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(54) **COMPOUNDS FOR THE MODULATION OF THE GLYKOLYSIS-ENZYME-AND/OR OF THE TRANSAMINASE-COMPLEX**

(76) Inventors: **Erich Eigenbrodt**, Linden (DE); **Hans Scheefers**, Wettenberg-Wissmar (DE); **Sybille Mazurek**, Linden (DE)

Correspondence Address:
Mayer Fortkort & Williams, PC
2nd Floor
251 North Avenue West
Westfield, NJ 07090 (US)

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(52) **U.S. Cl.** **514/506**; 435/7.1

(57) **ABSTRACT**

The invention relates to compounds for the modulation of the glycolysis enzyme complex and of the transaminase complex, pharmaceutical compositions containing such compounds as well as uses of such compounds for preparing pharmaceutical compositions for treating various diseases.

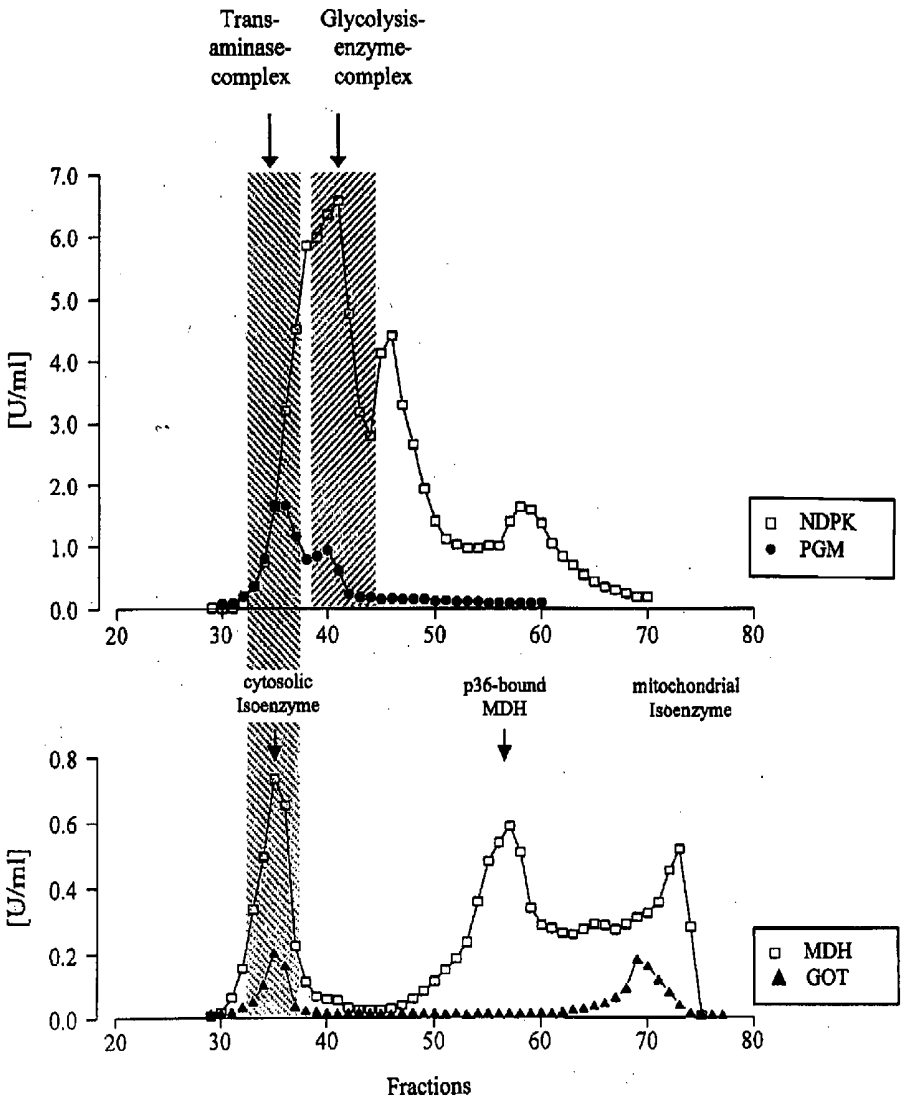


FIG.1a

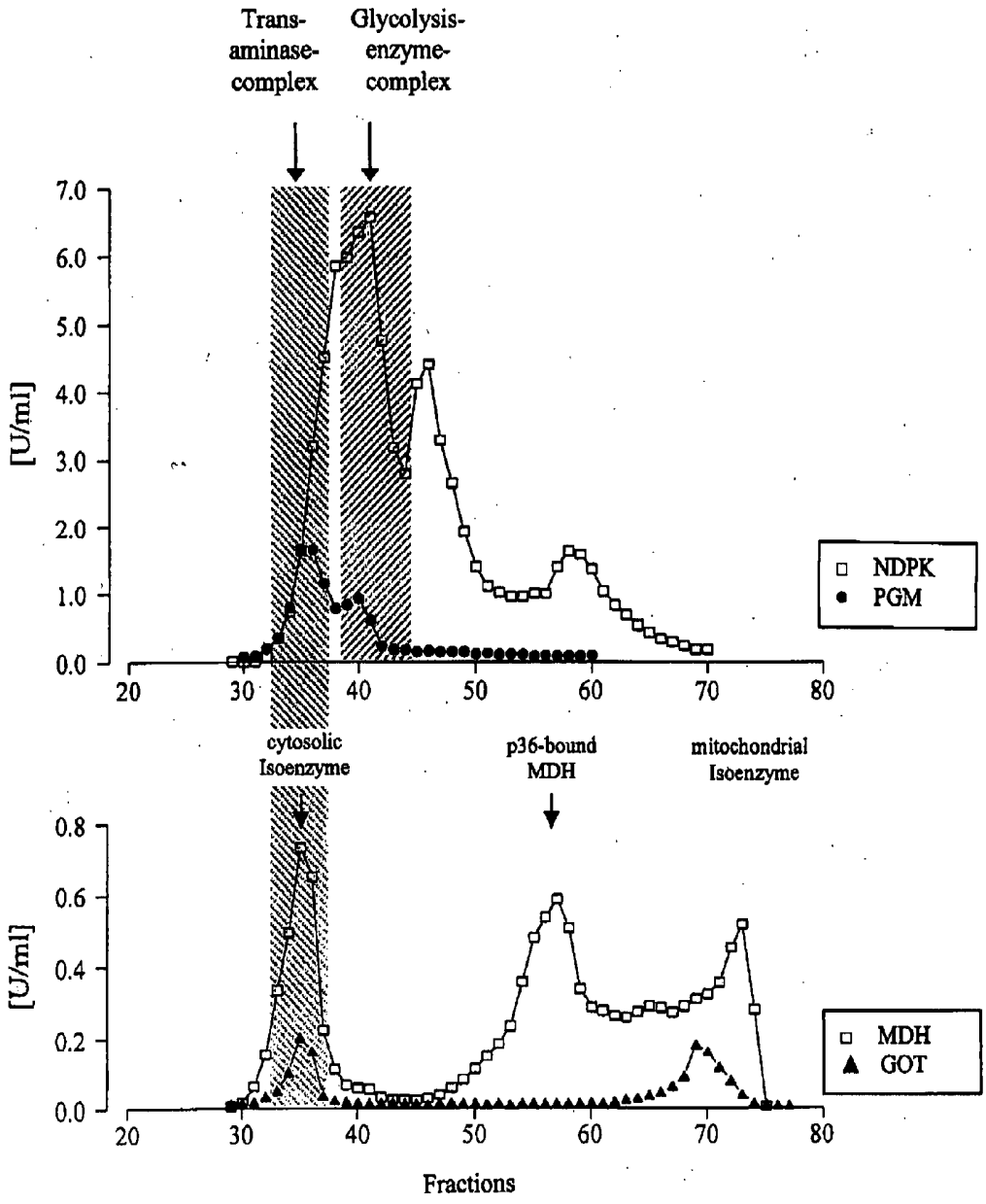

Trans-
aminase-
complex
Glycolysis-
enzyme-
complex
[U/ml]
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
20
30
40
50
60
70
80
NDPK
PGM
cytosolic
Isoenzyme
p36-bound
MDH
mitochondrial
Isoenzyme
[U/ml]
0.8
0.6
0.4
0.2
0.0
MDH
GOT
Fractions

FIG.1b

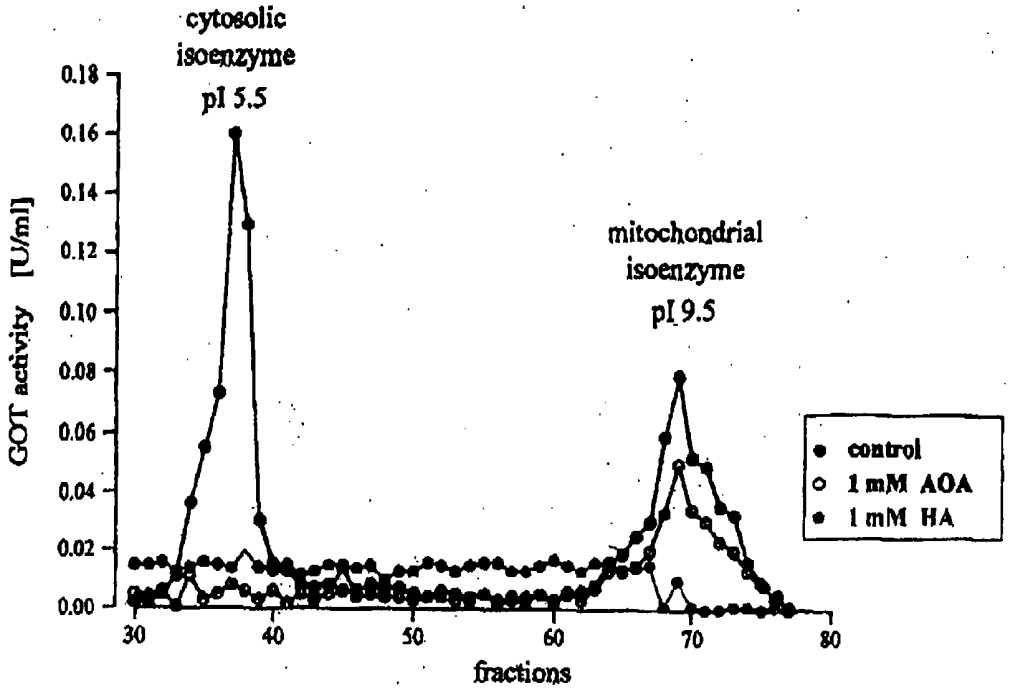

cytosolic
isoenzyme
pI 5.5
mitochondrial
isoenzyme
pI 9.5
GOT activity [U/ml]
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
30
40
50
60
70
80
fractions
control
1 mM AOA
1 mM HA

FIG.2a

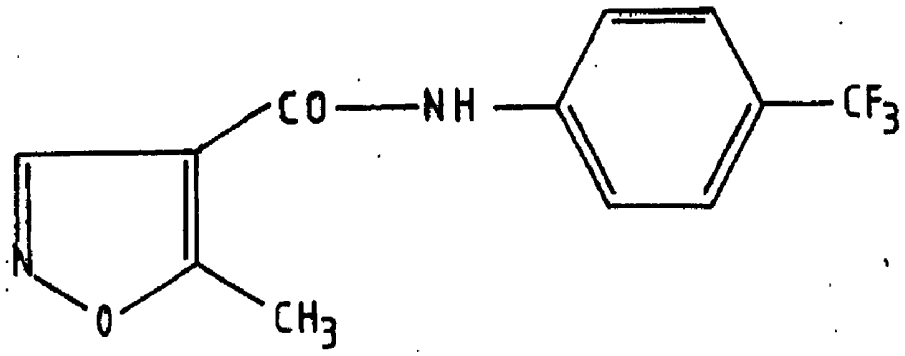


FIG.2b

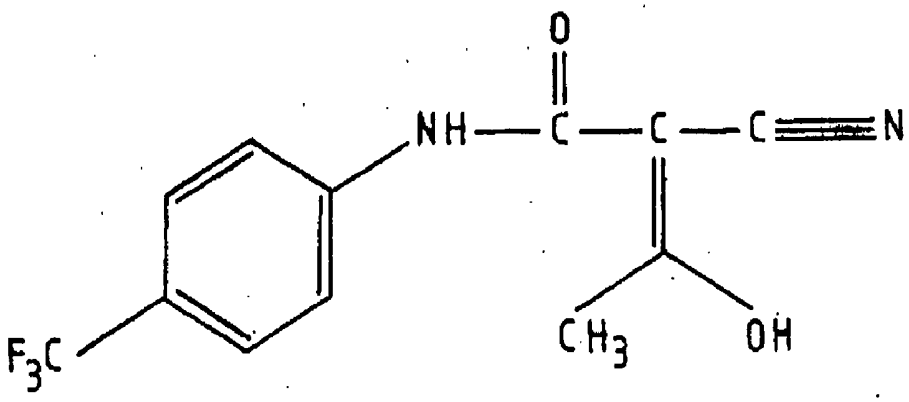


FIG.2c

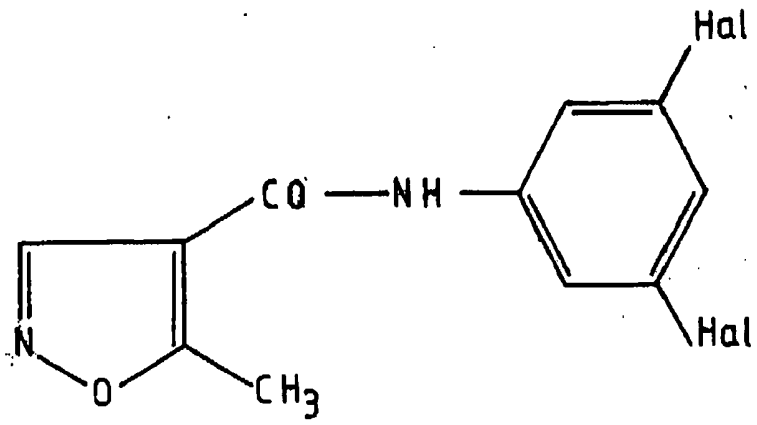


FIG.2d

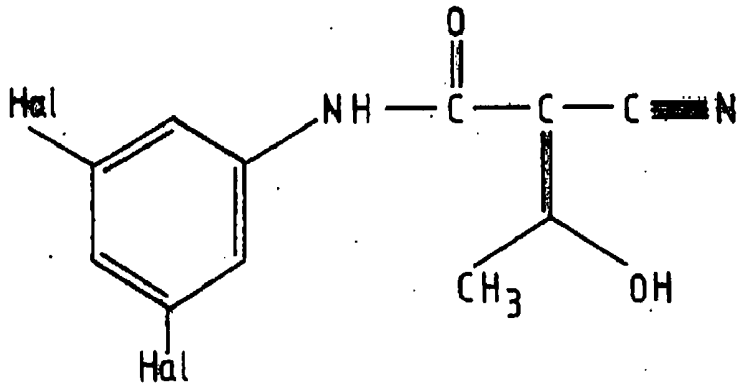


FIG.2e

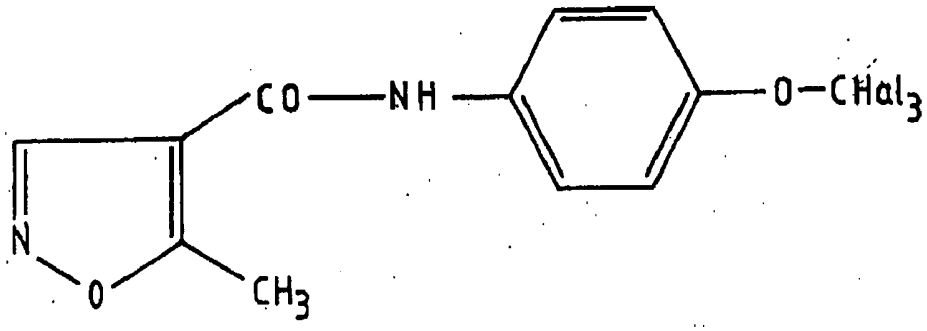


FIG.2f

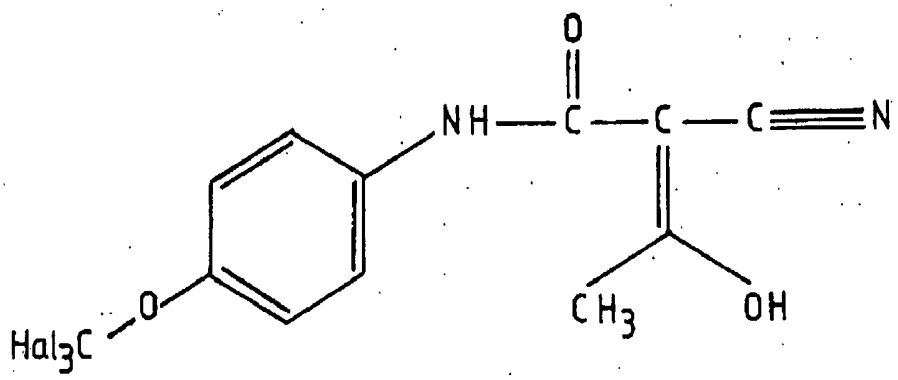


FIG.2g

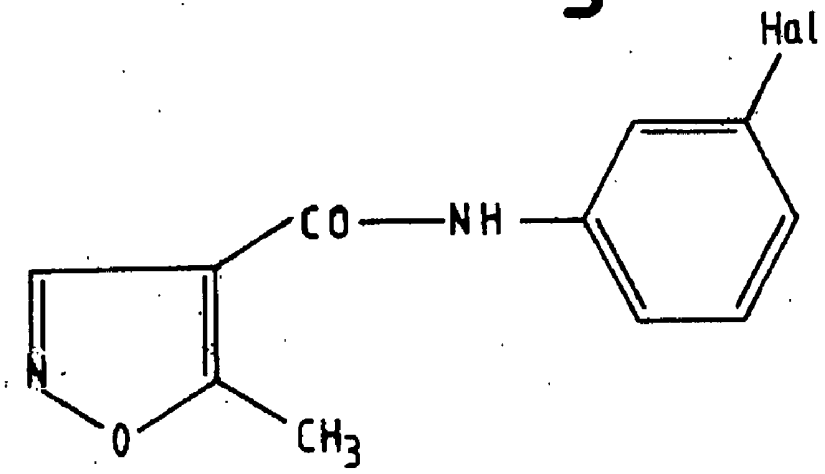
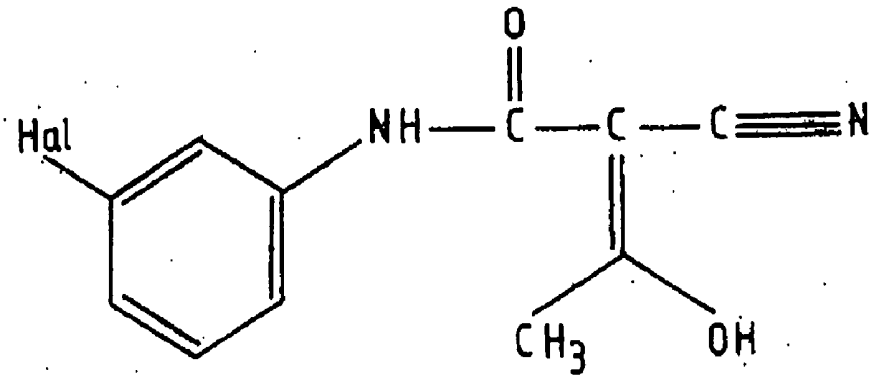


FIG.2h



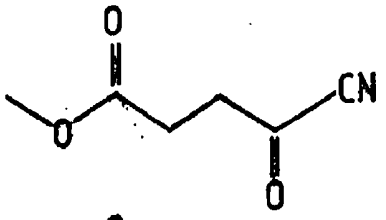


FIG.3a

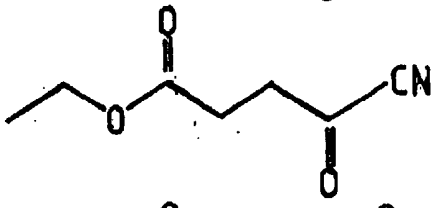


FIG.3b

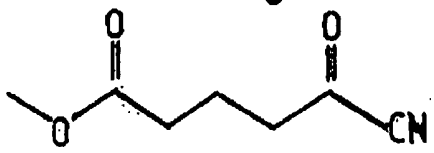


FIG.3c

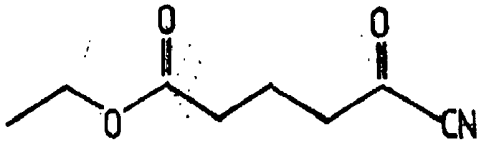


FIG.3d

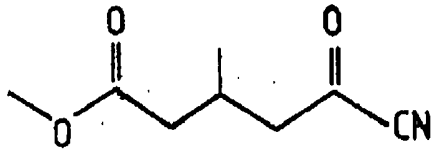


FIG.3e

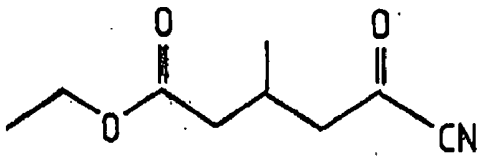


FIG.3f

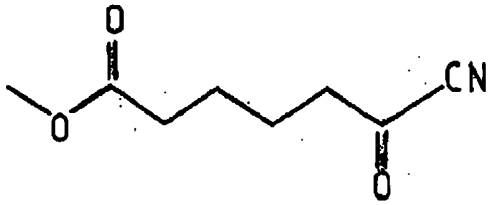


FIG.3g

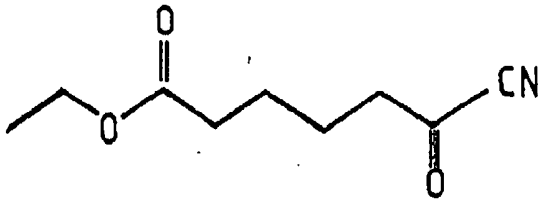


FIG.3h

FIG.4a

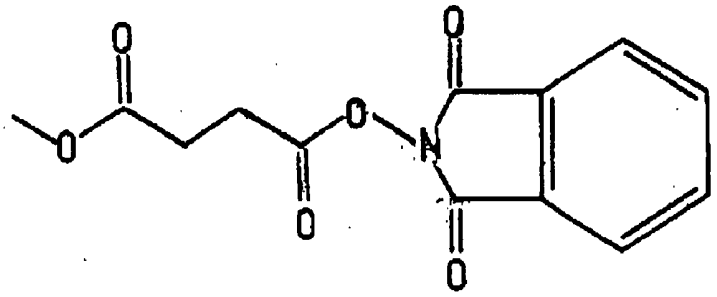


FIG.4b

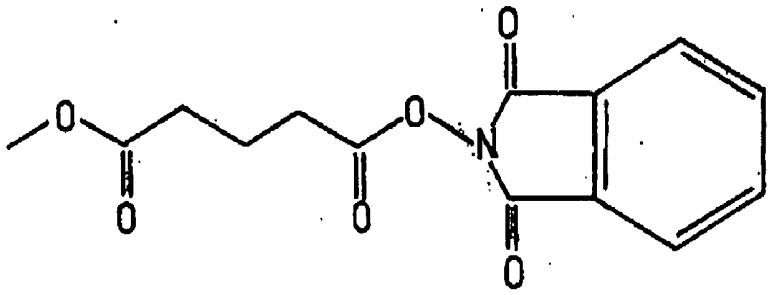
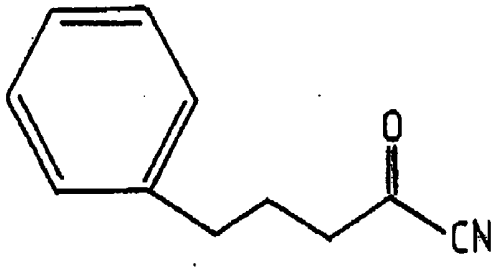


FIG.4c



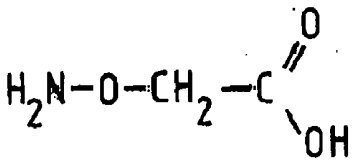


FIG.5a

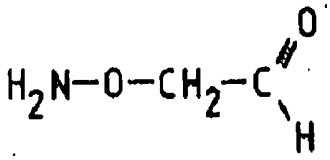


FIG.5b

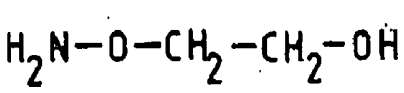


FIG.5c

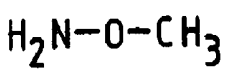


FIG.5d

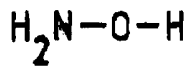


FIG.5e

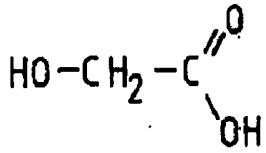


FIG.5f

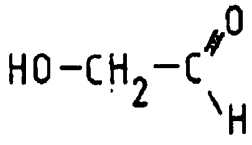


FIG.5g

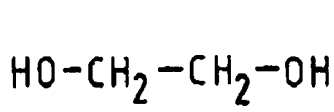


FIG.5h

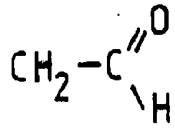


FIG.5i

FIG.6a

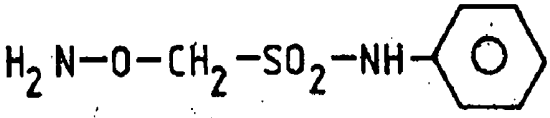


FIG.6b

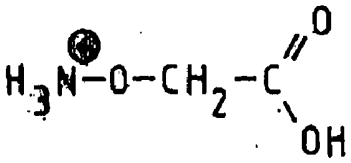


FIG.6c

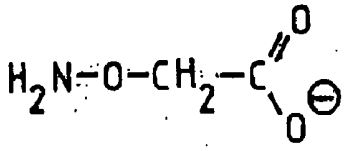


FIG.6d

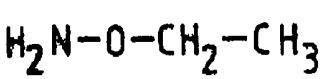


FIG.6e

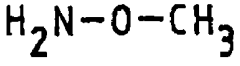


FIG.6f

COMPOUNDS FOR THE MODULATION OF THE GLYKOLYSIS-ENZYME-AND/OR OF THE TRANSAMINASE-COMPLEX

STATEMENT OF RELATED APPLICATION

[0001] This is a continuation of U.S. patent application Ser. No. 10/618,578, filed Jul. 11, 2003, entitled "Compounds For The Modulation Of The Glykolysis-Enzyme-And/Or Of The Transaminase-Complex, which is incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

[0002] The invention relates to compounds for the modulation of the glycolysis enzyme and/or transaminase complex and thus in particular to the growth inhibition of cells and/or bacteria, pharmaceutical compositions containing such compounds as well as uses of such compounds for preparing pharmaceutical compositions for treating various diseases.

BACKGROUND OF THE INVENTION

[0003] Cancer is one of the most prevalent causes of death today, and the number of cancer cases in industrialized countries continues to grow. This is mainly based on the fact that malignant tumors are diseases of advanced age and due to successes in controlling infectious diseases, it is likely that more people will reach this age.

[0004] In spite of much progress in the diagnostic and therapeutic fields, the odds for overcoming the most prevalent types of cancer types are seldom higher than 20%. A cancerous tumor nowadays can be destroyed or its growth inhibited. A re-conversion of a tumor cell into a normal cell is, however, not yet possible. Rather, the most important therapeutic measures, via operation and irradiation, remove cancer cells from the organism. The presently used chemotherapeutic agents for cancer, the cytostatics, also lead to the destruction or damaging of tumor cells only. In most cases, the effect is so non-specific that simultaneous heavy damage to healthy cells will occur.

[0005] In general, tumor cells have a metabolism differing from healthy cells, in particular glycolysis. Thus, a change in the isoenzyme system involved in glycolysis and a change in the transport of NADH is typical for tumor cells. Among other effects, the activity of the enzymes involved in glycolysis is increased. This permits high reaction rates under the aerobic conditions typical for tumor cells. For details, reference is made to E. Eigenbrodt et al., Biochemical and Molecular Aspects of Selected Cancers, Vol. 2, p. 311 ff (1994).

[0006] Various other diseases mentioned below are either characterized by an excessive metabolism by glycolysis enzyme complex and can be treated by the reduction or inhibition thereof.

PRIOR ART

[0007] From the document E. Eigenbrodt et al., Biochemical and Molecular Aspects of Selected Cancers, Vol. 2, p. 311 ff (1994), it is known that glucose analogs are used for inhibiting the glycolysis. Other approaches known here from are the use of inhibitors of glycolysis isoenzymes, for instance by suitable chelation or inhibition of chelations. As a result, the tumor cells are, in a manner of speaking, starved out. However, a problem with the above compounds is that many of them are genotoxic and/or not sufficiently specific for tumor cells.

SUMMARY OF THE INVENTION

[0008] It is the technical object of the present invention to specify active ingredients that are able to modulate or inhibit the glycolysis enzyme and transaminase complex, in particular the proliferation of cancer cells and to thus inhibit the growth of neoplastic tumors as well as defense over-reactions of the body, such as septic shock, autoimmune diseases, transplant rejections as well as acute and chronic inflammatory diseases, and that simultaneously have only slight to no cytotoxicity at all with regard to cells which have an intact glycolysis enzyme complex or other complex structures. In addition, it is intended to inhibit the growth of unicellular organisms.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] FIG. **1***a* is a graphical representation showing the migration of PGM from the glycolysis enzyme completed into the transaminase complex.

[0010] FIG. **1***b* is a graphical representation showing the effect of aminoacetate (AOA) and hydrozylamine (HA) on the activity of the cytosolic and mitochondrial isoenzyme of the GOT in vitro.

[0011] FIG. **2***a* is the structural formula of 5-methyl-N-[4-(trifluoromethyl)phenyl]isoxazole-4-carboxamide.

[0012] FIG. **2***b* is the structural formula of (2Z)-2-cyano-3-hydroxy-N-[4-(trifluoromethyl)phenyl]but-2-enamide.

[0013] FIG. **2***c* is the structural formula of N-(3,5-dihalogenophenyl)-5-methylisoxazole-4-carboxamide.

[0014] FIG. **2***d* is the structural formula (2Z)-2-cyano-N-(3,5-dihalogenophenyl)-3-hydroxybut-2-enamide.

[0015] FIG. **2***e* is the structural formula of 5-methyl-N-[4-(trihalogenomethoxy)phenyl]isoxazole-4-carboxamide.

[0016] FIG. **2***f* is the structural formula of (2Z)-2-cyano-3-hydroxy-N-[4-(trihalogenomethoxy)phenyl]but-2-enamide.

[0017] FIG. **2***g* is the structural formula of N-(3-halogenophenyl)-5-methylisoxazole-4-carboxamide.

[0018] FIG. **2***h* is the structural formula of (2Z)-2-cyano-N-(3-halogenophenyl)-3-hydroxybut-2-enamide.

[0019] FIG. **3***a* is the structural formula of methyl 4-cyano-4-oxobutanoate.

[0020] FIG. **3***b* is the structural formula of ethyl 4-cyano-4-oxobutanoate.

[0021] FIG. **3***c* is the structural formula of methyl 5-cyano-5-oxopentanoate.

[0022] FIG. **3***d* is the structural formula of ethyl 5-cyano-5-oxopentanoate.

[0023] FIG. **3***e* is the structural formula of methyl 5-cyano-3-methyl-5-oxopentanoate.

[0024] FIG. **3***f* is the structural formula of ethyl 5-cyano-3-methyl-5-oxopentanoate.

[0025] FIG. **3***g* is the structural formula of methyl 6-cyano-6-oxohexanoate.

[0026] FIG. **3***h* is the structural formula of ethyl 6-cyano-6-oxohexanoate.

[0027] FIG. **4***a* is the structural formula of methyl 4-[(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)oxy]-4-oxobutanoate.

[0028] FIG. **4***b* is the structural formula of methyl 5-[(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)oxy]-5-oxopentanoate.

[0029] FIG. **4***c* is the structural formula of 2-oxo-5-phenylpentanenitrile.

[0030] FIG. **5***a* is the structural formula of (aminooxy-)acetic acid.

[0031] FIG. **5***b* is the structural formula of (aminooxy)acetaldehyde.

[0032] FIG. **5***c* is the structural formula of 2-(aminooxy-)ethanol.

[0033] FIG. **5***d* is the structural formula of O-methylhydroxylamine.

[0034] FIG. **5***e* is the structural formula of hydroxylamine.

[0035] FIG. **5***f* is the structural formula of glycol acid.

[0036] FIG. **5***g* is the structural formula of glycolaldehyde.

[0037] FIG. **5***h* is the structural formula of ethylene glycol.

[0038] FIG. **5***i* is the structural formula of acetaldehyde.

[0039] FIG. **6***a* is the structural formula of 1-(aminooxy)methanesulfonamide.

[0040] FIG. **6***b* is the structural formula of 1-(aminooxy)-N-phenylmethanesulfonamide.

[0041] FIG. **6***c* is the structural formula of (carboxymethoxy)ammonium.

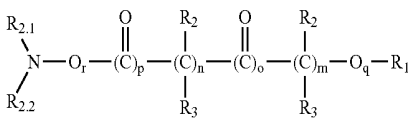
[0042] FIG. **6***d* is the structural formula of (aminooxy)acetate.

[0043] FIG. **6***e* is the structural formula of O-ethylhydroxylamine.

[0044] FIG. **6***f* is the structural formula of O-methylhydroxylamin.

DETAILED DESCRIPTION OF THE INVENTION

[0045] For achieving said technical object, the invention teaches a compound according to formula I



I

wherein R1=-H, —CN, —COO+, —COS+, —COOH, —COSH, —COOR1.1, —COSR1.1, N-phthalimidyl, wherein R1.1=-H, C1-10 alkyl, C1-10 aralkyl or aryl, wherein R2=-H, C1-C4 alkyl, —OR1.1, -Hal (—F, —Cl, —Br, -J), —NR2.1R2.2, —Am, —O—Am, —S—Am, wherein R3=-H, C1-C4 alkyl, —OR1.1, -Hal (—F —Cl, —Br, -J), —NR2.1R2.2, —Am, —O—Am, —S—Am, wherein R2.1=-H, C1-10 alkyl, C1-10 aralkyl or aryl, wherein R2.2=-H, C1-10 alkyl, C1-10 aralkyl or aryl, wherein R2.1 and R2.2 may be identical or different, wherein n and m may be identical or different and 0 to 10, wherein o and p may be identical or different and 0 to 3, wherein o>0, if n and m=0, wherein R2 and R3 may be identical or different for Cn and/or Cm, wherein R2 may be identical or different for every Cx=1 . . . n, wherein R3 may be identical or different for every Cy=1 . . . m, wherein —Am is an amino acid radical, wherein q and r=0 or 1 and identical or different, wherein —O_r— and/or —O_q— may also be replaced by —S_r— or —S_q—, resp., wherein —NR2.1R2.2 may be replaced by a linear or branched —C1-C20 alkyl, aralkyl or aryl, wherein a group —CN, —(CO) —CN, —(CO)—O—R1 or —(CO)—R1 or —C—O—R1 may be replaced by —SO_2—NR2.1R2.2, or a physiologically well tolerated salt of such a compound.

[0046] An amino acid radical is defined in an amino acid as follows: NH_2—CHAm—COOH. These are, in particular, amino acid radicals of the proteinogenic amino acids, especially of the essential amino acids. For compounds according to the invention which possess an optical activity (for instance according to embodiments of claim **3**), the various optical variants such as L and D types are also included. Corresponding considerations apply in the case of chiral centers.

[0047] Particularly suited are compounds according to the invention, wherein at least one of the R2 is —Am, wherein —Am preferably represents an amino acid radical of an essential amino acid, wherein in particular q=0 and r=1 or q=1 and r=0 or q=1 and r=1, m=1, R3=-H, n=o=p=0, R2.1=R2.2=-H.

[0048] Further, various specific groups are preferred, namely: i) wherein n=o=p=0, wherein m=0 to 4, wherein R2=R3=-H, wherein R2.1=R2.2=-H, wherein q=0 and r=1, ii) wherein m=p=0, wherein o=1, wherein n=0 to 4, wherein R2=H, wherein R3=-H or -Hal in the case Cx=1, wherein R3=-H for all Cx=n>1, wherein R2.1=R2.2=-H, wherein q=0 and r=1, iii) wherein m=1 to 4, wherein n=o=p=0, wherein R2=H, wherein R3=-H or -Hal in the case Cy=1, wherein R3=-H for all Cy=m>1, wherein R2.1=R2.2=-H, wherein q=0 and r=1, iv) wherein o=p=1, wherein m=0, wherein n=0 to 4, wherein R2=R3=-H, wherein R2.1=R2.2=-H, wherein q=0 and r=1, v) wherein n=p=0, wherein o=1, wherein m=0 to 4, wherein R2=R3=-H, wherein R2.1=R2.2=-H, wherein q=0 and r=1, or vi) wherein m=p=0, wherein o=1, wherein n=1 to 4, wherein R2=R3=-H, wherein R2.1=R2.2=-H, wherein q=0 and r=1.

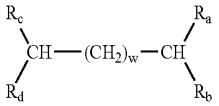
[0049] Generally, one R2 may be replaced by —Am.

[0050] Examples for compounds wherein —NR2.1R2.2 is replaced by —C1-C20 alkyl are: CH_3—O—$(CH_2)_m$—R1, CH_3—O—CO—$(CH_2)_m$—R1, CR5R6R7-O—$(CH_2)_m$—R1, CR5R6R7O—CO—$(CH_2)_m$—R1, wherein R5, R6 and R7 may be —C1-C10 alkyl, linear or branched, not substituted or substituted. (CH_2) may of course also be (CR2R3). —O— or =O may be replaced by —S— or =S. R1 is as specified above. CR5R6R7 may in particular be t-butyl.

[0051] Examples for the compounds according to the invention are: NH_2—O—$(CH_2)_m$—R1, NH_2—O—

$(CH_2)_n$—CO—R1, NH_2—O—CHHal-$(CO)_o$—R1, NH_2—O—CHHal-CH_2—$(CO)_o$—R1, NH_2—O—CHHal-$(CH_2)_2$—$(CO)_o$—R1, NH_2—O—CHHal-$(CH_2)_3$—$(CO)_o$—R1, NH_2—O—CHHal-$(CH_2)_4$—$(CO)_o$—R1, NH_2—O—CO—$(CH_2)_n$—CO—R1, NH_2—O—CO—$(CH_2)_n$—R1, NH_2—O—$(CH_2)_n$—CO—R1, NH_2—O—CO—$(CH_2)_n$—$CHNH_2$—R1, NH_2—O—$(CH_2)_n$—$CHNH_2$—R1, with R1=-CN or —COOH, m or n=0 to 4, o=0 or 1, wherein —O— may be replaced by S.

[0052] Another formula according to the invention is formula II



wherein R_a=—CN, R_b=—H, =O, —OH, —NH_2, R_c=—NH_2, —O—NH_2, —O—(C1-10)alkyl, R_d=—H, -Hal, =O, —OH, wherein the case of =O H the one CH is omitted, wherein w=0 to 10, e.g. 1 to 4.

[0053] Another formula according to the invention is formula III

wherein Rp=-R1, —O—R1, —O—(CR2R3)$_x$-R1, —(CR2R3)$_x$-O—R1, R_q=—NR2.1R2.2, —O—NR2.1R2.2, —O—(CR2R3)$_x$-NR2.1R2.2, —(CR2R3)$_x$-O—NR2.1R2.2, R_r=—Am, —O—Am, —O—(CR2R3)$_x$-Am, —(CR2R3)$_x$-O—Am, —R_s=—H, —C1-C10 alkyl, aryl or aralkyl, —C1-C10 hydroxyalkyl, aryl or aralkyl, or an ether of such a hydroxy radical, wherein —O— may be replaced by —S— and x=1 to 10, in particular 1 to 4. R1 is as specified above, in particular —CN or —COOH. Examples of such compounds are: NH_2—O—CHAm—R1, NH_2—CHAm—O—R1, NH_2—O—CHAm—O—R1, NH_2—CHR1-O—Am, Am—O—$CHNH_2$—O—R1, NH_2—O—(Am—O—CH—O—R1). On one side of one —O— or several —O— or on both sides of one —O— or several —O— immediately —$(CH_2)_x$— may be interposed.

[0054] Compounds according to the invention may be present in an ionized condition in a solution, depending on the pH value (e.g. as —COO^- in basic condition or —NH_3^+ in acid condition). Salts, such as hydrochlorides, may also be formed.

[0055] The invention is based on the finding that aside from the classic metabolic diseases, such as diabetes mellitus, adiposity, other diseases, such as cancer, autoimmune diseases and rheumatism are caused by metabolic defects. This explains the strong influence of diet on these diseases. A directly measurable biochemical parameter for these metabolic ketoacidoses is the increase of pyruvate kinase type M2 (M2-PK) growing in the blood of all diseases above and below. Depending on the respective disease, the M2-PK detectable in the blood of the patients originates from different cells: for cancer from tumor cells, for sepsis from immune cells, for rheumatism from immune and/or synovial cells. In healthy cells, there are tetrameric forms of the M2-PK in a high-order cytosolic complex, the glycolysis enzyme complex. By the over-activation of oncoproteins, there is a migration of the M2-PK out of the complex and typical changes in the tumor metabolism. Simultaneously, the phosphoglyceromutase (PGM) leaves the complex and migrates into another enzyme complex, where the cytosolic transaminases are associated (see example 2). This complex is therefore called transaminase complex. The substrate of the PGM, glycerate-3-P, is the first stage for the synthesis of the amino acids serine and glycine. Both amino acids are essential for DNA and polypholipid synthesis. By the migration of the PGM into the transaminase complex, the synthesis of serine from glutamate and thus glutaminolysis is activated. The same changes take place in immune cells, if the immune system fails, such as for instance in the case of rheumatism, sepsis or polytrauma. The integration of the metabolism of different cells in multi-cellular organisms takes place by organ-specific association of the enzymes in the cytosol: in the muscle, for instance, by association with contraction proteins. For this reason, the different organs are provided with respectively specific isoenzymes. The disruption of this order will necessarily lead to disease. Unicellular organisms, such as bacteria or yeasts which react based on sufficiency of nutrients with dissipated proliferation, do not have a complex organization of the cytosol. As a consequence, substances which inhibiting the failing metabolism of multi-cellular organisms, will also inhibit the proliferation of such uni-cellular organisms.

[0056] The invention further teaches the use of a compound according to the invention for preparing pharmaceutical compositions for treating one or several diseases of the group comprising "cancer, chronic inflammations, asthma, arthritis, osteoarthritis, chronic polyarthritis, rheumatic arthritis, inflammatory bowl disease, degenerative joint diseases, rheumatic diseases with cartilage disorders, sepsis, autoimmune diseases, type I diabetes, Hashimoto thyroidotis, autoimmune thrombocytopenia, multiple sclerosis, myasthenia gravis, chronically inflammatory intestinal diseases, Crohn's disease, uveitis, psoriasis, collagenosis, Goodpasture syndrome, diseases with disturbed leukocyte adhesion, cachexia, diseases by increased TNF-alpha concentration, diabetes, adiposity, bacterial infections, in particular with resistant bacteria". The term treatment also comprises the prophylaxis.

[0057] The invention further teaches a pharmaceutical composition, wherein a compound according to the invention is mixed with one or several physiologically well tolerated auxiliary substances and/or carrier substances and galenically prepared for local or systemic administration, in particular oral, parenteral, for infusion into a target organ, for injection (e.g. IV, IM, intracapsular or intralumbal), for application in tooth pockets (space between tooth root and gum).

[0058] The invention also teaches the use of a compound according to the invention for inhibiting in vitro the glycolysis enzyme complex, in particular of pyruvate kinase, asparaginase, serine dehydratases, transaminases, desaminases and/or glutaminases. In particular, transamination,

oxidative deamination, hydrolytic deamination, eliminating deamination, and reductive deamination are blocked.

[0059] It is understood that if applicable, there may exist stereoisomers for the compounds according to formula I, such stereoisomers all being covered by the invention. The term alkyl comprises linear and branched alkyl groups as well as cycloalkyl, if applicable also cycloalkyl groups having linear or branched alkyl substituents. The term aryl also comprises aralkyl groups, and alkyl substituents may be alkyl or cycloalkyl.

[0060] Surprisingly, it has been found that compounds according to the invention are able to competitively inhibit the above members of the glycolysis enzyme complex. The proliferation of cancer cells in therapeutically relevant concentrations can be inhibited. There are no cytotoxic effects to be expected in the respective dosage range. Because of their pharmacological properties, the compounds according to the invention are also very suitable for the treatment and prophylaxis of the above-listed diseases. In conjunction with the indications for the inhibition of inflammations or antirheumatic effects, it is of special relevance that the substances according to the invention are non-steroidal substances.

[0061] The inhibition of the glycolysis enzyme and of the transaminase complex in particular comprises the inhibition of the metabolic activity and the energy gain from serine, glutamine, ornithine, proline and arginine or from other amino acids of this and other families, but also the synthesis of such amino acids used for energy generation. These are important energy sources in tumor cells, but also in bacteria and yeast. The cells or bacteria or yeast are, in a manner of speaking, "starved out." In detail, substances according to the invention block, for instance, the following reactions: i) threonine to glycine, ii) threonine to α-amino-β-ketobutyrate, iii) α-amino-β-ketobutyrate to glycine, iv) serine pyridoxalphosphate (PLP) Schiff's base to aminoacrylate, in particular folic acid-dependent serine hydroxymethyltransferase, v) aminoacrylate to pyruvate (by displacement of the balance of the natural hydrolysis of the PLP Schiff's base to the Schiff's base), vi) transamination by means of PLP for the synthesis of an amino acid from an oxo acid, in particular of the branch-chained transaminase, the α-ketoglutarate, oxalacetate, 3-hydropyruvate and glyoxalate transaminase, the glutamate dehydrogenase. In particular, the formation of pyruvate from amino acids is inhibited by substances according to the invention. Important is the release of NH_2—OH or CH3-OH (—H to —C or —N if applicable replaced by other radicals, for instance alkyl) by glutaminase, arginase, asparaginase or serine hydroxymethyltransferase. This will lead to an increased specificity, since a feature of tumor cells is a high glutaminase and serine hydroxymethyltransferase activity. NH_2-OH (hydroxylamine, HA), for instance, can be phosphorylated by the high pyruvate kinase activities instead of the OH of the phosphate (e.g. of the ADP). This will lead to a decoupling of the pyruvate kinase reaction in tumor cells. Therefore, the invention generally also comprises all natural metabolites of the substances according to the invention, in particular of aminooxyacetate, i.e. fractions of these substances.

[0062] In the transaminase complex, in addition to the PGM and NDPK, the cytosolic isoforms of the glutamate oxalacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), glutamate dehydrogenase (GIDH) and malate dehydrogenase (MDH) are associated. GOT and MDH are components of the malate-aspartate shuttle, by which the hydrogen produced in the cytosol is transported into the mitochondria. NAD+ is recycled for the cytosolic glyceraldehyde 3-phosphate dehydrogenase reaction. The malate-aspartate shuttle is part of the glutaminolysis. For an active malate-aspartate shuttle, in addition to GOT, the presence of the p36-bound form of the MDH is important, as represented in example 3.

[0063] Various further embodiments of the invention are possible. For instance, a pharmaceutical composition according to the invention may comprise several different compounds of the above definitions. Furthermore, a pharmaceutical composition according to the invention may, in addition, comprise an active ingredient different from the compound of formula Ito forms a combination preparation. Therein, the various employed active ingredients may be prepared in a single type of administration, i.e. the active ingredients are mixed in the type of administration. It is, however, also possible to prepare the various active ingredients in spatially separated types of administration of identical or different species.

[0064] As counterions for ionic compounds according to formula I Na^+, K+, Li+, cyclohexylammonium or basic amino acids (e.g. lysine, argini, ornithine, glutamine) can be used.

[0065] Drugs prepared by the method according to the invention may be administered in an oral, intramuscular, periarticular, intraarticular, intravenous, intraperitoneal, subcutaneous or rectal manner.

[0066] The invention also relates to methods for preparing drugs which are characterized by the fact that at least one compound of formula I is brought into a suitable dosage form by using a pharmaceutically suitable and physiologically well tolerated carrier and if applicable further suitable active ingredients, or any additional or auxiliary substances.

[0067] Suitable solid or liquid dosage forms are for instance granulates, powders, dragées, tablets, (micro) capsules, suppositories, syrups, juices, suspensions, emulsions, drops or injectable solutions as well as preparations with protracted release of the active ingredient, for the preparation of which, standard means such as carrier substances, explosion, binding, coating, swelling, sliding or lubricating agents, flavoring substances, sweeteners and solution mediators are used.

[0068] Auxiliary substances are, for instance, magnesium carbonate, titanium dioxide, lactose, mannite and other sugars, talcum, milk protein, gelatin, starch, cellulose and its derivatives, animal and plant oils such as cod-liver oil, sunflower, peanut or sesame oil, polyethylene glycols and solvents, such as sterile water and one or poly-valent alcohols, e.g. glycerin.

[0069] Preferably the drugs are prepared and administered in dosage units, each unit containing as an active component a defined dose of the compound according to formula I of the invention. With solid dosage units such as tablets, capsules, dragées or suppositories, this dose may be 1 to 1,000 mg, preferably 50 to 300 mg, and for injection solutions in an ampule form 0.3 to 300 mg, preferably 10 to 100 mg.

[0070] For treating an adult patient of 50 to 100 kg weight, for instance 70 kg, for instance daily doses of 20 to 1,000 mg active ingredient, preferably 100 to 500 mg, are indicated. Under certain circumstances, higher or lower daily doses may be recommended. The administration of the daily dose may be a one-time administration in the form of a single dosage unit or several smaller dosage units as well as a multi-administration of separated doses in certain intervals.

[0071] In the following, the invention is explained in more detail with reference to examples representing embodiments only.

EXAMPLE 1

Quantification of the Effectivity of a Compound According to the Invention

[0072] Suitable Novikoff hepatoma cells are obtainable from the tumor bank of the Deutsches Krebsforschungszentrum, Heidelberg (Cancer Research 1951, 17, 1010). 100,000 cells each are sown out per 25 cm^2 cultivation bottle. A substance according to the invention, dissolved in a solvent suitable for use in cell cultures, for instance water, diluted ethanol, dimethylsulfoxide or the like, is added in an increasing concentration to the culture medium, e.g. in a concentration range of 80 μM-5,000 μM or of 100 μM-300 μM. After four days of cultivation, the number of cells per bottle is counted. In comparison to the control sample (without addition of a compound according to the invention but instead with addition of a reference compound), a measurement and dose dependence of the inhibitive effect of a particular compound on proliferation can be observed.

EXAMPLE 2

Migration of the PGM

[0073] In FIG. **1***a* is shown an isoelectric focusing of a tumor cell extract (MCF-7 cells). It can be seen that PGM leaves the glycolysis enzyme complex and migrates into a complex associated with the cytosolic transaminases, the transaminase complex. The transaminase complex comprises the following: cytosolic glutamate oxalacetate transaminase (GOT), c-malate dehydrogenase (MDH), phosphoglyceromutase (PGM). Not shown are: c-glutamate pyruvate transaminase (GPT), c-glutamate hydroxypyruvate transaminase, c-alanine hydroxypyruvate transaminase, c-serine hydroxymethyl transferase and c-glutamate dehydrogenase (GIDH). The PGM and the nucleotide diphosphate kinase (NDPK) may be associated in the transaminase as well as in the glycolysis enzyme complex.

EXAMPLE 3

Inhibition of the Malate-Aspartate Shuttle

[0074] In FIG. **1***b* is shown the effect of aminoacetate (AOA) and hydroxylamine (HA) on the activity of the cytosolic and mitochondrial isoenzyme of the GOT in vitro. The isoenzymes of the GOT were dissociated by an isoelectric focusing. It can be seen that aminooxyacetate mainly inhibits the cytosolic isoenzyme, and hydroxylamine inhibits both isoenzymes of the GOT. The inhibition of the GOT leads to an inhibition of the malate-aspartate shuttle. As a consequence, NAD cannot be recycled, and the glycolysis is inhibited at the stage of the GAPDH.

[0075] The following explanations are independent from the above examples. The invention further teaches the use of N-(4'-trifluoromethylphenyl)-5-methylisoxazole-4-carboxamide ($C_{12}H_9F_3N_2O_2$; MW 270.2, see also FIG. **2***a*) and/or its natural active metabolites A 77 1726 according to FIG. **2***b* for preparing a pharmaceutical composition for treating tumor diseases, in particular solid tumors. The benzene ring may, alternatively to the shown substitution with —CF3, generally be singly, doubly, triply, quadruply or quintuply substituted with -Chal3 or —O-Chal3 or -Hal at an arbitrary position. The pharmaceutical composition according to the invention is particularly suited for treating large tumors, i.e. beginning from 0.1 to 1 cm^3 tumor size. A pharmaceutical composition according to the invention is, for instance, prepared for oral administration, for instance with the following auxiliary and carrier substances: colloidal SiO_2, crospovidone, hydroxypropylmethyl cellulose, lactose monohydrate, magnesium stearate, polyethylene glycol, povidone, starch, talcum, TiO_2 and/or yellow iron oxide. The dosage is 1 to 50 mg per day, preferably 10 to 30 mg. It may be recommended to administer in a therapy initially at a starting dose of 20 to 500 mg, in particular 50 to 150 mg, for the first 1 to 10 days, in particular the first 1 to 3 days. In another embodiment of the invention, the substance mentioned above is combined with one or several sugar phosphates, for instance fructose-1,6-biphosphate, glycerate-2,3-biphosphate, glycerate-3-phosphate, ribose-1,5-biphosphate, ribulose-1,5-biphosphate, and the combination of substances in a dosage form, for instance a tablet, may be mixed. It is however also possible to provide the components separately in identical or different dosage forms. The sugar phosphate may be administered in a dosage of 20 to 5,000 mg per day, for instance 100 to 500 mg.

[0076] These variants of the invention surprisingly lead to an inhibition of the growth of tumor cells and tumor tissue, since these substances or the metabolite can bind to the pyruvate kinase and inhibit or reverse the failure of energy metabolism in tumor cells. This results in the beneficial effect that these substances specifically influence the metabolism of tumor cells and they do not or to a lesser extent influence the metabolism of normal cells, having only slight side effects, if at all.

[0077] The effectiveness of these substances is surprising because the known effect of a pyrimidine synthesis inhibitor relates to a completely different effective mechanism, and the empirical observation of an anti-proliferative effect is substantially directed toward immune cells and cells related to inflammatory diseases.

[0078] Of special importance is a combination of one or several of the active ingredients mentioned on the previous page with one or several of the active ingredients mentioned further above or aminooxyacetate (AOA, NH2-O—CH2-COOH, salts or esters thereof, for instance C1-C10 alkyl or hydoxyalkyl esters). For instance, AOA is particularly effective for small tumors (<0.1 to 1 cm^3) or prevents the development thereof, in particular, the development of metastases, whereas compounds of the formulas 10 or 11, if applicable in combination with sugar phosphate, is effective for large tumors. The reason for this is due to the different metabolisms of small and large tumors. The above explanations for combinations apply in an analogous manner.

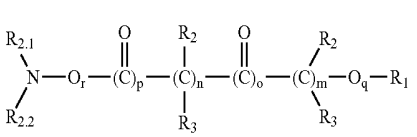
[0079] Substances according to the invention can further be used for preparing a pharmaceutical composition for

treating heart insufficiency or the chronic cardiac failure (CCF). These are the variants defined by the New York Heart Association (NYHA) Classification or grades from NYHA I to NYHA IV. All of these diseases are marked by an acute and/or chronic failure of the heart muscle to provide for blood circulation or the transportation capacity required for the metabolism of the organism under load or at rest. The reasons are: insufficient glycolysis by glucose deficiency in the heart muscle and/or its insufficient oxygen supply and complex coronary inflammation processes (activation of cells of the immune system and complement). This aspect of the invention is based on the finding that the substances according to the invention provide modulation of alternative energy-generating biochemical processes, and thus make it possible to produce replacement pathways for the above insufficiently operating processes. For instance, this is achieved by activation of the serinolysis and glutaminolysis or to displacement by the substances according to the invention, the existing dynamic balance between glycolysis on the one hand and glutaminolysis on the other hand in favor of the glycolysis, under simultaneous administration of oxygen (increase in the oxygen partial pressure in the blood by, for instance, breathing). In this context, the administration of anti-inflammatory substances according to the invention can prevent imminent highly dangerous acidosis (by lactate production). Compared to prior art measures, such as administration of ACE inhibitors, diuretics, digitalis, positive inotropic substances or isosorbide dinitrate, the substances according to the invention directly influence energy metabolism, and the latter is improved. Side effects are as a consequence, comparatively weak.

[0080] In this context, it has been found by the invention that at least in the cases of the NYHA grade II to IV, the concentration of tumor M2-PK (=M2-PK dimeric in contrast to standard M2-PK being tetrameric) in cells and/or the blood increases, and can be detected in a routine manner, as an alternative to other methods typically utilized to date. Therefore the invention further teaches the use of a tumor M2-PK detecting test system for preparing a diagnostic substance for the in vitro diagnosis of a heart insufficiency, in particular, the grade or the inflammatory processes connected therewith. If increased M2-PK values (sick collective) are found in the blood plasma of a patient compared to standard values (defined maximum limits; normal collective), this is indicative of the existence of a heart insufficiency and/or for inflammatory processes correlated therewith, or at least identifies a risk of heart insufficiency. Such a blood plasma analysis can easily and quickly be performed. Compared thereto, previous standard methods (gold standard, blood gas analysis) are not suitable for routine tests and are expensive. For this aspect of the invention, any known test systems can be used which detect tumor M2-PK, e.g. immunological test systems with antibodies. In particular, per se known test systems can be used which detect tumor M2-PK as a tumor metabolism marker, for instance monoclonal antibodies specific herefor.

[0081] Various substances which can be used according to the invention are shown in the further figures, FIGS. **2***a*-**2***h*, **3***a*-**3***h*, **4***a*-**4***c*, **5***a*-**5***i*, and **6***a*-**6***f*. In particular, the essential variation possibilities are represented in an exemplary manner, the permutations which are easily deduced not being shown for the sake of simplicity. The invention finally also comprises all natural metabolites of the described substances.

1. A method for treating cancer comprising administering a pharmaceutical composition comprising a compound that is mixed with one or several physiologically well tolerated auxiliary substances and/or carrier substances and galenically prepared for local, oral, or systemic administration comprising intravenous administration, wherein the compound is according to formula I



I

wherein R1=-H, —CN, —COO+, —COS+, —COOH, —COSH, —COOR1.1, —COSR1.1, N-phthalimidyl,

wherein R1.1=-H, C1-10 alkyl, C1-10 aralkyl or aryl,

wherein R2=-H, C1-C4 alkyl, —OR1.1, -Hal (—F —Cl, —Br, -J), —NR2.1R2.2, —Am, —O—Am, —S—Am,

wherein R3=-H, C1-C4 alkyl, —OR1.1, -Hal (—F —Cl, —Br, -J), —NR2.1R2.2, —Am, —O—Am, —S—Am,

wherein R2.1=-H, C1-10 alkyl, C1-10 aralkyl or aryl,

wherein R2.2=-H, C1-10 alkyl, C1-10 aralkyl or aryl,

wherein R2.1 and R2.2 may be identical or different,

wherein n and m may be identical or different and 0 to 10,

wherein o and p may be identical or different and 0 to 3,

wherein o>0, if n and m=0,

wherein R2 and R3 may be identical or different for Cn and/or Cm,

wherein R2 may be identical or different for every Cx=1 . . . n,

wherein R3 may be identical or different for every Cy=1 . . . m,

wherein —Am is an amino acid radical,

wherein q and r=0 or 1 and identical or different,

wherein $—O_r—$ and/or $—O_q—$ may also be replaced by $—S_r—$ or $—S_q—$, resp.,

wherein —NR2.1R2.2 may be replaced by a linear or branched —C1-C20 alkyl, aralkyl or aryl,

wherein a group —CN, —(CO)—CN, —(CO)—O—R1 or —(CO)—R1 or —C—O—R1 may be replaced by $—SO_2—$NR2.1R2.2, or a physiologically well tolerated salt of such a compound.

2. The method according to claim 1, wherein the cancer comprises tumor cells which overexpress a tumor marker comprising glycolytic enzyme comprising pyruvate kinase M2 as compared to non-cancerous cells.

3. The method according to claim 2, wherein the tumor cells comprise hepatoma cells.

4. The method according to claim 1, wherein the cancer comprises cancer of the liver.

5. The method according to claim 1, wherein R1=-CN.

6. The method according to claim 1, wherein at least one of the R2 is —Am, wherein —Am preferably represents an

amino acid radical of an essential amino acid, wherein in particular q=0 and r=1 or q=1 and r=0 or q=1 and r=1, m=1, R3=-H, n=o=p=0, R2.1=R2.2=-H.

7. The method according to claim 1, wherein n=o=p=0, wherein m=0 to 4, wherein R2=R3=-H, or for at least one R2, R2=-Am, wherein R2.1=R2.2=-H, wherein q=0 and r=1.

8. The method according to claim 1, wherein m=p=0, wherein o=1, wherein n=0 to 4, wherein R2=H, or for at least one R2, R2=-Am, wherein R3=-H or -Hal in the case Cx=1, wherein R3=-H for all Cx=n>1, wherein R2.1=R2.2=-H, wherein q=0 and r=1.

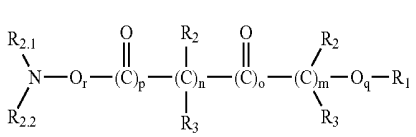
9. The method according to claim 1, wherein m=1 to 4, wherein n=o=p=0, wherein R2=H, or for at least one R2, R2=-Am, wherein R3=-H or -Hal in the case Cy=1, wherein R3=-H for all Cy=m>1, wherein R2.1=R2.2=-H, wherein q=0 and r=1.

10. The method according to claim 1, wherein o=p=1, wherein m=0, wherein n=0 to 4, wherein R2=R3=-H, or for at least one R2, R2=-Am, wherein R2.1=R2.2=-H, wherein q=0 and r=1.

11. The method according to claim 1, wherein n=p=0, wherein o=1, wherein m=0 to 4, wherein R2=R3=-H, or for at least one R2, R2=-Am, wherein R2.1=R2.2=-H, wherein q=0 and r=1.

12. The method according to claim 1, wherein m=p=0, wherein o=1, wherein n=1 to 4, wherein R2=R3=-H, or for at least one R2, R2=-Am, wherein R2.1=R2.2=-H, wherein q=0 and r=1.

13. A method of treating cancer comprising tumor cells which overexpress a tumor market comprising pyruvate kinase M2 compared to non-cancerous cells, comprising administering a pharmaceutical composition comprising a compound according to formula I



I

wherein R1=-H, —CN, —COO+, —COS+, —COOH, —COSH, —COOR1.1, —COSR1.1, N-phthalimidyl,

wherein R1.1=-H, C1-10 alkyl, C1-10 aralkyl or aryl,

wherein R2=-H, C1-C4 alkyl, —OR1.1, -Hal (—F —Cl, —Br, -J), —NR2.1R2.2, —Am, —O—Am, —S—Am,

wherein R3=-H, C1-C4 alkyl, —OR1.1, -Hal (—F —Cl, —Br, -J), —NR2.1R2.2, —Am, —O—Am, —S—Am,

wherein R2.1=-H, C1-10 alkyl, C1-10 aralkyl or aryl,

wherein R2.2=-H, C1-10 alkyl, C1-10 aralkyl or aryl,

wherein R2.1 and R2.2 may be identical or different,

wherein n and m may be identical or different and 0 to 10,

wherein o and p may be identical or different and 0 to 3,

wherein o>0, if n and m=0,

wherein R2 and R3 may be identical or different for Cn and/or Cm,

wherein R2 may be identical or different for every Cx=1 . . . n,

wherein R3 may be identical or different for every Cy=1 . . . m,

wherein —Am is an amino acid radical,

wherein q and r=0 or 1 and identical or different,

wherein —O_r— and/or —O_q— may also be replaced by —S_r— or —S_q—, resp.,

wherein —NR2.1R2.2 may be replaced by a linear or branched —C1-C20 alkyl, aralkyl or aryl,

wherein a group —CN, —(CO)—CN, —(CO)—O—R1 or —(CO)—R1 or —C—O—R1 may be replaced by —SO_2—NR2.1R2.2, or a physiologically well tolerated salt of such a compound.

14. The method according to claim 2, wherein the compound inhibits glycolysis of the tumor cells.

15. The method according to claim 2, wherein the compound inhibits the activity of the pyruvate kinase M2 of the tumor cells.

16. The method according to claim 1, wherein the cancer comprises solid tumors.

17. A method for in vitro detection of heart insufficient or chronic cardiac failure comprising comparing a level of pyruvate kinase type M2 in a blood plasma sample of a patient with a predetermined standard level, wherein the level of pyruvate kinase type M2 in the blood plasma sample is measured by an immunological assay using monoclonal antibodies targeted to detect pyruvate kinase type M2.

18. A method for treating one or several diseases from the group comprising cancer, chronic inflammations, asthma, arthritis, osteoarthritis, chronic polyarthritis, rheumatic arthritis, inflammatory bowl disease, degenerative joint diseases, rheumatic diseases with cartilage disorders, sepsis, autoimmune diseases, type I diabetes, Hashimoto thyreoiditis, autoimmune thrombocytopenia, multiple sclerosis, myasthenia gravis, chronically inflammatory intestinal diseases, Crohn's disease, uveitis, psoriasis, collagenoses, Goodpasture syndrome, diseases with disturbed leukocyte adhesion, cachexia, diseases by increased TNF-alpha concentration, diabetes, adiposity, bacterial infections including those by antibiotic resistant bacteria comprising administering a pharmaceutical composition prepared comprising the compound according to claim 1.

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摘要(译)

本发明涉及用于调节糖酵解酶复合物和转氨酶复合物的化合物，含有这些化合物的药物组合物以及这些化合物在制备用于治疗各种疾病的药物组合物中的用途。