorg Med Chem Lett. 2005 Feb 15;15(4):1111-4.

25 Lember N, Joos HC, Idahl LA, Ammon HP, Wahl MA.

Methyl pyruvate initiates membrane depolarization and insulin release by metabolic factors other than ATP.

Biochem J. 2001 Mar 1;354(Pt 2):345-50

30 Lewis BC, Prescott JE, Campbell SE, Shim H, Orlowski RZ, Dang CV.

Tumor induction by the c-Myc target genes rcl and lactate dehydrogenase A.

Cancer Res. 2000 Nov 1;60(21):6178-83.

35

Lluís C, Bozal 4.

[LDH and structural analogues of pyruvate (author's transl)]

Rev Esp Fisiol. 1976 Mar;32(1):9-13

Mannervik B, Aronsson AC, Tibbelin G.
Glyoxalase I from human erythrocytes.

5 Methods Enzymol. 1982;90 Pt E:535-41.

Martins AM, Cordeiro C, Freire AP.

Glyoxalase II in *Saccharomyces cerevisiae*: in situ kinetics
using the 5,5'-dithiobis(2-nitrobenzoic acid) assay.

10 Arch Biochem Biophys. 1999 Jun 1;366(1):15-20.

Marx E, Mueller-Klieser W, Vaupel P.

Lactate-induced inhibition of tumor cell proliferation.
Int J Radiat Oncol Biol Phys. 1988 May;14(5):947-55.

15

McLellan AC, Thornalley PJ.

Glyoxalase activity in human red blood cells fractioned by
age.

Mech Ageing Dev. 1989 Apr;48(1):63-71

20

Miyaji T, Hu X, Yuen PS, Muramatsu Y, Iyer S, Hewitt SM, Star
RA.

Ethyl pyruvate decreases sepsis-induced acute renal failure
and multiple organ damage in aged mice.

25 Kidney Int. 2003 Nov;64(5):1620-31.

Mulier KE, Beilman GJ, Conroy MJ, Taylor JH, Skarda DE,
Hammer BE.

Ringer's ethyl pyruvate in hemorrhagic shock and resuscitation
does not improve early hemodynamics or tissue energetics.
Shock. 2005 Mar;23(3):248-252.

30

Murray MJ, Barbose JJ, Cobb CF.

Serum D(-)-lactate levels as a predictor of acute intestinal
ischemia in a rat model.

35

J Surg Res. 1993 May;54(5):507-9.

Nesterova M, Cho-Chung YS.

Killing the messenger: antisense DNA and siRNA.

Curr Drug Targets. 2004 Nov;5(8):683-9.

5 Pemberton KD, Barrett J.

The detoxification of xenobiotic compounds by *Onchocerca gutturosa* (Nematoda:

Filarioidea). Int J Parasitol. 1989 Dec;19(8):875-8.

10 Ranganathan S, Walsh ES, Tew KD.

Glyoxalase I in detoxification: studies using a glyoxalase I transfectant cell line.

Biochem J. 1995 Jul 1;309 (Pt 1):127-31.

15 Roth DA, Brooks GA.

Lactate and pyruvate transport is dominated by a pH gradient-sensitive carrier in rat skeletal muscle sarcolemmal vesicles.

Arch Biochem Biophys. 1990 Jun; 279(2):386-94

20

Schwock J, Kirchberger J, Edelmann A, Kriegel TM, Kopperschlager G.

Interaction of 6-phosphofructokinase with cytosolic proteins of *Saccharomyces cerevisiae*.

25 Yeast. 2004 Apr 30;21(6):483-94.

Sharkey EM, O'Neill HB, Kavarana MJ, Wang H, Creighton DJ, Sentz DL, Eiseman JL.

Pharmacokinetics and antitumor properties in tumor-bearing mice of an enediol analogue inhibitor of glyoxalase I.

30 Cancer Chemother Pharmacol. 2000;46(2):156-66.

Stanko RT, Mullick P, Clarke MR, Contis LC, Janosky JE, Ramasastry SS.

35 Pyruvate inhibits growth of mammary adenocarcinoma 13762 in rats. Cancer Res. 1994 Feb 15;54(4):1004-7.

Sugden MC, Holness MJ.

Therapeutic potential of the mammalian pyruvate dehydrogenase kinases in the prevention of hyperglycaemia.

Curr Drug Targets Immune Endocr Metabol Disord. 2002

5 Jul;2(2):151-65.

Thornalley PJ.

Pharmacology of methylglyoxal: formation, modification of proteins and nucleic acids, and enzymatic detoxification--a
10 role in pathogenesis and antiproliferative chemotherapy.

Gen Pharmacol. 1996 Jun;27(4):565-73.

Thornalley PJ.

The glyoxalase system in health and disease.

15 Mol Aspects Med. 1993;14(4):287-371

Thornalley PJ, Edwards LG, Kang Y, Wyatt C, Davies N, Ladan MJ, Double J.

Antitumour activity of S-p-bromobenzylglutathione cyclopentyl
20 diester in vitro and in vivo. Inhibition of glyoxalase I and induction of apoptosis.

Biochem Pharmacol. 1996 May 17;51(10):1365-72.

Thornalley PJ, Strath M, Wilson RJ.

25 Antimalarial activity in vitro of the glyoxalase I inhibitor diester, S-p-bromobenzylglutathione diethyl ester.

Biochem Pharmacol. 1994 Jan 20;47(2):418-20.

Ulloa L, Ochani M, Yang H, Tanovic M, Halperin D, Yang R,
30 Czura CJ, Fink MP, Tracey KJ.

Ethyl pyruvate prevents lethality in mice with established lethal sepsis and systemic inflammation.

Proc Natl Acad Sci U S A. 2002 Sep 17;99(19):12351-6. Epub
2002 Sep 03

35

Valverde I, Cancelas J, Villanueva-Penacarrillo ML, Malaisse WJ.

Potentialiation by methyl pyruvate of GLP-1 insulintropic action in normal rats.

Int J Mol Med. 2001 Jun;7(6):621-3.

- 5 Vander Jagt DL, Hunsaker LA, Campos NM, Baack BR.
D-lactate production in erythrocytes infected with Plasmodium falciparum.
Mol Biochem Parasitol. 1990 Sep-Oct;42(2):277-84.
- 10 Varma SD, Devamanoharan PS, Ali AH.
Prevention of intracellular oxidative stress to lens by pyruvate and its ester.
Free Radic Res. 1998 Feb;28(2):131-5.
- 15 Vince R, Daluge S. Glyoxalase inhibitors. A possible approach to anticancer agents. J Med Chem. 1971 Jan;14(1):35-7.
- 20 Yang R, Uchiyama T, Watkins SK, Han X, Fink MP.
Ethyl pyruvate reduces liver injury in a murine model of extrahepatic cholestasis.
Shock. 2004 Oct;22(4):369-75

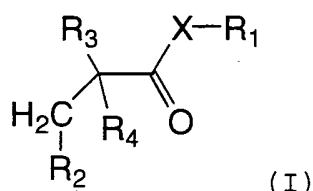
List of abbreviations

	2-DG	2-deoxyglucose
	ATP	Adenosine triphosphate
5	BBB	Blood Brain Barrier
	BL	butyl lactate
	BMP	bone morphogenic protein
	BP	butyl pyruvate
	CNS	Central nervous system
10	CSF	colony stimulation factor
	DBL	butyl D-lactate
	DEL	Ethyl D-lactate
	DHT	dihydro testosterone
	DMEM	Dulbecco's modified Eagle Medium
15	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)
20	ECACC	European Collection of Cell Cultures
	E.C.	Enzyme Commission
	EDTA	ethylene diamine tetraacetate
	EGF	epidermal growth factor
	EOB	ethyl -2-oxo-butyrate
25	EP	ethyl pyruvate
	EPO	erythropoietin
	FGF	fibroblast growth factor
	FCS	fetal calf serum
	GLO 1	Glyoxalase 1
30	HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
	HGF	hepatocyte growth factor
	IC-50	Inhibitor concentration to reach 50% of the maximal effect
	IU	international unit
35	IGF	insulin-like growth factor
	IL	interleukine
	INF	interferon

	LBL	Butyl L-lactate
	LEL	Ethyl L-lactate
	MES	4-morpholine ethanesulfonic acid
	MCT	monocarboxylate transporter
5	MIC	Minimal inhibition concentration
	MRSA	Methicillin-resistant <i>Staphylococcus Aureus</i>
	MSSA	Methicillin-sensitive <i>Staphylococcus Aureus</i>
	NFkB	nuclear factor kappa B
	NGF	nerve growth factor
10	INF	interferon
	O.D.	optical density
	PBS	phosphate buffered saline
	PDGF	platelet-derived growth factor
	PET	positron emission tomography
15	RPMI	Rosswell Park Memorial Institute
	SF	serum free
	TGF-	transforming growth factor beta
	TNF	Tumor necrosis factor
	VEGF	Vascular endothelial growth factor
20	YPD	yeast peptone dextrose medium

Claims

1. **Substance** according to the general formula (I)



for the inhibition of one or more selected from the group
 10 comprising glyoxalase I and glyoxalase II; 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-
 15 branched alkynyl, cycloalkynyl, alkoxyalkyl,
 alkoxycarbonylalkyl, aryl or a sugar residue; and

R2 is H or a branched or non-branched alkyl, cycloalkyl,
 branched or non-branched alkenyl, cycloalkenyl, branched or
 non-branched alkynyl, cycloalkynyl, alkoxyalkyl,
 20 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.

2. The substance according to claim 1, wherein R1 comprises
 25 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 or 2
 30 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.

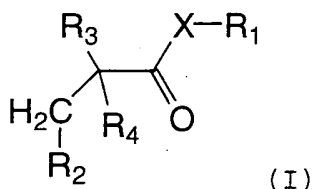
5. The substance according to any one of claims 1 to 4,
 5 wherein said substance is one or more selected from methyl pyruvate, ethyl pyruvate, isopropyl pyruvate, butyl pyruvate, isobutyl pyruvate, ethyl 2-oxobutyrate, butyl-2-oxobutyrate, cyclohexylmethyl pyruvate, or the said compound wherein X =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

10

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.

15

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



20

for the treatment, prophylaxis, or treatment and prophylaxis of a disease associated with increased glycolytic metabolism;

25

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-
 30 branched alkynyl, cycloalkynyl, alkoxyalkyl, alkoxycarbonylalkyl, aryl or a sugar residue; and

R₂ is H or a branched or non-branched alkyl, cycloalkyl, branched or non-branched alkenyl, cycloalkenyl, branched or

non-branched alkynyl, cycloalkynyl, alkoxyalkyl, alkoxycarbonylalkyl or aryl residue; and R3 and R4 together are =O; or R3 is OH and R4 is H; or R3 is H and R4 is OH.

5

8. The pharmaceutical composition according to claim 7, wherein R1 comprises 1 to 8 carbon atoms and R2 is H or comprises 1 to 8 carbon atoms.

10

9. The pharmaceutical composition according to claim 7, wherein R1 comprises 1 to 4 carbon atoms and R2 is H or comprises 1 or 2 carbon atoms.

15

10. The pharmaceutical composition according to any one of claims 7 to 9, wherein R1, R2 or R1 and R2 is selected from the group comprising methyl, ethyl, propyl, isopropyl, butyl, or isobutyl.

20

11. The pharmaceutical composition according to any one of claims 7 to 10, 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 =

25

S.

30

12. The pharmaceutical composition according to any one of claims 7 to 10, 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.

35

13. The pharmaceutical composition according to any one of claims 7 to 12, wherein said substance according to formula (I) is present in a therapeutically effective concentration.

14. The pharmaceutical composition according to any one of claims 7 to 13, which comprises one or more additional pharmaceutically active ingredients.

5 15. The pharmaceutical composition according to claim 14, wherein said additional pharmaceutically active ingredient is selected from chemotherapeutics.

10 16. The pharmaceutical composition according to claim 15, wherein said chemotherapeutic is one or more selected from azathioprine, bleomycin, cyclophosphamide, doxorubicin, mitomycin, taxol, vinblastine, 5-fluorouracil or cisplatin.

15 17. The pharmaceutical composition according to any one of claims 7 to 16, which further comprises one or more auxiliary substances, selected from the group comprising fillers, flavouring agents and stabilizers.

20 18. The pharmaceutical composition according to any one of claims 7 to 17, wherein said composition is in the form of a sustained release or controlled release galenic formulation.

25 19. The pharmaceutical composition according to any one of claims 7 to 18, which is for topic or systemic administration.

30 20. The pharmaceutical composition according to any one of claims 7 to 19, which is for one or more selected from the group comprising oral, intravenous, subcutaneous, intramuscular, intradermal, intraperitoneal, rectal, intranasal, epidural, percutaneous, transdermal, pulmonary or intratumoral administration, or administration as an aerosol, via mini-pumps, as mouth lavage, gel, plaster, and via microbubbles.

21. The pharmaceutical composition according to any one of claims 7 to 20, which is for use as a food supplement and/or beverage supplement.

5 22. The pharmaceutical composition according to any one of claims 7 to 21, wherein said disease is associated with increased formation of methylglyoxal.

10 23. The pharmaceutical composition according to any one of claims 7 to 22, wherein said disease is associated with increased activity of one or more selected from glyoxalase I and glyoxalase II.

15 24. The pharmaceutical composition according to any one of claims 7 to 23, wherein said disease is associated with one or more selected from increased cell proliferation and increased cell growth.

20 25. The pharmaceutical composition according to any one of claims 7 to 24 wherein said disease is cancer in a mammal including humans.

25 26. The pharmaceutical composition according to any one of claims 7 to 25, for the treatment, prophylaxis, or treatment and prophylaxis of malignant or benignant tumors.

30 27. The pharmaceutical composition according to any one of claims 7 to 26, for the treatment, prophylaxis, or treatment and prophylaxis of solid or non-solid tumors.

28. The pharmaceutical composition according to any one of claims 7 to 27, which is for the ex-vivo purging of cancer cells.

35 29. The pharmaceutical composition according to any one of claims 7 to 28, wherein said disease is resistant to one or

more selected from the group comprising chemotherapeutic agents and radiation therapy.

30. The pharmaceutical composition according to any one of
5 claims 7 to 29, wherein said disease is one or more selected
from carcinomas including breast, lung, bladder, thyroid
gland, prostate, intestine, rectum, pancreas, stomach, liver,
uterus, and ovary carcinomas; plasmacytoma; lymphomas
including non-Hodgkin-Lymphoma, Hodgkin-Lymphoma, myeloma;
10 leukemia including acute and chronic lymphoblastic leukemia,
acute and chronic myeloblastic leukaemia; brain tumors
including astrocytoma, glioma, medulloblastoma, glioblastoma,
oligodendroglioma, neuroblastoma; sarcomas including
fibrosarcoma, liposarcoma, angiosarcoma, mesothelioma,
15 chrondrosarcoma, osteosarcoma, and melanoma.

31. The pharmaceutical composition according to any one of
claims 7 to 30, which is for the treatment of a mammal
concomitantly suffering from an infectious disease.

20

32. The pharmaceutical composition according to claim 31,
wherein said infectious disease is one or more selected from
a bacterial, protozoal or fungal infection and helminthes.

25 33. The pharmaceutical composition according to claim 31 or
32, wherein said infectious disease is caused by one or more
of Candida, Aspergillus, Cryptococcus, Zygomycetes,
Dermatophytes, Blastomyces, Histoplasma, Coccidioides,
Sporothrix, Trypanosoma, Leishmania, Plasmodium, Toxoplasma,
30 helminthes, Acrobacter, Actinobacillus, Actinomyces,
Bacteroides, Brucella, Chlamydia, Clostridium, Campylobacter,
Escherichia, Enterobacter, Enterococcus, Eubacterium,
Fusobacterium, Helicobacter, Hemophilus, Legionella,
Listeria, Mycobacteria, Mycoplasma, Neisseria, Pasteurella,
35 Peptostreptococcus, Pneumococcus, Pneumocystis,
Porphyromonas, Prevotella, Pseudomonas, Salmonella, Shigella,

Spirochetes, Staphylococcus, Streptococcus, Treponema, Vibrio, Yersinia, Escherichia coli or Pneumocystis carinii.

34. The pharmaceutical composition according to any one of
5 claims 31 to 33, wherein said infectious disease is an opportunistic infection.

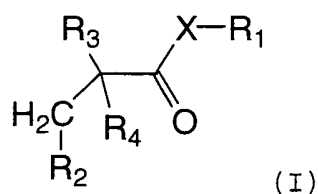
35. The pharmaceutical composition according to any one of
10 claims 31 to 34, wherein said infectious disease is characterized by antibiotic resistance.

36. The pharmaceutical composition according to any one of
15 claims 7 to 35, which is for use in a mammal having a reduced blood glucose level.

37. The pharmaceutical composition according to any one of
20 claims 25 to 36, wherein said mammal is going to receive, is currently receiving, or has received conventional cancer therapy.

38. The pharmaceutical composition according to claim 37,
wherein said conventional cancer therapy is one or more of chemotherapy, surgery, radiotherapy or brachytherapy.

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



30 for the preparation of a medicament for the treatment and/or prophylaxis of a disease associated with increased glycolytic metabolism; wherein

X is O or S; and

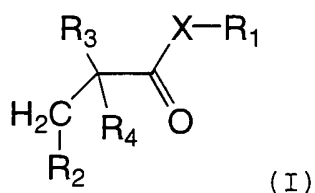
R1 is a branched or non-branched alkyl, cycloalkyl, branched or non-branched alkenyl, cycloalkenyl, branched or non-branched alkynyl, cycloalkynyl, alkoxyalkyl,

5 alkoxycarbonylalkyl, aryl or a sugar residue; and

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

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

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



20

wherein X is O or S; and

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

25 alkoxycarbonylalkyl, aryl or a sugar residue; and

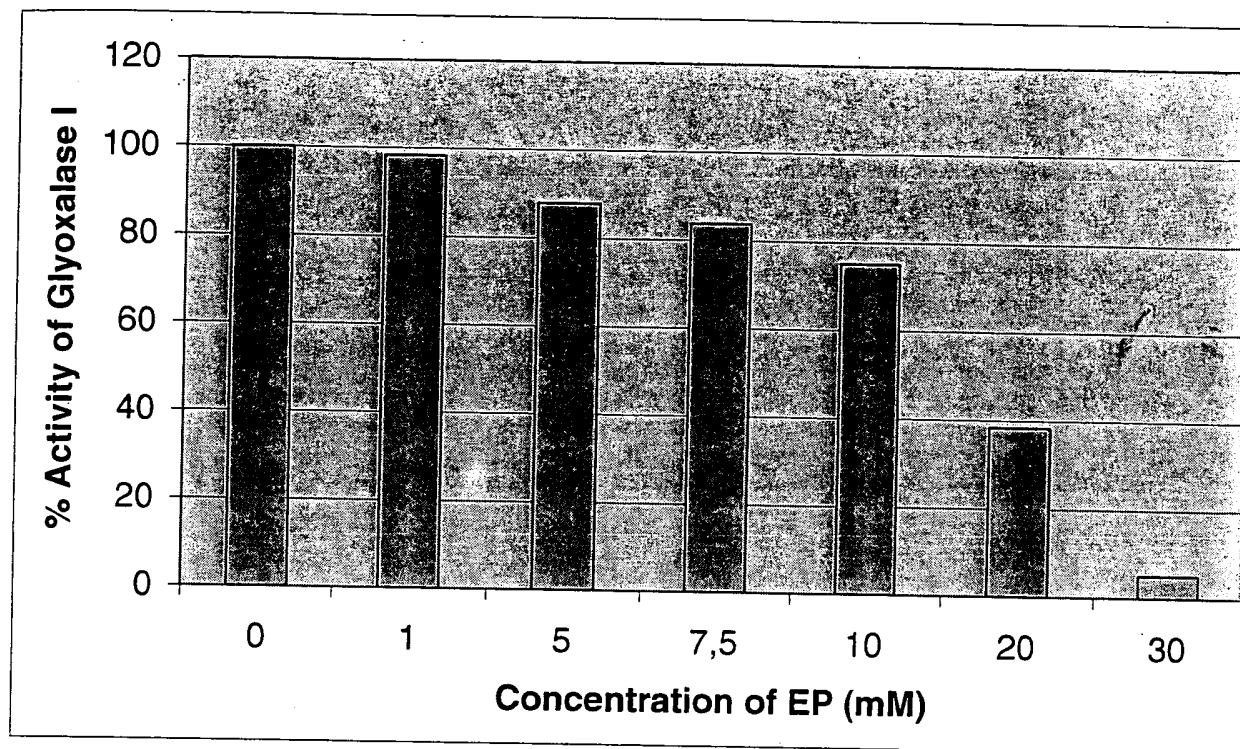
R2 is H or a branched or non-branched alkyl, cycloalkyl, branched or non-branched alkenyl, cycloalkenyl, branched or non-branched alkynyl, cycloalkynyl, alkoxyalkyl, alkoxycarbonylalkyl or aryl residue; and

30 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 disease associated with increased glycolytic metabolism.

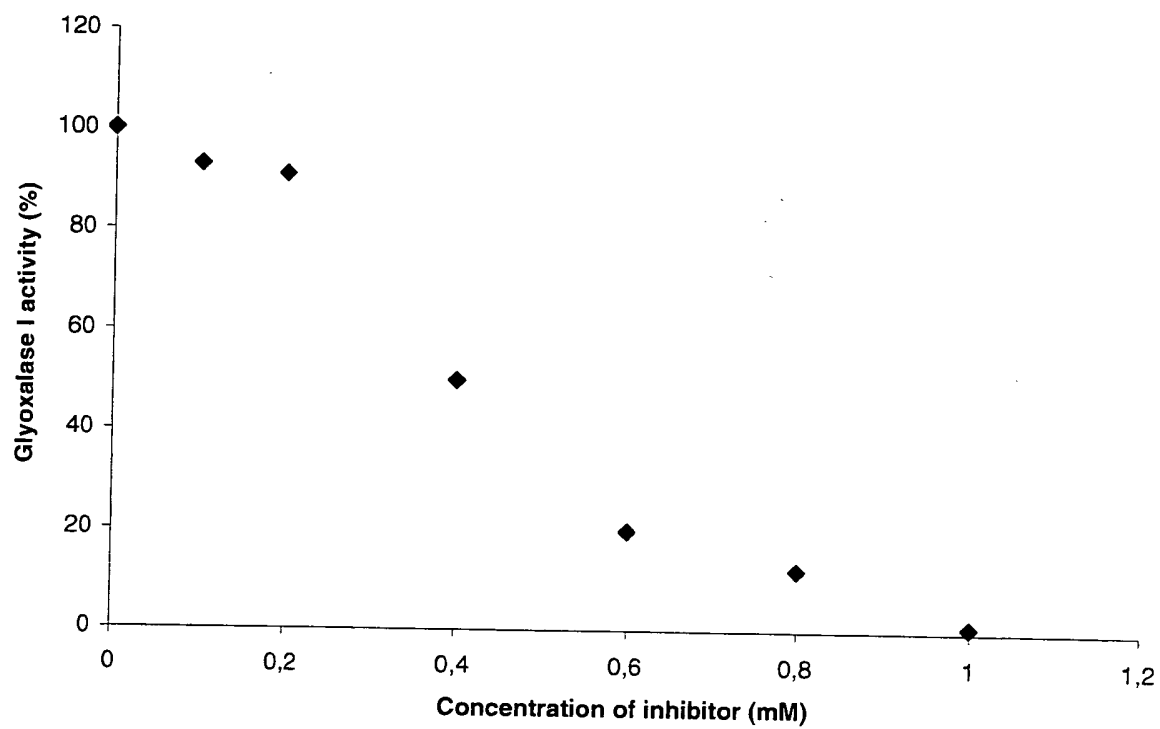
1/17

Fig. 1



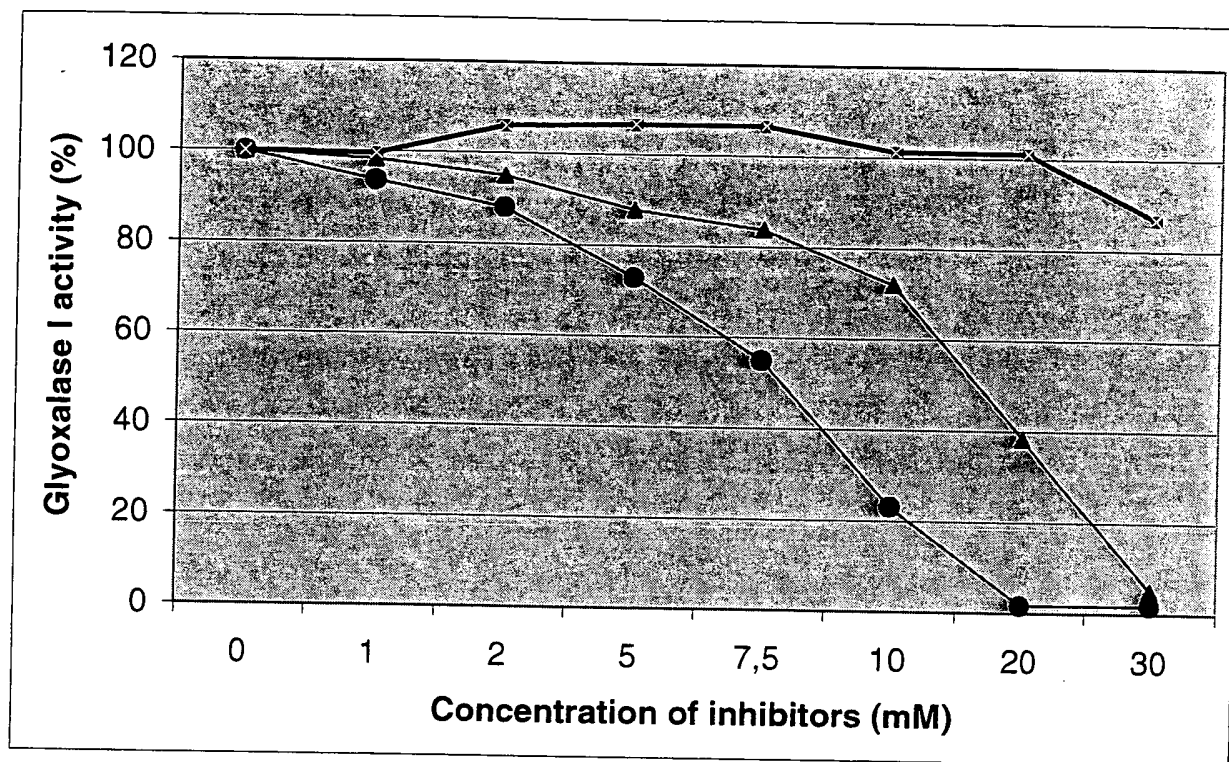
2/17

Fig. 2



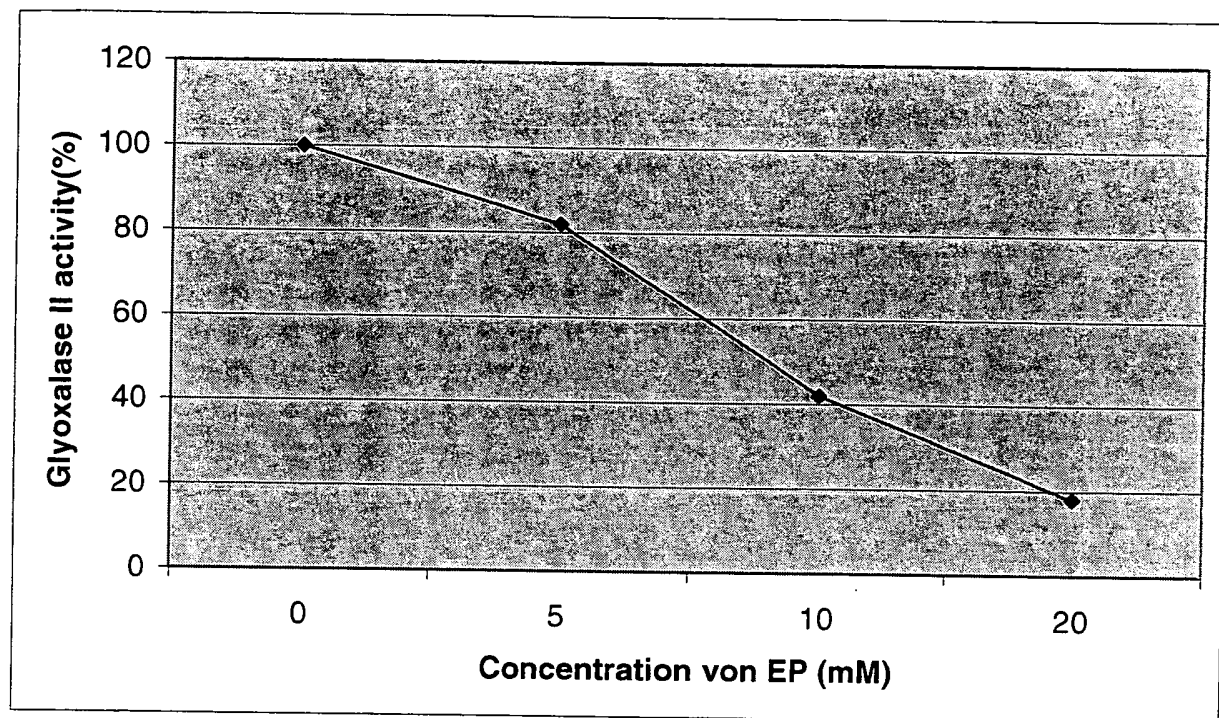
3/17

Fig. 3



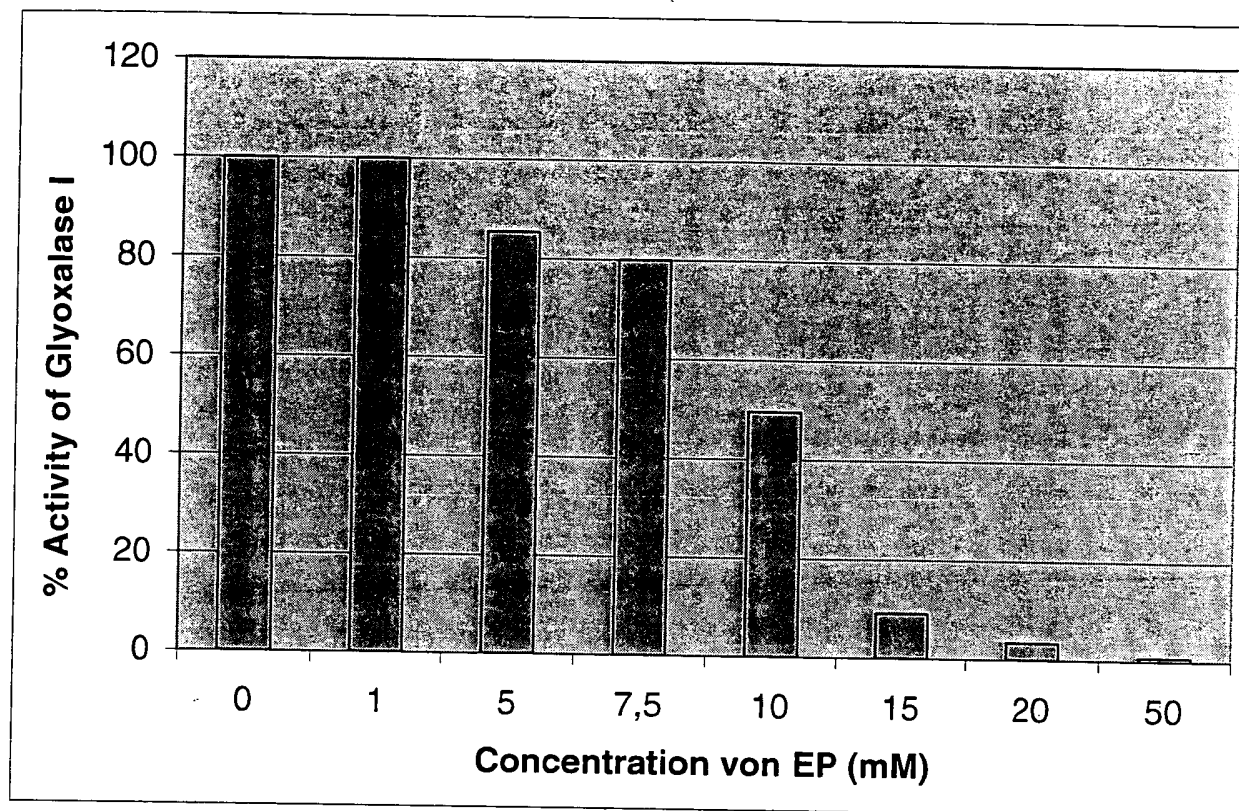
4/17

Fig. 4



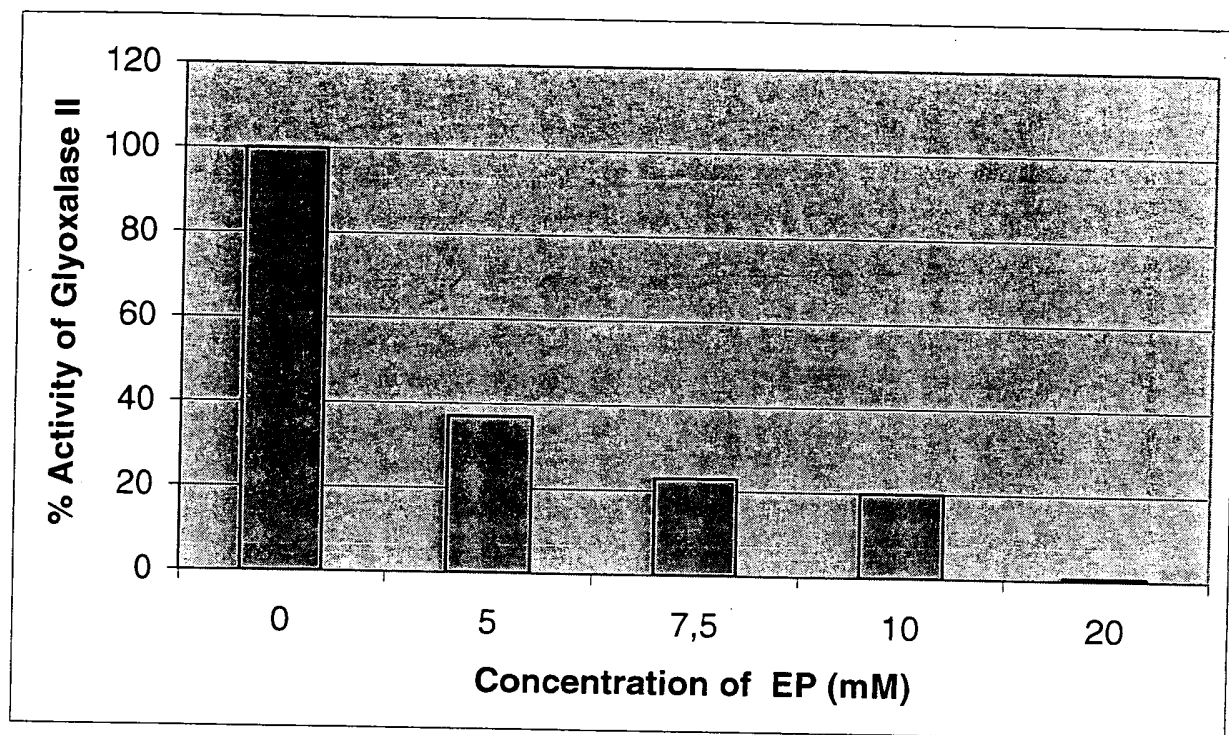
5/17

Fig. 5



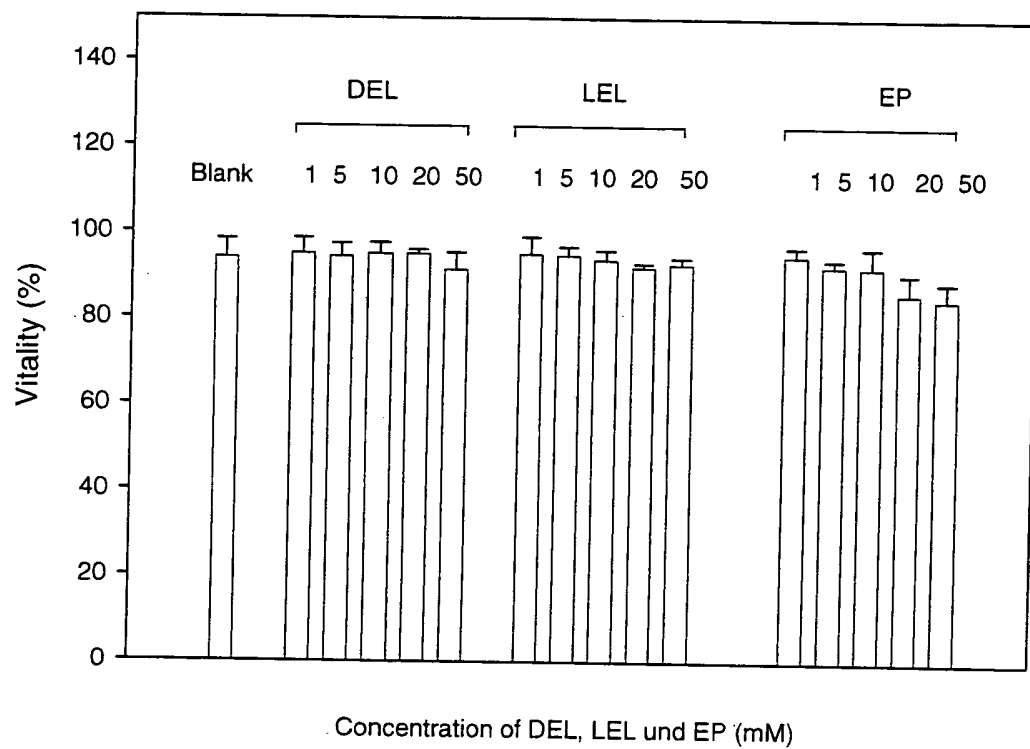
6/17

Fig. 6



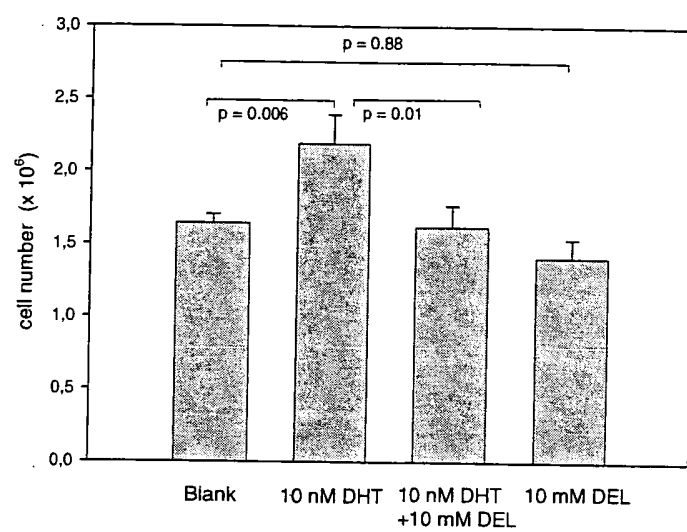
7/17

Fig. 7



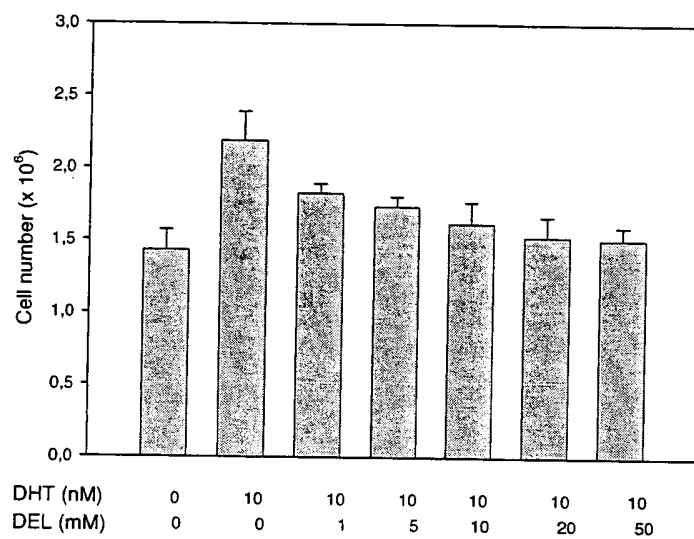
8/17

Fig. 8



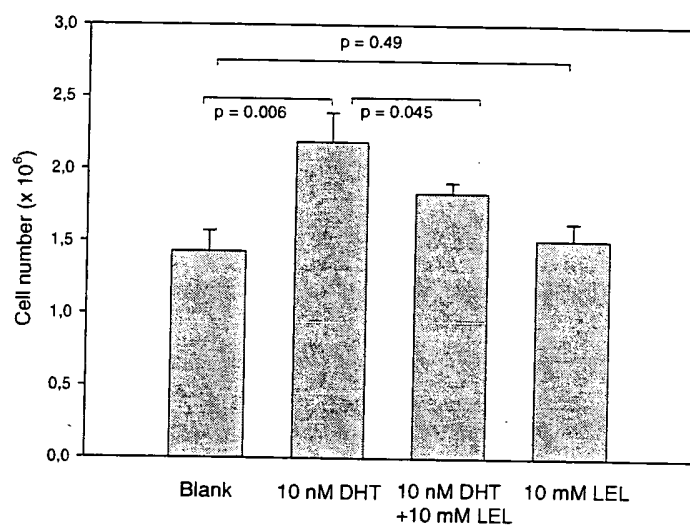
9/17

Fig. 9



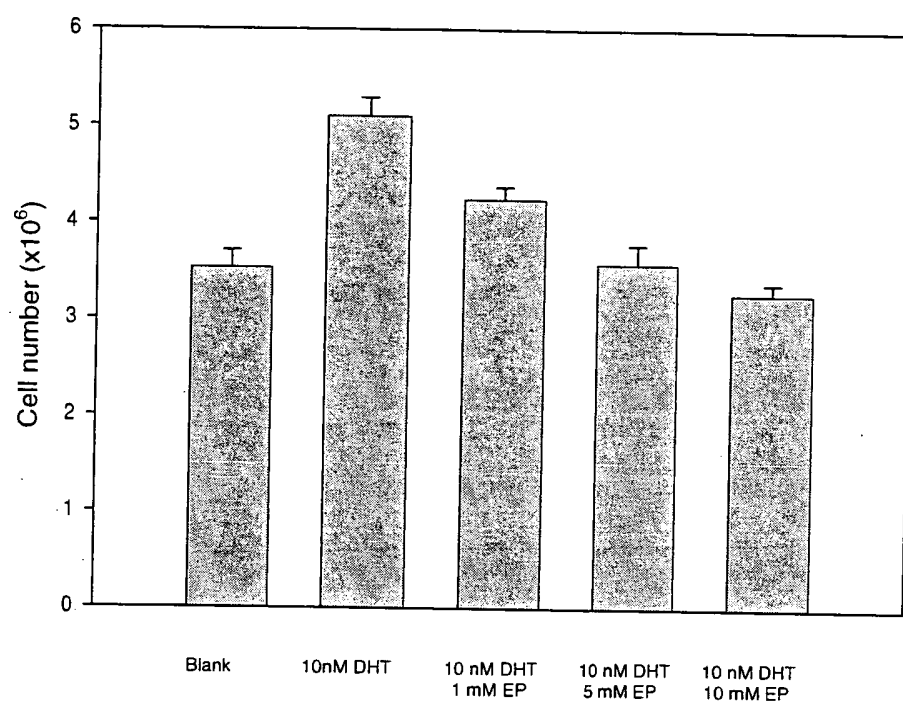
10/17

Fig. 10



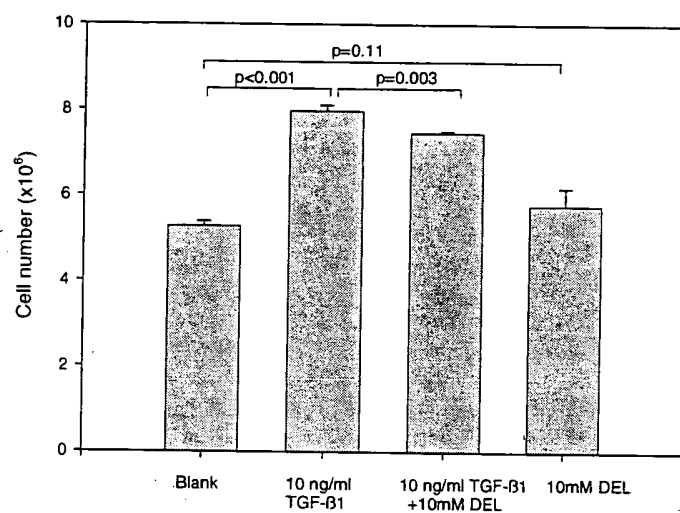
11/17

Fig. 11



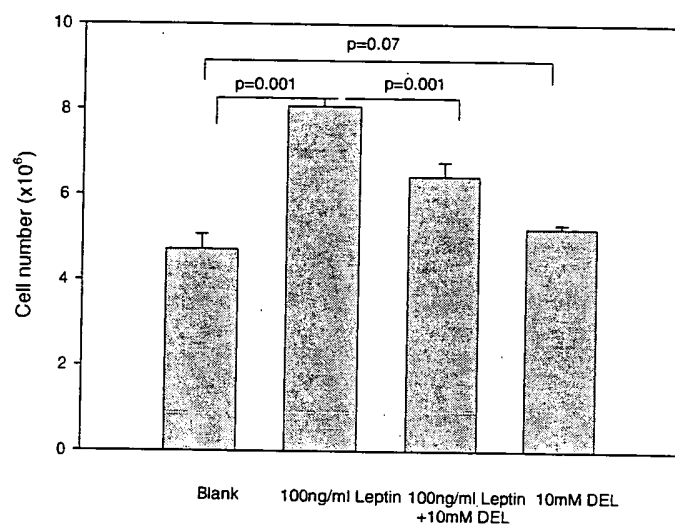
12/17

Fig. 12



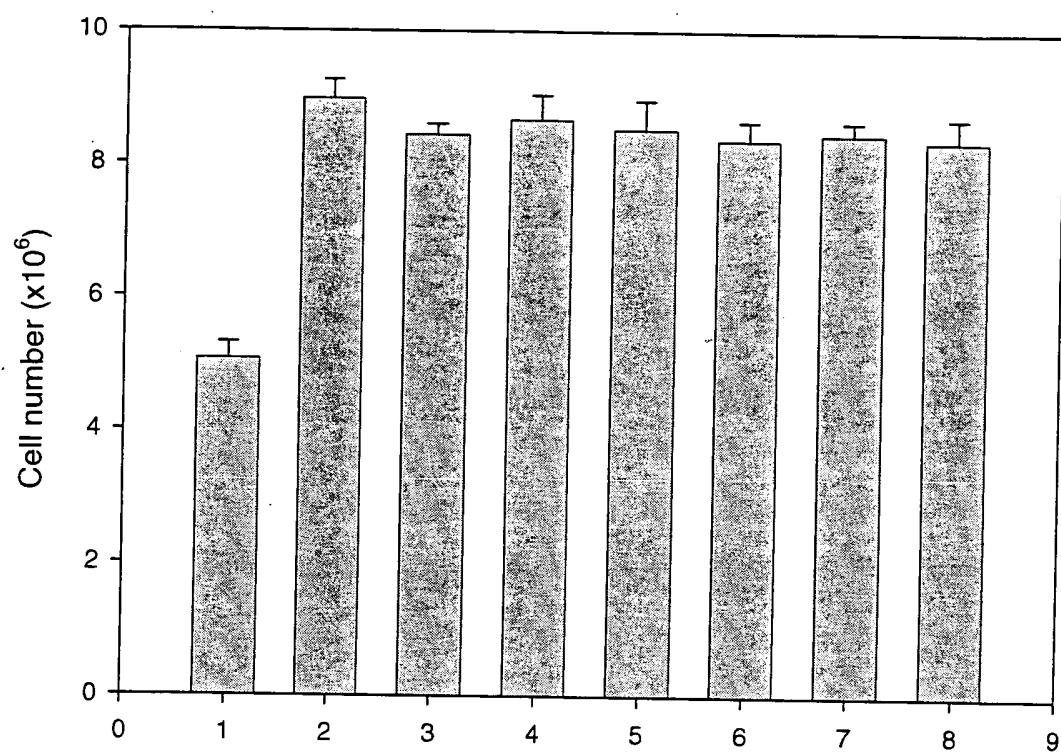
13/17

Fig. 13



14/17

Fig. 14



15/17

Fig. 15A

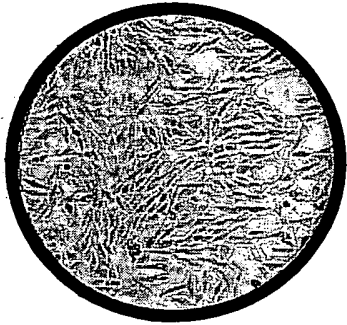


Fig. 15C

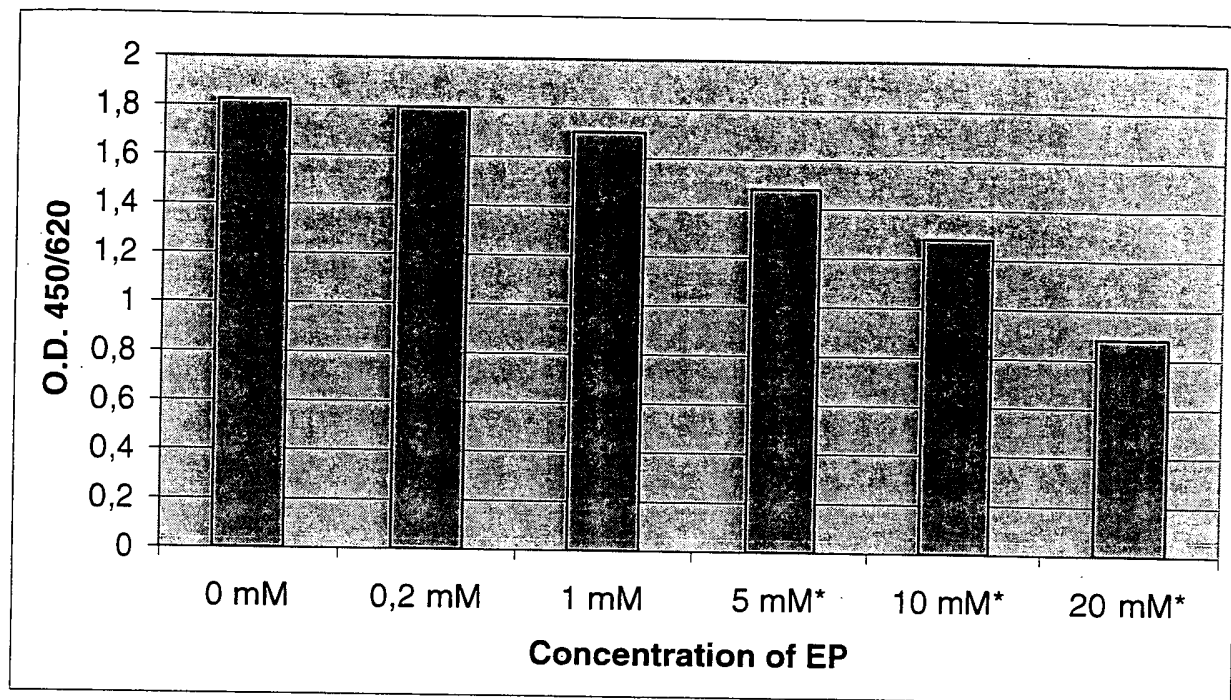


Fig. 15B



16/17

Fig. 16



17/17

Fig.17

