(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
10 November 2005 (10.11.2005)

PCT

(10) International Publication Number
WO 2005/105145 A1

(51) International Patent Classification[7]: A61K 45/06, A61P 31/00, 33/00

(21) International Application Number: PCT/DK2005/000294

(22) International Filing Date: 29 April 2005 (29.04.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PA 2004 00680 30 April 2004 (30.04.2004) DK
PA 2004 01716 8 November 2004 (08.11.2004) DK

(71) Applicant *(for all designated States except US)*: **BKG PHARMA APS** [DK/DK]; c/o Birgit Kjældgaard Giwercman, Vintervej 2, DK-2920 Charlottenlund (DK).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **BIRGIT, kJældgaard Giwercman** [DK/DK]; Vintervej 2, DK-2920 Charlottenlund (DK).

(74) Agent: **PLOUGMANN & VINGTOFT A/S**; Sundkrogsgade 9, P.O. Box 831, DK-2100 Copenhagen Ø (DK).

(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, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, 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, 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:
— *with international search 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: TREATMENT OF INFECTIOUS DISEASES

(57) Abstract: The present invention is directed to the use of chemosensitising compounds, in particular thioxanthene derivatives and phenothiazine derivatives, for treatment of infectious diseases in combination with an anti-infective agent. The invention furthermore relates to compositions comprising said chemosensitising compounds and anti-infective agents.

WO 2005/105145 A1

TREATMENT OF INFECTIOUS DISEASES

FIELD OF THE INVENTION

The present invention is directed to the use of chemosensitising compounds, in particular thioxanthene derivatives and phenothiazine derivatives, for treatment of infectious diseases in combination with an anti-infective agent.

BACKGROUND OF THE INVENTION

Resistance to chemotherapy is a common clinical problem in patients with infectious diseases. During treatment of infections the drug targets of prokaryotic or eukaryotic microorganisms cells are often found to be refractory to a variety of drugs that have different structures and functions. This phenomenon has been termed multidrug resistance (MDR). Organisms ranging from bacteria to human beings possess transmembrane transporters which confer resistance to toxic compounds. Underlining their biological significance, prokaryotic and eukaryotic multidrug transport proteins are very similar in structure and function. In addition, other kinds of important resistance mechanisms in combination with efflux pumps may co-operate thereby giving rise to high levels of resistance. There is an urgent need for drugs that can inhibit (reverse) or circumvent the resistance mechanisms and improve the effectiveness of commonly used anti-infective agents.

The incidence of the multiple antimicrobial resistance of bacteria which cause infections in hospitals/intensive care units is increasing, and finding microorganisms insensitive to more than 10 different antibiotics is not unusual. Examples of such resistant bacteria include methicillin-resistant and methicillin-vancomycin-resistant *Staphylococcus aureus*; vancomycin-resistant enterococci, such as *Enterococcus faecalis* and *Enterococcus faecium*; penicillin-resistant *Streptococcus pneumoniae*, and cephalosporin and quinolone resistant gram-negative rods (coliforms), such as *E. coli, Salmonella* species, *Klebsiella pneumoniae, Pseudomonas* species and *Enterobacter* species. More recently, pan antibiotic resistant gram-negative and gram-positive bacilli have emerged.

The rapidity of emergence of these multiple antibiotic-resistance is not being reflected by the same rate of development of new antibiotics and it is, therefore, conceivable that patients with serious infections soon will no longer be treatable with currently available anti-infective agents. Several international reports have highlighted the potential problems associated with the emergence of antimicrobial resistance in many areas of medicine and also outlined the difficulties in the management of patients with infections caused by these microorganisms.

CONFIRMATION COPY

Although most of the hardier microorganisms are present in hospitals, strains of multidrug resistant bacteria, such as *Streptococcus pneumoniae* and *Mycobacterium tuberculosis* have also caused serious community-acquired infections. The prevalence of drug-resistant *Streptococcus pneumoniae* has increased 60-fold since 1980 with 51% and 8% of isolates demonstrating intermediate- or high-level resistance to penicillin or third-generation cephalosporins, respectively. Thus, pneumococcal pneumonia is becoming more difficult to treat with first-line anti-infective agents. Resistant bacteria from hospitals can be introduced into the community via patients discharged for continued treatment at home taking with them, for example, multidrug resistant *Staphylococcus aureus* and vancomycin resistant enterococci.

Mechanisms of resistance in bacteria can be mediated either via chromosomes or via plasmids. Studies of emerging resistance show that resistance in bacteria can arise in steps progressing from low level to high level, unless a plasmid is acquired on which full-blown resistance is already present. For example, the initial penicillin resistant pneumococci appeared with slightly decreased susceptibility to the antibiotic but over time evolved high-level resistance. Penicillin and tetracycline resistance among gonococci emerges in a similar way. The phenomenon has also been observed with pan resistant *E. coli*, where multiple steps are required to reach a clinically relevant level of resistance.

Anti-infective agents may be rendered inactive by three major mechanisms: i) destruction or modification of the antibiotic (for example, by production of beta-lactamases and aminoglycoside-inactivating enzymes), ii) alteration of the target site, and iii) prevention of access to the target (for example, alteration of permeability or efflux).

All of the three major resistance mechanisms alone or in combination are clinically important, leading to treatment failure. However, recently efflux-related multidrug resistance (MDR) has become appreciated as a significant complicating factor in the chemotherapy of bacterial infections.

Efflux mechanisms that account for resistance to a variety of anti-infective agents are commonly found in a wide range of bacteria. The term MDR system refers to a group of transporters, which are able to expulse a wide range of quite different substrates. While this type of system was first described in eukaryotic cells in the late 1980s, the presence of MDR efflux pumps in bacteria showing resistance to several drugs has been increasingly reported in the literature. MDR cell lines in general are associated with decreased drug accumulation due to enhanced efflux as well as diminished influx of chemotherapeutic drugs.

Over expression of MDR efflux pumps, after induction or because of the emergence of mutations in their regulatory elements, is a major mechanism in acquired bacterial resistance to multiple anti-infective agents. Two major groups of efflux systems are known, specific exporters and transporters conferring multidrug resistance (MDR). The MDR systems are able to remove anti-infective agents of different classes from the bacterial cell and occasionally play a role in the intrinsic resistance of some bacteria to certain anti-infective agents. Their genes are commonly located on the bacterial chromosome. The mechanisms leading to MDR are frequently caused by trans-membrane xenobiotic transport molecules belonging to the superfamily of ATP-binding cassette (ABC) transporters. MDR efflux has mainly been identified with resistance to compounds such as tetracyclines, fluoroquinolones, macrolides, lincosamides rifampicin, chlorphenicol, and aminoglycosides. In contrast, the genes coding for specific efflux systems are often associated with mobile genetic elements, which can easily be interchanged between bacteria. Specific efflux systems have mainly been identified with resistance to compounds such as macrolides, lincosamides and/or streptogramins, tetracyclines, as well as chloramphenicol in Gram positive and Gram negative bacteria.

Resistance to anti-viral agents is a worldwide problem in a variety of vira, such as HIV in AIDS patients and Herpes vira. Drug resistance is not restricted to the well-known mutations in the virus but may also develop on the human cellular level. It has been shown in the prior art that AZT (3'-Azido-3'-deoxythymidine)–resistance in HIV-1 is due to a cellular mechanism for AZT-resistance. The cellular AZT efflux is increased remarkably, leading to resistance. Thus the cellular efflux resistance mechanism is an important factor limiting the efficiency of antiviral chemotherapeutic agents used in the treatment of viral-infected patients, such as HIV infected patients, and inhibition of such cellular efflux mechanisms would reverse the resistance and enhance the effect of the antiviral compound.

Prokaryotic and eukaryotic multidrug transport proteins are very similar in structure and function. For eukaryotic cells, drug efflux pumps have been viewed by many authors as complementing enzyme-based detoxification systems.

In prokaryotic cells, several kinds of resistance mechanisms in combination with efflux pumps may co-operate, resulting in high levels of resistance and therapeutic failure.

Inhibition of resistance mechanisms can restore the activities of anti-infective agents that are substrates for these mechanisms. The recent demonstration and elucidation of the phenomenon of developing resistance has led to the search for drugs that could improve the effectiveness of anti-infective agents.

Two potential groups of MDR inhibitors are the phenothiazines and thioxanthenes. Phenothiazines and thioxanthenes are used clinically as neuroleptic and antiemetic agents. Phenothiazines, and structurally related antipsychotic agents, inhibit several cellular enzymes and block the function of critical cellular receptors. The extrapyramidal side effects associated with antipsychotic therapy are attributed to dopamine receptor binding. In general these extrapyramidal side effects have proven to be dose limiting in clinical trials using phenothiazines and thioxanthenes in non-psychotic areas, such as anti-cancer treatment.

Phenothiazines and thioxanthenes have been shown to inhibit the function of eukaryotic MDR efflux pumps and certain prokaryotic MDR efflux pumps.

Phenothiazines have been shown to be among the group of drugs known to modify resistance to one or more antibacterial agents in certain bacteria. Although the mechanism by which phenothiazines and other drugs modulate MDR is not yet clear, it has been suggested that their pharmacological properties may be mediated at least in part by inhibition of efflux pumps. Also, promethazine has been recognised as an effective antiplasmid agent in cultures containing bacterial species such as *Escherichia coli, Yersinia enterocolitica, Staphylococcus aureus and Agrobacterium tumefaciens.* The concentrations used, however, are generally high above clinically relevant concentrations.

The fact that effective concentrations are above clinically relevant concentrations was emphasised by Kaatz et al. (2003). Although inhibition of efflux pumps in certain types of *S. aureus* by selected phenothiazines and the two geometric stereoisomers *cis*- and *trans*-flupenthixol were shown, the authors still concluded that "Unfortunately, the IC_{50} values of inhibitors for EtBr, acriflavine and pyronin Y efflux are above those employed in clinical practice".

Phenothiazines and thioxanthenes have modest, but broad, antimicrobial activities. MICs are generally above clinically relevant concentrations inasmuch as the minimum effective concentrations *in vitro* are in the order from approximately 20 mg/l to several hundreds mg/l and the relevant serum levels range from approximately 0.3 μg/l to 0.5 mg/l (0.3 ng/ml to 0.5 μg/ml).

The thioxanthenes demonstrate geometric stereoisomerism. The *cis* and *trans* forms have previously been shown to have roughly equal modest antibacterial potency. MICs are generally far above clinically relevant concentrations. In 1998 Kristiansen et al. suggested a synergistic effect between penicillin and *trans*-clopenpenthixol in a study using a non-

resistant mouse pathogen isolate of Streptococcus pneumoniae highly sensitive to penicillin (MIC 0.02 μg/ml penicillin). However, the authors did not show significant differences of MIC values using penicillin alone and penicillin in combination with *trans*-clopenthixol, and concentration/response/ time correlation studies were not performed, i.e. the concentration of drugs in the infected mice were unknown. Also the response of the infecting bacteria in the mice were unknown.

Johnstone et al. showed in a clinical trial of the anti-psychotic effect of *cis*-flupenthixol versus *trans*-flupenthixol versus placebo, that while *cis*-flupenthixol was a potent neuroleptic (especially for "positive" symptoms), *trans*-flupenthixol had no activity as an anti-psychotic. Since *trans*-flupenthixol is a far less potent dopamine antagonist, and the extrapyramidal side effects associated with antipsychotic therapy are attributed to dopamine receptor binding, *trans*-flupenthixol lacks these side effects.

The apparent lack of anti-psychotic activity or extrapyramidal side effects of the *trans*-forms such as trans-flupenthixol and trans-clopenthixol makes them particularly attractive for use as anti-resistance agents.

Several phenothiazine and thioxanthene derivatives are disclosed in US 6,569,853.

Flupenthixol is disclosed in UK Patent 925,538 as having utility as tranquilliser, ataractic, antiemetic, antihistamine, antispasmodic and general central nervous system depressant. No mention is made of any anti-infective or anti-resistance activity.

Several thioxanthene derivatives are disclosed in UK Patent 863,699 as tranquillisers. No mention is made of any of anti-infective or anti-resistance activity.

From the discussion above it is clear that the increase in resistance to anti-infective agents, such as antibiotics, present a major impediment to the treatment of infections. Thus, there is an urgent need for drugs that can inhibit or circumvent the resistance mechanisms and improve the effectiveness of the currently available anti-infective agents.

The object of the present invention is to provide chemosensitising compounds capable of sensitising resistant, including multidrug resistant, cells or microorganisms to an anti-infective agent by administration of clinically relevant amounts of such chemosensitising compounds to a subject in need thereof. Another, but related, object of the invention is improve the effectiveness of anti-infective agents for treatment of infectious diseases, in particular when such infectious diseases are caused by resistant, including multidrug resistant, microorganisms.

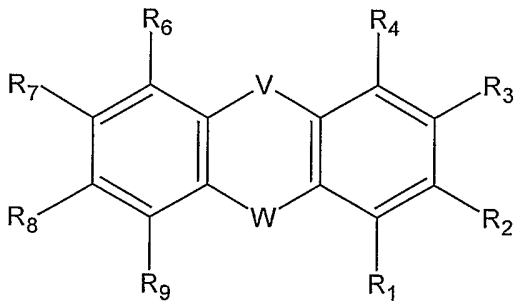
SUMMARY OF THE INVENTION

As will be understood from the above discussion, the prior art has hitherto deemed thioxanthenes and phenothiazines unsuitable for treatment of infectious diseases in combination with anti-infective agents, since the necessary therapeutic amount of such chemosensitising compounds would cause severe side effects.

The present inventor realised that the above conclusions in general were based on studies carried out on artificial resistant or multidrug resistant microorganisms or *in vitro* selected microorganisms. Thus, despite the prejudice in the art that thioxanthene and phenothiazine concentrations far above the clinical relevant level would be necessary in order to combat resistant or multidrug resistant microorganisms, the present inventor decided to investigate this matter in further detail by studying the effect of such chemosensitising compounds in combination with anti-infective agents on clinically relevant resistant and multidrug resistant isolates using clinically relevant amounts of the chemosensitising compounds described herein.

Surprisingly, it was found that by applying clinically relevant amounts of the chemosensitising compounds described herein in combination with anti-infective agents, effective killing of resistant and multidrug resistant clinically relevant isolates was achieved. Contrary to what was previously believed, this surprising finding opens up the possibility to effectively combat resistant and multidrug resistant microorganisms by a combination of the chemosensitising compounds described herein and commonly used anti-infective agents.

Accordingly, in a first aspect the present invention relates to the use of a compound of the general formula (I)



(I)

wherein

V is selected from the group consisting of S, SO_2, SO, O and NH;

W is N-$(CHX)_n$-$N(R_{10})(R_{11})$ or W is C=CH-$(CHX)_m$-$N(R_{10})(R_{11})$;

n is an integer in the range of from 2 to 6;

m is an integer in the range of from 1 to 5;

each X is individually selected from the group consisting of hydrogen, halogen, hydroxy, amino, nitro, optionally substituted C_{1-6}-alkyl and optionally substituted C_{1-6}-alkoxy;

R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8 and R_9 are each individually selected from the group consisting of hydrogen, halogen, hydroxy, amino, nitro, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted C_{2-6}-alkynyl and optionally substituted C_{1-6}-alkoxy, optionally substituted C_{2-6}-alkenyloxy, carboxy, optionally substituted C_{1-6}-alkoxycarbonyl, optionally substituted C_{1-6}-alkylcarbonyl, fomyl, optionally substituted C_{1-6}-alkylsulphonylamino, optionally substituted aryl, optionally substituted aryloxycarbonyl, optionally substituted aryloxy, optionally substituted arylcarbonyl, optionally substituted arylamino, arylsulphonylamino, optionally substituted heteroaryl, optionally substituted heteroaryloxycarbonyl, optionally substituted heteroaryloxy, optionally substituted heteroarylcarbonyl, optionally substituted heteroarylamino, heteroarylsulphonylamino, optionally substituted heterocyclyl, optionally substituted heterocyclyloxycarbonyl, optionally substituted heterocyclyloxy, optionally substituted heterocyclylcarbonyl, optionally substituted heterocyclylamino, heterocyclylsulphonylamino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)aminocarbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, amino-C_{1-6}-alkyl-carbonylamino, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-carbonylamino, amino-C_{1-6}-alkyl-amino, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-amino, cyano, guanidino, carbamido, C_{1-6}-alkanoyloxy, C_{1-6}-alkylsulphonyl, C_{1-6}-alkylsulphinyl, C_{1-6}-alkylsulphonyloxy, aminosulfonyl, mono- and di(C_{1-6}-alkyl)aminosulfonyl, and optionally substituted C_{1-6}-alkylthio; and

R_{10} and R_{11} are each independently selected from the group consisting of hydrogen, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted C_{2-6}-alkynyl, optionally substituted C_{1-6}-alkoxycarbonyl, optionally substituted C_{1-6}-alkylcarbonyl, optionally substituted aryl, optionally substituted

aryloxycarbonyl, optionally substituted arylcarbonyl, optionally substituted heteroaryl, optionally substituted heteroaryloxycarbonyl, optionally substituted heteroarylcarbonyl, aminocarbonyl, mono- and di(C_{1-6}-alkyl)aminocarbonyl; or R_{10} and R_{11} together with the nitrogen atom to which they are attached form an optionally substituted nitrogen-containing heteroaryl or optionally substituted heterocyclyl;

or a metabolite or salt thereof for the manufacture of a medicament for the treatment or prophylaxis of an infectious disease in combination with an anti-infective agent.

Other aspect of the present invention will be apparent from the below description and the appended claims.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a series of bars depicting the effect of chemosensitising compounds against drug resistant and multidrug resistant microorganisms (values from Tables 1-4 below). The Y-axis represents the DR Ratio and is defined as the ratio of the MIC for anti-infective agent alone divided by the MIC for the anti-infective agent in the presence of chemo-sensitising compound. This ratio represents the apparent increase in potency of the anti-infective agent produced by the individual chemosensitising compounds. Anti-infective agent: Coprofloxacin. Chemosensitising compounds: 1. promazine; 2. 1-chlorpromazine; 3. chlorpromazine; 4. 7-hydroxychlorpromazine; 5. 7,8-dihydroxychlorpromazine; 6. thiomethylpromazine; 7. trifluopromazine; 8. chlorpromazine sulfoxide; 9. desmethyl-chlorpromazine; 10. perphenazine; 11. prochlorperazine; 12. fluphenazine; 13. trifluo-perazine; 14. 2-chloro-10-(2-dimethylaminoethyl)phenothiazine; 15. promethazine; 16. *cis*-flupenthixol; 17. *trans*-flupenthixol; 18. *trans*-clopenthixol.

Figures 2A and 2B show a series of bars depicting the strong synergistic effect of *cis*-flupenthixol, *trans*-flupenthixol and *trans*-clopenthixol on ciprofloxacin, gentamycin and piperacillin, respectively (values from Table 5 below). The tested microorganisms were *E. coli* 331 ME (Figure 2A) and *P. aeruginosa* 432b (Figure 2B). The Y-axis represents the Fractional Inhibitory Concentration (FIC) index. Synergy was defined for FIC indices less than 0.5.

Figure 3 shows the enhancement of the effect of ciprofloxacin by Trans-Clopenthixol in a mouse peritonitis model.

Figure 4 shows serum levels of Trans-clopenthixol in mice after a single dose of 0.3 mg per mouse.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

In the present context, the term "C_{1-6}-alkyl" is intended to mean a linear or branched saturated hydrocarbon group having from one to six carbon atoms, such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, *sec*-butyl, *tert*-butyl, n-pentyl, isopentyl, neopentyl and n-hexyl.

In the present context the term "C_{3-6}-cycloalkyl" is intended to cover three-, four-, five- and six-membered rings comprising carbon atoms only, whereas the term "heterocyclyl" is intended to mean three-, four-, five- and six-membered rings wherein carbon atoms together with from 1 to 3 heteroatoms constitute said ring. The heteroatoms are independently selected from oxygen, sulphur, and nitrogen. C_{3-6}-cycloalkyl and heterocyclyl rings may optionally contain one or more unsaturated bonds situated in such a way, however, that an aromatic π-electron system does not arise.

Illustrative examples of "C_{3-6}-cycloalkyl" are the carbocycles cyclopropane, cyclobutane, cyclopentane, cyclopentene, cyclopentadiene, cyclohexane, cyclohexene, 1,3-cyclohexadiene and 1,4-cyclohexadiene.

Illustrative examples of "heterocyclyls" are the nitrogen-containing heterocycles 2-pyrrolinyl, 3-pyrrolinyl, pyrrolidinyl, 2-imidazolinyl, imidazolidinyl, 2-pyrazolinyl, 3-pyrazolinyl, pyrazolidinyl, piperidinyl, morpholinyl, thiomorpholinyl and piperazinyl. Binding to the heterocycle may be at the position of the heteroatom or via a carbon atom of the heterocycle.

In the present context, the term "C_{2-6}-alkenyl" is intended to mean a linear or branched hydrocarbon group having from two to six carbon atoms and containing one or more double bonds. Illustrative examples of C_{2-6}-alkenyl groups include allyl, homo-allyl, vinyl, crotyl, butenyl, pentenyl and hexenyl. Illustrative examples of C_{2-6}-alkenyl groups with more than one double bond include butadienyl, pentadienyl and hexadienyl. The position of the double bond(s) may be at any position along the carbon chain.

In the present context the term "C_{2-6}-alkynyl" is intended to mean a linear or branched hydrocarbon group containing from two to six carbon atoms and containing one or more triple bonds. Illustrative examples of C_{2-6}-alkynyl groups include acetylene, propynyl,

butynyl, pentynyl and hexynyl. The position of the triple bond(s) may be at any position along the carbon chain. More than one bond may be unsaturated so that the "C_{2-6}-alkynyl" is a di-yne or enedi-yne as is known to the person skilled in the art.

When used herein the term "C_{1-6}-alkoxy" is intended to mean C_{1-6}-alkyl-oxy, such as methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, isobutoxy, *sec*-butoxy, *tert*-butoxy, n-pentoxy, isopentoxy, neopentoxy and n-hexoxy.

The term "halogen" includes fluorine, chlorine, bromine and iodine.

In the present context the term "aryl" is intended to mean a carbocyclic aromatic ring or ring system. Moreover, the term "aryl" includes fused ring systems wherein at least two aryl rings, or at least one aryl and at least one C_{3-6}-cycloalkyl, or at least one aryl and at least one heterocyclyl, share at least one chemical bond. Illustrative examples of "aryl" rings include phenyl, naphthalenyl, phenanthrenyl, anthracenyl, acenaphthylenyl, tetralinyl, fluorenyl, indenyl, indolyl, coumaranyl, coumarinyl, chromanyl, isochromanyl, and azulenyl.

In the present context, the term "heteroaryl" is intended to mean an aryl group where one or more carbon atoms in an aromatic ring have been replaced with one or more heteroatoms selected from the group consisting of nitrogen, sulphur, phosphorous and oxygen. Furthermore, in the present context, the term "heteroaryl" comprises fused ring systems wherein at least one aryl ring and at least one heteroaryl ring, at least two heteroaryls, at least one heteroaryl and at least one heterocyclyl, or at least one heteroaryl and at least one C_{3-6}-cycloalkyl share at least one chemical bond.

Illustrative examples of a heteroaryl include furanyl, thienyl, pyrrolyl, phenoxazonyl, oxazolyl, thiazolyl, isothiazolyl, imidazolyl, pyrazolyl, isoxazolyl, imidazolyl isothiazolyl, oxadiazolyl, furazanyl, triazolyl, thiadiazolyl, piperidinyl, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl, pyrazolyl and triazinyl, isoindolyl, indolinyl, benzofuranyl, benzothiophenyl, benzopyrazolyl,indazolyl, benzimidazolyl, benzthiazolyl, purinyl, quinolizinyl, quinolinyl, isoquinolinyl, cinnolinyl, phthalazinyl, quinazolinyl, quinoxalinyl, naphthyridinyl, pteridinylthienofuranyl, carbazolyl, acridinyl, phenazinyl, phenothiazinyl, phenoxazinyl and thianthrenyl.

In the present context the term "optionally substituted" is intended to mean that the group in question may be substituted one or several times, such as 1 to 5 times, preferably 1 to 3 times, most preferably 1 to 2 times, with one or more groups selected from the group consisting of C_{1-6}-alkyl, C_{1-6}-alkoxy, oxo (which may be represented in the tautomeric enol

form), carboxyl, amino, hydroxy (which when present in an enol system may be represented in the tautomeric keto form), nitro, sulphono, sulphanyl, C_{1-6}-carboxyl, C_{1-6}-alkoxycarbonyl, C_{1-6}-alkylcarbonyl, formyl, aryl, aryloxy, aryloxycarbonyl, arylcarbonyl, heteroaryl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)aminocarbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkylcarbonylamino, cyano, guanidino, carbamido, C_{1-6}-alkanoyloxy, C_{1-6}-alkylsulphonyloxy, dihalogen-C_{1-6}-alkyl, trihalogen-C_{1-6}-alkyl and halogen, where aryl and heteroaryl substituents may themselves be substituted 1-3 times with C_{1-6}-alkyl, C_{1-6}-alkoxy, nitro, cyano, hydroxy, amino or halogen. In general, the above substituents may be susceptible to further optional substitution.

The term "infectious agent" is intended to mean pathogenic microorganisms, such as bacteria, viruses, fungi and intra- or extra-cellular parasites.

In a particular embodiment of the invention, the term "infectious agent" does not mean an agent selected from the group consisting of the *Staphylococcus aureus* strains ATCC 2593 and strains derived therefrom, SA-1199, SA-1199B, SA-K1712, SA-K1748, SA 8325-4, and SA-K2068.

In another embodiment of the invention, the term "infectious agent" does not mean *Plasmodium falciparum*.

Analogously, the term "infectious disease" is used about a disease caused by an infectious agent.

In the present context, the term "anti-infective agent" covers compounds, such as commercially available antibiotics, that are capable of killing, inhibiting or otherwise slowing the growth of the infectious agent.

Specific examples of antibiotics commonly used for treating bacterial and fungal infections include, but is not limited to, aminoglycosides, such as amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin and tobramycin; cabecephems, such as loracarbef; carbapenems, such as ertapenem, imipenem/cilastatin and meropenem; cephalosporins, such as cefadroxil, cefazolin, cephalexin, cefaclor, cefamandole, cephalexin, cefoxitin, cefprozil, cefuroxime, cefixime, cefdinir, cefditoren, cefoperazone, cefotaxime, cefpodoxime, ceftazidime, ceftibuten, ceftizoxime, ceftriaxone and cefepime; macrolides, such as azithromycin, clarithromycin, dirithromycin, erythromycin and troleandomycin; monobactam; penicillins, such as amoxicillin, ampicillin, carbenicillin, cloxacillin, dicloxacillin, nafcillin, oxacillin, penicillin G, penicillin V, piperacillin and ticarcillin;

polypeptides, such as bacitracin, colistin and polymyxin B; quinolones, such as ciprofloxacin, enoxacin, gatifloxacin, levofloxacin, lomefloxacin, moxifloxacin, norfloxacin, ofloxacin and trovafloxacin; sulfonamides, such as mafenide, sulfacetamide, sulfamethizole, sulfasalazine, sulfisoxazole and trimethoprim-sulfamethoxazole; tetracyclines, such as demeclocycline, doxycycline, minocycline, oxytetracycline and tetracycline;

Specific examples of anti-viral compounds commonly used for treating viral infections include, but is not limited to, acyclovir, amantadine, cidofovir famciclovir, fomivirsen, foscarnet, ganciclovir, interferon alpha, oseltamivir, penciclovir, ribavirin, rimantadine, trifluridine, valacyclovir, valganciclovir, vidarabine and zanamivir.

Specific examples of anti-fungal compounds commonly used for treating severe fungal infections include, but is not limited to, amphotericin B, caspofungin, fluconazole, flucytosine, itraconazole, ketoconazole and voriconazole.

In the present context, an infectious agent is said to be "resistant" or "drug resistant" if the infectious agent has undergone a change which reduces or eliminates the effectiveness of an anti-infective agent which is normally used to cure infections caused by the infectious agent. Analogously, the term "drug resistance" means a circumstance when a disease, e.g. an infectious disease, does not respond to a therapeutic agent, such as an anti-infective agent. Drug resistance can be intrinsic, which means that the disease has never been responsive to the therapeutic agent, or acquired, which means that the disease ceases responding to the therapeutic agent to which the disease had previously been responsive.

In the present context, an infectious agent is said to be "multidrug resistant" if the infectious agent has undergone a change which reduces or eliminates the effectiveness of two or more anti-infective agents which are normally used to cure infections caused by the infectious agent. Analogously, "multidrug resistance" is a type of drug resistance wherein a disease, e.g. an infectious disease, is resistant to a variety of drugs, such as a variety of anti-infective agents.

The term "clinically relevant amount" is intended to mean that the chemosensitising compound is administered to a patient in an amount, which, on the one hand, is capable of reducing the symptoms of the infectious disease or curing the infectious disease for which the patient is treated, but, on the other hand, is not toxic to the patient and does not lead to unacceptable side effects. As indicated above, many, if not all, of the chemosensitising compounds described herein are known to cause severe side effects in patients when administered in too high concentrations, i.e. in amounts which are not "clinically relevant".

In the present context, the term "naturally occurring" when used in connection with the term "infectious agent", i.e. in connection with pathogenic microorganisms, means that the infectious agent giving rise to the infectious disease is a microorganism that can be found in nature, including in human beings. It will be understood that infectious agents, such as gen-manipulated laboratory strains, or infectious agents which by other means have been changed and/or manipulated by human intervention, are not considered to be covered by the term "naturally occurring".

The term "serum" is used in its normal meaning, i.e. as blood plasma without fibrinogen and other clotting factors.

In order to combat or prevent the infectious disease, the chemosensitising compounds disclosed herein should be administered together with, or in combination with, an anti-infective agent. When used in this context the terms "together with" and "in combination with" should not be interpreted narrowly in the sense that the chemosensitising compound and the anti-infective agent should necessarily be administered simultaneously and/or form part of the same pharmaceutical composition, although this is one embodiment of the present invention. Thus, it should be understood that the terms "together with" and "in combination with" mean that the dosage (including the dosage form) and the administration frequency of each compound, i.e. the chemosensitising compound and the anti-infective agent, may be controlled individually. For example, one compound may be administered orally three times per day during the treatment period, while the other compound may be administered intravenously once per day during the treatment period. Likewise, one compound may be administered every day in the treatment period and the other compound may be administered only once or a few days in the treatment period. As explained above, the chemosensitising compound and the anti-infective compound may be administered simultaneously and they may be comprised in the same pharmaceutical composition. Accordingly, the terms "together with" and "in combination with" mean that the chemosensitising compound is administered to the patient at least once during the treatment period and that the anti-infective agent is also administered to the patient at least once during the treatment period.

Herein, the term "steady state serum concentration" (of a chemosensitising compound) is defined as those values that recur with each dose and represent a state of equilibrium between the amount of chemosensitising compound administered and the amount being eliminated in a given time interval.

In the present context, the term "treatment" refers to the administration of a drug to a subject and includes *i)* preventing an infectious disease (i.e. causing the clinical symptoms of the infectious disease not to develop), *ii)* inhibiting an infectious disease (i.e. arresting the development of the clinical symptoms of the infectious disease) and *iii)* relieving the disease (i.e. causing regression of the clinical symptoms of the infectious disease) as well as combinations thereof.

The terms "prophylaxis" or "prophylactic treatment" refers to the treatment of a subject who is not yet infected, but who may be susceptible to, or at risk of getting an infection.

The term "subject", as used herein, means a living vertebrate animal, e.g., a mammal, such as a human being.

"Pharmaceutically acceptable" means suitable for use in a mammal, in particular suitable for use in a human being.

When used herein, the term "chemosensitising compound" is intended to mean an agent which reverses a microorganism's or cell's resistance to a given anti-infective agent. Thus, a "chemosensitising compound", as used herein, sensitises resistant or multidrug resistant microorganisms or cells to the action of anti-infective agents.

Chemosensitising compounds

Concerning the general formula (I) above, the substituents R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8 and R_9 are each individually selected from the group consisting of hydrogen, halogen, hydroxy, amino, nitro, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted C_{2-6}-alkynyl and optionally substituted C_{1-6}-alkoxy, optionally substituted C_{2-6}-alkenyloxy, carboxy, optionally substituted C_{1-6}-alkoxycarbonyl, optionally substituted C_{1-6}-alkylcarbonyl, fomyl, optionally substituted C_{1-6}-alkylsulphonylamino, optionally substituted aryl, optionally substituted aryloxycarbonyl, optionally substituted aryloxy, optionally substituted arylcarbonyl, optionally substituted arylamino, arylsulphonylamino, optionally substituted heteroaryl, optionally substituted heteroaryloxycarbonyl, optionally substituted heteroaryloxy, optionally substituted heteroarylcarbonyl, optionally substituted heteroarylamino, heteroarylsulphonylamino, optionally substituted heterocyclyl, optionally substituted heterocyclyloxycarbonyl, optionally substituted heterocyclyloxy, optionally substituted heterocyclylcarbonyl, optionally substituted heterocyclylamino, heterocyclylsulphonylamino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)aminocarbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkylcarbonylamino, amino-C_{1-6}-alkyl-carbonylamino, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-carbonylamino, amino-C_{1-6}-alkyl-amino, mono- and di(C_{1-6}-

alkyl)amino-C_{1-6}-alkyl-amino, cyano, guanidino, carbamido, C_{1-6}-alkanoyloxy, C_{1-6}-alkylsulphonyl, C_{1-6}-alkylsulphinyl, C_{1-6}-alkylsulphonyloxy, aminosulfonyl, mono- and di(C_{1-6}-alkyl)aminosulfonyl, and optionally substituted C_{1-6}-alkylthio.

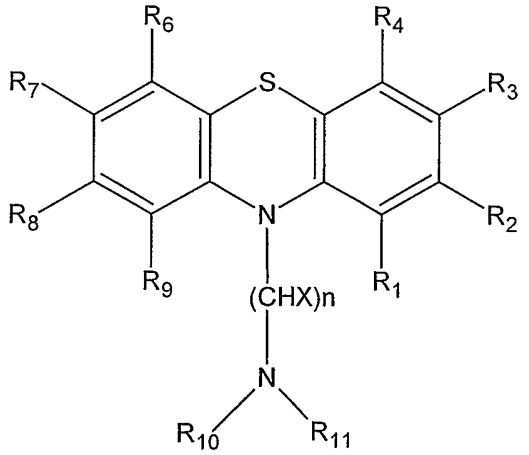
In a preferred embodiment of the invention, the R_2 substituent is an electron-withdrawing group, such as halogen, nitro or halogen-substituted C_{1-6}-alkyl. More preferably, R_2 is selected from the group consisting of F, Cl, Br, I, CH_2Y, CHY_2 and CY_3 (wherein Y represents a halogen atom), such as CH_2Cl, CH_2F, $CHCl_2$, CHF_2, CCl_3 or CF_3, in particular CCl_3 or CF_3. Most preferably, R_2 is Cl or CF_3.

The substituents R_1, R_3, R_4, R_5, R_6, R_7, R_8 and R_9 are preferably each individually selected from the group consisting of hydrogen, optionally substituted C_{1-6}-alkyl and optionally substituted C_{1-6}-alkoxy. More preferably, all of R_1, R_3, R_4, R_5, R_6, R_7, R_8 and R_9 are hydrogen.

Accordingly, in a highly preferred embodiment of the invention, R_2 is Cl or CF_3 and each of R_1, R_3, R_4, R_5, R_6, R_7, R_8 and R_9 are hydrogen.

As mentioned above, V is selected from the group consisting of S, SO_2, SO, O and NH, such as S or SO. In a highly preferred embodiment of the invention, V is S.

As will be understood, in case W is N-$(CHX)_n$-$N(R_{10})(R_{11})$ and V is S, the chemosensitising compound of the general formula (I) becomes a phenothiazine of the general formula (II)



(II)

wherein n is an integer in the range of from 2 to 6, such as 2, 3, 4, 5 or 6, and each X is individually selected from the group consisting of hydrogen, halogen, hydroxy, amino, nitro, optionally substituted C_{1-6}-alkyl and optionally substituted C_{1-6}-alkoxy. R_{10} and R_{11}

are each independently selected from the group consisting of hydrogen, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted C_{2-6}-alkynyl, optionally substituted C_{1-6}-alkoxycarbonyl, optionally substituted C_{1-6}-alkylcarbonyl, optionally substituted aryl, optionally substituted aryloxycarbonyl, optionally substituted arylcarbonyl, optionally substituted heteroaryl, optionally substituted heteroaryloxycarbonyl, optionally substituted heteroarylcarbonyl, aminocarbonyl, mono- and di(C_{1-6}-alkyl)aminocarbonyl; or R_{10} and R_{11} together with the nitrogen atom to which they are attached form an optionally substituted nitrogen-containing heteroaryl or optionally substituted heterocyclyl.

In a preferred embodiment of the invention n is 2 or 3 and X is hydrogen or CH_3. Thus in a preferred embodiment of the invention, W is N-$(CH_2)_3$-N(R_{10})(R_{11}) or N-CH_2-CH(CH_3)-N(R_{10})(R_{11}). In particular, the structure where W is N-$(CH_2)_3$-N(R_{10})(R_{11}) is preferred.

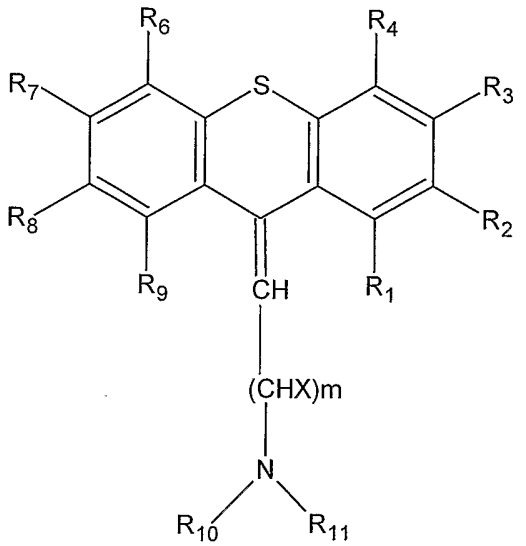
In one interesting embodiment of the invention, R_{10} and R_{11} are each individually selected from the group consisting of hydrogen and optionally substituted C_{1-6}-alkyl. According to this embodiment, it is preferred that both of R_{10} and R_{11} are optionally substituted C_{1-6}-alkyl. Most preferably both of R_{10} and R_{11} are CH_3.

In another interesting embodiment of the invention, R_{10} and R_{11}, together with the nitrogen atom to which they are attached, form an optionally substituted heterocyclyl, such as optionally substituted 2-pyrrolinyl, optionally substituted 3-pyrrolinyl, optionally substituted pyrrolidinyl, optionally substituted 2-imidazolinyl, optionally substituted imidazolidinyl, optionally substituted 2-pyrazolinyl, optionally substituted 3-pyrazolinyl, optionally substituted pyrazolidinyl, optionally substituted piperidinyl, optionally substituted morpholinyl, optionally substituted thiomorpholinyl or optionally substituted piperazinyl, According to this embodiment, it is preferred that R_{10} and R_{11}, together with the nitrogen atom to which they are attached, form an optionally substituted piperidinyl or an optionally substituted piperazinyl, in particular an optionally substituted piperazinyl. The piperazinyl ring may be unsubstituted, but is preferably substituted with an optionally substituted C_{1-6} alkyl group, in particular in the *para* position, i.e. an optionally substituted C_{1-6} alkyl group is covalently attached to the second nitrogen atom of the piperazinyl ring. In a highly preferred embodiment of the invention, the optionally substituted C_{1-6}-alkyl is selected from the group consisting of -CH_3, -CH_2OH, -CH_2-CH_3 and -CH_2-CH_2OH, such as -CH_3 or -CH_2-CH_2OH, in particular -CH_2-CH_2OH.

Specific examples of the above-mentioned phenothiazines include promazine, 1-chlorpromazine, chlorpromazine, 7-hydroxychlorpromazine, 7,8-dihydroxychlorpromazine,

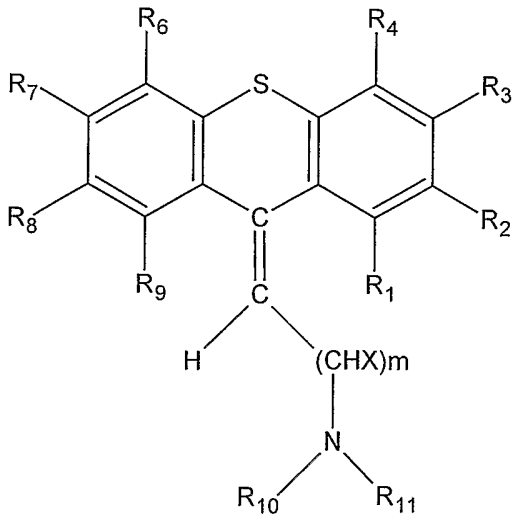
thiomethlypromazine, trifluorpromazine, desmethylclorpromazine, perphenazine, prochlorperazine and 2-chloro-10-(2-dimethylaminoethyl)phenothiazine.

As will also be understood, in case W is C=CH-$(CHX)_m$-$N(R_{10})(R_{11})$ and V is S, the chemo-sensitising compound of the general formula (I) becomes a thioxanthene of the general formula (III)

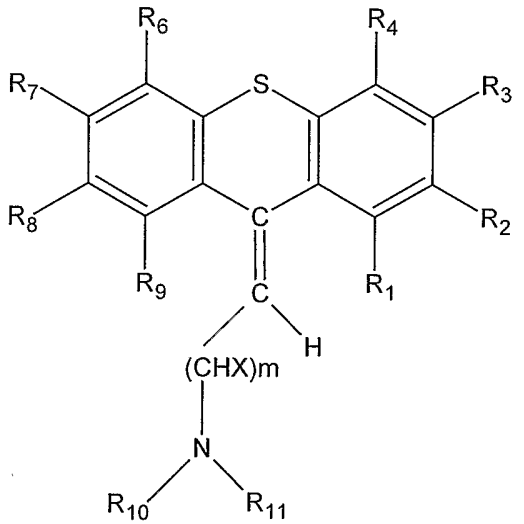


(III)

A thioxanthene of the general formula (III) gives rise to *cis* and *trans* isomerism. In the present context, compounds of the general formula (IIIa) are said to be in the *cis* configuration, whereas compounds of the general formula (IIIb) are said to be in the *trans* configuration:



(IIIa)



(IIIb)

wherein m is an integer in the range of from 1 to 5, such as 1, 2, 3, 4, or 5, and each X is individually selected from the group consisting of hydrogen, halogen, hydroxy, amino, nitro, optionally substituted C_{1-6}-alkyl and optionally substituted C_{1-6}-alkoxy. R_{10} and R_{11} are each independently selected from the group consisting of hydrogen, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted C_{2-6}-alkynyl, optionally substituted C_{1-6}-alkoxycarbonyl, optionally substituted C_{1-6}-alkylcarbonyl, optionally substituted aryl, optionally substituted aryloxycarbonyl, optionally substituted arylcarbonyl, optionally substituted heteroaryl, optionally substituted heteroaryloxycarbonyl, optionally substituted heteroarylcarbonyl, aminocarbonyl, mono- and di(C_{1-6}-alkyl)aminocarbonyl; or R_{10} and R_{11} together with the nitrogen atom to which

they are attached form an optionally substituted nitrogen-containing heteroaryl or optionally substituted heterocyclyl.

It is generally preferred that the compounds of the general formula (III) have the *trans* configuration, i.e. the structure shown in the general formula (IIIb).

In a preferred embodiment, X is hydrogen and m is 2 or 3, in particular 3. Thus, in a preferred embodiment of the invention, W has the structure $C=CH-(CH_2)_2-N(R_{10})(R_{11})$.

In one interesting embodiment of the invention, R_{10} and R_{11} are each individually selected from the group consisting of hydrogen and optionally substituted C_{1-6}-alkyl. According to this embodiment, it is preferred that both of R_{10} and R_{11} are optionally substituted C_{1-6}-alkyl. Most preferably both of R_{10} and R_{11} are CH_3.

In another interesting embodiment of the invention, R_{10} and R_{11}, together with the nitrogen atom to which they are attached, form an optionally substituted heterocyclyl, such as optionally substituted 2-pyrrolinyl, optionally substituted 3-pyrrolinyl, optionally substituted pyrrolidinyl, optionally substituted 2-imidazolinyl, optionally substituted imidazolidinyl, optionally substituted 2-pyrazolinyl, optionally substituted 3-pyrazolinyl, optionally substituted pyrazolidinyl, optionally substituted piperidinyl, optionally substituted morpholinyl, optionally substituted thiomorpholinyl or optionally substituted piperazinyl, According to this embodiment, it is preferred that R_{10} and R_{11}, together with the nitrogen atom to which they are attached, form an optionally substituted piperidinyl or an optionally substituted piperazinyl, in particular an optionally substituted piperazinyl. The piperazinyl ring may be unsubstituted, but is preferably substituted with an optionally substituted C_{1-6} alkyl group, in particular in the *para* position, i.e. an optionally substituted C_{1-6} alkyl group is covalently attached to the second nitrogen atom of the piperazinyl ring. In a highly preferred embodiment of the invention, the optionally substituted C_{1-6}-alkyl is selected from the group consisting of $-CH_3$, $-CH_2OH$, $-CH_2-CH_3$ and ,CH_2-CH_2OH, such as $-CH_3$ or $-CH_2-CH_2OH$, in particular $-CH_2-CH_2OH$.

Specific examples of the above-mentioned phenothiazines include *trans*-flupenthixol, *cis*-flupenthixol, *trans*-clopenthixol and *cis*- clopenthixol. Particularly preferred chemo-sensitising compounds for the use according to the invention are *trans*-flupenthixol and *trans*-clopenthixol. Most preferred is *trans*-clopenthixol.

As is evident from the formulae shown herein and the definitions associated therewith, certain of the chemosensitising compounds described herein are chiral. Moreover, the presence of certain unsaturated or cyclic fragments or multiple stereogenic atoms provides

for the existence of diastereomeric forms of some of the chemosensitising compounds. The invention is intended to include all stereoisomers, including optical isomers, and mixtures thereof, as well as pure, partially enriched, or, where relevant, racemic forms. In particular, many of the chemosensitising compounds described herein may be in the form of *E*- or *Z*-stereoisomers, or mixtures of such isomers.

It should furthermore be understood that the chemosensitising compounds described herein include possible salts thereof, of which pharmaceutically acceptable salts are of course especially relevant for the therapeutic applications. Salts include acid addition salts and basic salts. Examples of acid addition salts are hydrochloride salts, fumarate, oxalate, etc. Examples of basic salts are salts where the (remaining) counter ion is selected from alkali metals, such as sodium and potassium, alkaline earth metals, such as calcium salts, potassium salts, and ammonium ions ($^{+}N(R')_4$, where the R's independently designate optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted aryl, or optionally substituted heteroaryl). Pharmaceutically acceptable salts are, e.g., those described in Remington's - The Science and Practice of Pharmacy, 20th Ed. Alfonso R.Gennaro (Ed.), Lippincott, Williams & Wilkins; ISBN: 0683306472, 2000, and in Encyclopedia of Pharmaceutical Technology.

The effect of the chemosensitising compounds for reversing drug resistance or multiple drug resistance may be assayed as described herein and the efficiency of the chemosensitising compound in combination with selected anti-infective agents against selected microorganisms may be expresses as the MIC value, DR ratio and/or the FIC index.

The Minimal Inhibitory Concentration, (MIC) is defined as the lowest inhibitory concentration showing no visible growth according to the NCCLS Guidelines.

The Drug Resistance (DR) ratio is defined as the ratio between the MIC value for anti-infective agent alone divided by the MIC for the anti-infective agent in the presence of the chemosensitising compound. This ratio represents the increase in apparent potency of the anti-infective agent caused by the chemosensitising compound, and may be expressed as

$$\text{DR ratio} = (MIC_{\text{anti-infective agent}})/(MIC_{\text{anti-infective agent + chemosensitising compound}})$$

The Fractional Inhibitory Concentration (FIC) index may be calculated for each anti-infective agent alone and in combination with chemosensitising according to the following formulae:

$$FIC = FIC_{\text{chemosensitising compound}} + FIC_{\text{anti-infective agent}}$$

where:

$$FIC_{\text{chemosensitising compound}} = (MIC_{\text{chemosensitising compound + anti-infective agent}})/(MIC_{\text{chemosensitising compound}})$$

$$FIC_{\text{anti-infective agent}} = (MIC_{\text{anti-infective agent + chemosensitising compound}}/(MIC_{\text{anti-infective agent}})$$

The synergistic effects of the chemosensitising compounds described herein, i.e. their ability to reverse drug resistance or multiple drug resistance in a microorganism, may be assessed by any of the methods available to those skilled in the art, including the *in vitro* assays described in the examples herein. In a preferred embodiment of the invention, the chemosensitising compound, the anti-infective agent and the infectious agent (and hence the infectious disease to be treated) exhibit a FIC index of at the most 0.5 when determined as described in the examples herein. More preferably, the FIC index is at the most 0.4, such as at the most 0.3, e.g. at the most 0.2. Even more preferably, the FIC index is at the most 0.1, such as at the most 0.075, at the most 0.05 or even at the most 0.025.

As described previously, the chemosensitising compounds described herein typically have a high MIC value. For chemosensitising compounds, which are effective inhibitors, this means that the ratio $(MIC_{\text{chemosensitising compound + anti-infective agent}})/(MIC_{\text{chemosensitising compound}})$ becomes close to zero, which, in turn, means that $FIC_{\text{chemosensitising compound}} \approx 0$. This also means that $FIC \approx FIC_{\text{anti-infective agent}} = (MIC_{\text{anti-infective agent + chemosensitising compound}}/(MIC_{\text{anti-infective agent}}) \approx 1/DR$.

Accordingly, in another preferred embodiment of the invention, the chemosensitising compound, the anti-infective agent and the infectious agent (and hence the infectious disease to be treated) exhibit a DR ratio of at least 2 when determined as described in the examples herein. More preferably, the DR ratio is at least 5, such as at least 10, e.g. at least 20. Even more preferably, the MIC value is at least 30, such as at least 50, at least 75 or even at least 100.

Therapy, pharmaceutical compositions and dosages

As explained above, the chemosensitising compounds described herein are useful for treatment of infectious diseases in combination with an anti-infective agent. Thus, the chemosensitising compounds described herein may be used for the manufacture of a medicament for the treatment of an infectious disease in combination with an anti-infective agent.

In addition, the chemosensitising compounds described herein are useful for prophylactic treatment of infectious diseases in combination with an anti-infective agent. This may be particularly relevant in situations where a person has a high risk of getting infections, such as immunosuppressed patients or patients undergoing surgery. Thus, the chemosensitising compounds described herein may also be used for the manufacture of a medicament for the prophylactic treatment of an infectious disease in combination with an anti-infective agent.

Also, the chemosensitising compounds described herein may be used, in combination with an anti-infective agent, for the manufacture of a medicament for the treatment or prophylaxis of infectious diseases.

In a further aspect, the present invention is directed to the chemosensitising compounds described herein for use as medicaments in combination with an anti-infective agent, or to the chemosensitising compounds described herein, in combination with an anti-infective agent, for use as medicaments.

In another, but related, aspect, the chemosensitising compounds described herein are useful for decreasing resistance, in particular multidrug resistance, of an infectious agent. Thus, the chemosensitising compounds described herein may be used for the manufacture of a medicament for decreasing resistance of an infectious agent against an anti-infective agent.

In still another, but also related aspect, the chemosensitising compounds described herein are useful for sensitising susceptible, resistant or multidrug resistant cells, preferably resistant and multidrug resistant cells, more preferably multidrug resistant cells, to an anti-infective agent. Thus, the chemosensitising compounds described herein may be used for the manufacture of a medicament for sensitising susceptible, resistant or multidrug resistant cells, preferably resistant and multidrug resistant cells, more preferably multidrug resistant cells, to an anti-infective agent.

A further aspect of the present invention relates to a method for treating or preventing an infectious disease in a subject, said method comprising administering to said subject a chemosensitising compound as described herein in combination with an anti-infective agent.

A still further aspect of the present invention relates to a method for decreasing resistance, in particular multidrug resistance, of an infectious agent.

An even further aspect of the present invention relates for a method for sensitising susceptible, resistant or multidrug resistant microorganisms or cells, preferably resistant and multidrug resistant microorganisms or cells, more preferably multidrug resistant microorganisms or cells, to an anti-infective agent.

Still another aspect of the present invention relates to a method for prevention of development of resistance or multidrug resistance in an infectious agent, said method comprising administering to a subject a chemosensitising compound as described herein.

Yet a further aspect of the invention relates to a kit comprising a first dosage unit comprising a chemosensitising compound as described herein and a further dosage unit comprising an antimicrobial agent.

Therapy

As will be understood from the disclosure herein, the infectious disease to be treated is one that is normally treated with an anti-infective agent. The infectious disease is normally caused by an infectious agent, such as a bacterium, a virus, a fungi or an intra- or extra-cellular parasite. The infectious agent is typically naturally-occurring, i.e. a naturally-occurring bacterium, a naturally occurring virus, a naturally occurring fungi or a naturally occurring intra- or extra-cellular parasite.

More particularly, the infectious agent may be Gram negative or Gram positive bacteria.

Specific examples include Gram negative bacteria of a genus selected from the group consisting of Escherichia, Proteus, Salmonella, Klebsiella, Providencia, Enterobacter, Burkholderia, Pseudomonas, Acinetobacter, Aeromonas, Haemophilus, Yersinia, Neisseria, Erwinia, Rhodopseudomonas and Burkholderia.

Specific examples of Gram positive bacteria include bacteria from a genus selected from the group consisting of Lactobacillus, Azorhizobium, Streptococcus, Pediococcus, Photobacterium, Bacillus, Enterococcus, Staphylococcus, Clostridium, Butyrivibrio, Sphingomonas, Rhodococcus and Streptomyces.

In other embodiments, the infectious agent is, e.g., from a genus selected from the group consisting of Methanobacierium, Sulfolobus, Archaeoglobu, Rhodobacter and Sinorhizobium.

In still other embodiments, the infectious agent is fungi, such as from the genus Mucor or

Candida, e.g., Mucor racemosus or Candida albicans; from genus Crytococcus e.g., Cr. Neoformans; or from Genus Aspergillus, e.g., A. fumingatus.

In additional embodiments, the infectious agent is virus, such as picornaviridae, reoviridae, orthomyxoviridae, paramyxoviridae, adenoviridae, coronaviridae, Human Immunedefiency Virus, hepatitisvira, herpesviridae, oncovira, cytomegalovira, papovaviridae or prions.

In yet other embodiments, the infectious agent is protozoa, such as a malaria or cryptosporidium parasite.

In a particular embodiment of the invention, the infectious agent is not a *Staphylococcus aureus* strain selected from the group consisting of ATCC 2593 and strains derived therefrom, SA-1199, SA-1199B, SA-K1712, SA-K1748, SA 8325-4, and SA-K2068.

In another embodiment of the invention, the infectious agent is not *Plasmodium falciparum*.

Toxicity and therapeutic efficacy of the chemosensitising compounds described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., by determining the LD_{50} (the dose lethal for 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio between LD_{50} and ED_{50} (LD_{50}/ED_{50}). Chemosensitising compounds which exhibit large therapeutic indices are preferred. The data obtained from these cell culture assays or animal studies can be used in formulating a range of dosage for use in human subjects. The dosage of such chemosensitising compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilised.

<u>Pharmaceutical compositions</u>

The chemosensitising compounds described herein are typically formulated in a pharmaceutical composition prior to use as a drug substance.

Accordingly, in a further aspect the present invention relates to a pharmaceutical composition comprising a chemosensitising compound as described herein and at least one pharmaceutically acceptable carrier or exipient.

The pharmaceutical composition may contain a single or two or more chemosensitising compound(s) as described herein. In an interesting embodiment of the invention the pharmaceutical composition comprises, in addition to a chemosensitising compound, one or more anti-infective agent(s).

The administration route of the chemosensitising compounds described herein may be any suitable route that leads to a concentration in the blood or tissue corresponding to a clinically relevant concentration. Thus, e.g., the following administration routes may be applicable although the invention is not limited thereto: the oral route, the parenteral route, the cutaneous route, the percutaneous route, the nasal route, the topical route, the rectal route, the vaginal route and the ocular route. It should be clear to a person skilled in the art that the administration route is dependant on the particular chemosensitising compound in question, particularly, the choice of administration route depends on the physico-chemical properties of the chemosensitising compound together with the age and weight of the patient and on the particular disease or condition and the severity of the same. In general, however, the oral and the parenteral routes are preferred.

The chemosensitising compounds described herein may be contained in any appropriate amount in the pharmaceutical composition, and are generally contained in an amount of about 0.1-95% by weight of the total weight of the composition. The composition may be presented in a dosage form, such as a unit dosage form, which is suitable for the oral, parenteral, rectal, cutaneous, percutaneous, nasal, topical, vaginal and/or ocular administration route. Thus, the composition may be in form of, e.g., tablets, capsules, pills, powders, granulates, suspensions, emulsions, solutions, gels including hydrogels, pastes, ointments, creams, plasters, drenches, delivery devices, suppositories, enemas, injectables, implants, sprays, aerosols and in other suitable form.

The pharmaceutical compositions may be formulated according to conventional pharmaceutical practice, see, e.g., "Remington's Pharmaceutical Sciences" and "Encyclopedia of Pharmaceutical Technology", edited by Swarbrick, J. & J. C. Boylan, Marcel Dekker, Inc., New York, 1988. Typically, the chemosensitising compounds described herein are formulated with (at least) a pharmaceutically acceptable carrier or exipient. Pharmaceutically acceptable carriers or exipients are those known by the person skilled in the art.

Oral formulations

Pharmaceutical compositions for oral use include tablets which contain a chemosensitising compound as described herein, optionally in combination with at least one anti-infective

agent, in admixture with non-toxic pharmaceutically acceptable excipients. These excipients may be, for example,

inert diluents or fillers, such as sucrose, sorbitol, sugar, mannitol, microcrystalline cellulose, starches including potato starch, calcium carbonate, sodium chloride, lactose, calcium phosphate, calcium sulfate or sodium phosphate;

granulating and disintegrating agents, for example, cellulose derivatives including microcrystalline cellulose, starches including potato starch, croscarmellose sodium, alginates or alginic acid;

binding agents, for example, sucrose, glucose, sorbitol, acacia, alginic acid, sodium alginate, gelatin, starch, pregelatinized starch, microcrystalline cellulose, magnesium aluminum silicate, carboxymethylcellulose sodium, methylcellulose, hydroxypropyl methylcellulose, ethylcellulose, polyvinylpyrrolidone or polyethylene glycol; and

lubricating agents, including glidants and antiadhesives, for example, magnesium stearate, zinc stearate, stearic acid, silicas, hydrogenated vegetable oils or talc.

Other pharmaceutically acceptable excipients can be colorants, flavouring agents, plasticisers, humectants, buffering agents, etc.

The tablets may be uncoated or they may be coated by known techniques, optionally to delay disintegration and absorption in the gastrointestinal tract and thereby providing a sustained action over a longer period. The coating may be adapted to release the chemo-sensitising compound in a predetermined pattern, e.g., in order to achieve a controlled release formulation (see below) or it may be adapted not to release the active drug substance until after passage of the stomach (enteric coating). The coating may be a sugar coating, a film coating (e.g. based on hydroxypropyl methylcellulose, methylcellulose, methyl hydroxyethylcellulose, hydroxypropylcellulose, carboxymethylcellulose, acrylate copolymers (Eudragit E®), polyethylene glycols and/or polyvinylpyrrolidone) or an enteric coating (e.g. based on methacrylic acid copolymer (Eudragit® L and S), cellulose acetate phthalate, hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate succinate, polyvinyl acetate phthalate, shellac and/or ethylcellulose).

Furthermore, a time delay material such as, e.g., glyceryl monostearate or glyceryl distearate may be employed.

In addition, the solid tablet compositions as mentioned above may be provided with a coating adapted to protect the composition from unwanted chemical changes, e.g. chemical degradation, prior to the release of the chemosensitising compound.

The coating may be applied on the solid dosage form in a similar manner as that described in "Aqueous film coating" by James A. Seitz in "Encyclopedia of Pharmaceutical Technology", Vol 1, pp.337-349 edited by Swarbrick, J. & J. C. Boylan, Marcel Dekker, Inc., New York, 1988.

Formulations for oral use may also be presented as chewing tablets, or as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, potato starch, lactose, microcrystalline cellulose, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin, or olive oil.

Powders and granulates may be prepared using the ingredients mentioned above under tablets and capsules in a conventional manner using, e.g., a mixer, a fluid bed apparatus or a spray drying equipment.

Controlled release compositions for oral use may, e.g., be constructed to release the active drug substance by controlling the dissolution and/or the diffusion of the active drug substance.

Dissolution or diffusion controlled release can be achieved by appropriate coating of a tablet, capsule, pellet or granulate formulation of the chemosensitising compound, or by incorporating the chemosensitising compound in question in, e.g., an appropriate matrix.

A controlled release coating may comprise one or more of the coating substances mentioned above and/or, e.g., shellac, beeswax, glycowax, castor wax, carnauba wax, stearyl alcohol, glyceryl monostearate, glyceryl distearate, glycerol palmitostearate, ethylcellulose, acrylic resins, dl-polylactic acid, cellulose acetate butyrate, polyvinyl chloride, polyvinyl acetate, vinyl pyrrolidone, polyethylene, polymethacrylate, methylmethacrylate, 2-hydroxymethacrylate, methacrylate hydrogels, 1,3-butylene glycol, ethylene glycol methacrylate and/or polyethylene glycols.

In a controlled release matrix formulation of the chemosensitising compound, the matrix material may comprise, e.g., hydrated metylcellulose, carnauba wax and stearyl alcohol, carbopol 934, silicone, glyceryl tristearate, methyl acrylate-methyl methacrylate, polyvinyl chloride, polyethylene and/or halogenated fluorocarbon.

A controlled release composition of the chemosensitising compounds described herein, may also be in the form of a buoyant tablet or capsule, i.e. a tablet or capsule which upon oral administration floats on top of the gastric content for a certain period of time. A buoyant tablet formulation of the chemosensitising compound in question can be prepared by granulating a mixture of the chemosensitising compound, excipients and 20-75% w/w of hydrocolloids, such as hydroxyethylcellulose, hydroxypropylcellulose and hydroxypropylmethylcellulose. The obtained granules can then be compressed into tablets. On contact with the gastric juice, the tablet can form a substantially water-impermeable gel barrier around its surface. This gel barrier takes part in maintaining a density of less than one, thereby allowing the tablet to remain buoyant in the gastric juice.

Fluid/liquid compositions for oral use

Powders, dispersible powders or granules suitable for preparation of an aqueous suspension by addition of water are also convenient dosage forms. Formulation as a suspension, an emulsion or a dispersion provides the active substance in admixture with a dispersing or wetting agent, suspending agent, and/or one or more preservatives. Such formulations may also be suitable for use in of an active substance to e.g. a mucosa such as the gastrointestinal, buccal, nasal, rectal, or vaginal mucosa, or for administration to intact or damaged skin, or wounds.

Suitable dispersing or wetting agents are, for example, naturally occurring phosphatides, e.g., lecithin, or soybean lecithin; condensation products of ethylene oxide with e.g. a fatty acid, a long chain aliphatic alcohol, or a partial ester derived from fatty acids and a hexitol or a hexitol anhydride, for example polyoxyethylene stearate, polyoxyethylene sorbitol monooleate, polyoxyethylene sorbitan monooleate etc.

Suitable suspending agents are, e.g., naturally occurring gums such as, e.g., gum acacia, xanthan gum, or gum tragacanth; celluloses such as, e.g., sodium carboxymethylcellulose, microcrystalline cellulose (e.g, Avicel® RC 591, methylcellulose; alginates such as, e,g., sodium alginate, etc.

Suitable examples of preservatives for use in formulations according to the invention are parabens, such as methyl or propyl p-hydroxybenzoate, and benzalkonium chloride.

Rectal and/or vaginal formulations

For application to the rectal or vaginal mucosa suitable formulations for use according to the invention include suppositories (emulsion or suspension type), enemas, and rectal

gelatin capsules (solutions or suspensions). Appropriate pharmaceutically acceptable suppository bases include cocoa butter, esterified fatty acids, glycerinated gelatin, and various water-soluble or dispersible bases like polyethylene glycols and polyoxyethylene sorbitan fatty acid esters.

Various additives like, e.g., enhancers or surfactants may be incorporated.

Nasal formulations

For application to the nasal mucosa, nasal sprays and aerosols for inhalation are suitable compositions for use according to the invention. In a typically nasal formulation, the active substance is present in the form of a particulate formulation optionally dispersed in a suitable vehicle. The pharmaceutically acceptable vehicles and excipients and optionally other pharmaceutically acceptable materials present in the composition such as diluents, enhancers, flavouring agents, preservatives etc. are all selected in accordance with conventional pharmaceutical practice in a manner understood by the persons skilled in the art of formulating pharmaceuticals.

Nasal administration may be employed in those cases where an immediate effect is desired, Furthermore, after administration of a nasal formulation according to the invention, the active substance may be adsorped on the nasal mucosa. The adsorption to the mucosa is believed to lead to a less irritative effect than when e.g. a liquid vehicle e.g. containing a penetration enhancer or promoter is employed.

Topical formulations

For application to the skin, the formulations according to the invention may contain conventionally non-toxic pharmaceutically acceptable carriers and excipients including microspheres and liposomes. The formulations include creams, ointments, lotions, liniments, gels, hydrogels, solutions, suspensions, sticks, sprays, pastes, plasters, and other kind of transdermal drug delivery systems. The pharmaceutically acceptable excipients may include emulsifying agents, antioxidants, buffering agents, preservatives, humectants, penetration enhancers, chelating agents, gelforming agents, ointment bases, perfumes, and skin protective agents.

Examples of emulsifying agents are naturally occurring gums, e.g. gum acacia or gum tragacanth, naturally occurring phosphatides, e.g. soybean lecithin, and sorbitan monooleate derivatives.

Examples of antioxidants are butylated hydroxy anisole (BHA), ascorbic acid and derivatives thereof, tocopherol and derivatives thereof, butylated hydroxy anisole, and cysteine.

Examples of preservatives are parabens, such as methyl or propyl p-hydroxybenzoate, and benzalkonium chloride.

Examples of humectants are glycerin, propylene glycol, sorbitol, and urea.

Examples of penetration enhancers are propylene glycol, DMSO, triethanolamine, N,N-dimethylacetamide, N,N-dimethylformamide, 2-pyrrolidone and derivatives thereof, tetrahydrofurfuryl alcohol, and Azone®.

Examples of chelating agents are sodium EDTA, citric acid, and phosphoric acid.

Examples of other excipients are edible oils like almond oil, castor oil, cacao butter, coconut oil, corn oil, cottonseed oil, linseed oil, olive oil, palm oil, peanut oil, poppyseed oil, rapeseed oil, sesame oil, soybean oil, sunflower oil, and teaseed oil; and of polymers such as carmelose, sodium carmelose, hydroxypropylmethylcellulose, hydroxyethylcellylose, hydroxypropylcellulose, chitosane, pectin, xanthan gum, carragenan, locust bean gum, acacia gum, gelatin, and alginates, Examples of ointment bases are beeswax, paraffin, cetyl palmitate, vegetable oils, sorbitan esters of fatty acids (Span), polyethylene glycols, and condensation products between sorbitan esters of fatty acids and ethylene oxide, e.g'. polyoxyethylene sorbitan monooleate (Tween).

The formulations mentioned above for topical administration may also be applied to wounds or they may be suitable for direct application or for introduction into relevant orifice(s) of the body, e.g. the rectal, urethral, vaginal or oral orifices. The formulation may simply be applied directly on the part to be treated such as, e.g., the mucosa.

Parenteral formulations

The pharmaceutical composition may also be administered parenterally by injection, infusion or implantation (intravenous, intramuscular, intraarticular, subcutaneous or the like) in dosage forms, formulations or e.g. suitable delivery devices or implants containing conventional, non-toxic pharmaceutically acceptable carriers and adjuvants.

The formulation and preparation of such compositions is well-known to those skilled in the art of pharmaceutical formulation. Specific formulations can be found in the textbook entitled "Remington's Pharmaceutical Sciences".

Compositions for parenteral use may be presented in unit dosage forms, e.g. in ampoules, or in vials containing several doses and in which a suitable preservative may be added (see below). The composition may be in form of a solution, a suspension, an emulsion, an infusion device or a delivery device for implantation or it may be presented as a dry powder to be reconstituted with water or another suitable vehicle before use. Apart from the chemosensitising compounds described herein, the compositions may comprise suitable parenterally acceptable carriers and/or excipients or the active drug substance may be incorporated into microspheres, microcapsules, nanoparticles, liposomes or the like for controlled release. Furthermore, the composition may, in addition, conveniently comprise suspending, solubilising, stabilising, pH-adjusting agents and/or dispersing agents.

In another interesting embodiment of the invention, the pharmaceutical composition is a solid dosage form, such as a tablet, prepared from the particulate material described in WO 03/004001 and WO 2004/062643.

As indicated above, the pharmaceutical compositions may contain the chemosensitising compound in the form of a sterile injection. To prepare such a composition, the chemosensitising compound is dissolved or suspended in a parenterally acceptable liquid vehicle. Among acceptable vehicles and solvents that may be employed are water, water adjusted to a suitable pH by addition of an appropriate amount of hydrochloric acid, sodium hydroxide or a suitable buffer, 1,3-butanediol, Ringer's solution and isotonic sodium chloride solution. The aqueous formulation may also contain one or more preservatives, for example, methyl, ethyl or n-propyl p-hydroxybenzoate. In cases where chemosensitising compound is only sparingly or slightly soluble in water, a dissolution enhancing or solubilising agent can be added or the solvent may apart from water comprise 10-60% w/w of propylene glycol or the like.

Dosages

As discussed in detail previously, an important aspect of the present invention is the realisation that the chemosensitising compounds described herein are capable of reversing resistance or multidrug resistance when administered in clinical relevant amounts, i.e. in amounts sufficiently small to avoid the severe side effects normally associated with the chemosensitising compounds described herein.

It will be understood that the dosage to be administered will be dependent on the administration form (see below). Independently, of the administration form, the chemosensitising compound should be administered in clinically relevant amounts, i.e. in amounts which on the one hand exert the relevant therapeutic effect, but on the other hand does not provide severe side effects.

Preferably, a chemosensitising compound as described herein is administered in a clinically relevant amount giving rise to a steady state serum concentration of less than 8.0 mg/l. More preferably, the chemosensitising compound is administered in a clinically relevant amount giving rise to a steady state serum concentration of less than 7.0 mg/l, such as less than 6.0 mg/l, e.g. less than 5.0 mg/l. Even more preferably, the chemosensitising compound is administered in a clinically relevant amount giving rise to a steady state serum concentration of less than 4.0 mg/l, such as less than 3.0 mg/l, e.g. less than 2.0 mg/l. Most preferably, the chemosensitising compound is administered in a clinically relevant amount giving rise to a steady state serum concentration of less than 1.5 mg/l, e.g. about 1.0 mg/l or about 0.5 mg/l.

In other words, the chemosensitising compound is preferably administered in a clinically relevant amount giving rise to a steady state serum concentration in the interval of from 0.01 μg/l to less than 8.0 mg/l, such as in the interval of from 0.02 μg/l to 7.0 mg/l, e.g. in the interval of from 0.04 μg/l to 6.0 mg/l. More preferably, the steady state serum concentration of the chemosensitising compound is in the interval of from 0.06 μg/l to 5.0 mg/l, such as is in the interval of from 0.08 μg/l to 4.0 mg/l, e.g. in the interval of from 0.1 μg/l to 3.0 mg/l. Even more preferably, the steady state serum concentration of the chemosensitising compound is in the interval of from 0.2 μg/l to 2.0 mg/l, such as in the interval of from 0.4 μg/l to 2.0 mg/l, e.g. in the interval of from 0.5 μg/l to 2.0 mg/l. Still more preferably, the steady state serum concentration of the chemosensitising compound is in the interval of from 0.6 μg/l to 2.0 mg/l, such as in the interval of from 0.8 μg/l to 2.0 mg/l, e.g. in the interval of from 0.9 μg/l to 2.0 mg/l. Most preferably, the steady state serum concentration of the chemosensitising compound is in the interval of from 1.0 μg/l to 2.0 mg/l, such as in the interval of from 1.5 μg/l to 2.0 mg/l, e.g. in the interval of from 1.5 μg/l to 1.5 mg/l.

The chemosensitising compound is preferably administered in an amount of about 0.1 to 3000 mg per day, such as about 0.5 to 2000 mg per day. As will be understood by the skilled person, the actual amount to be administered will *inter alia* be dependent on the administration route, i.e. whether the chemosensitising compound is administered orally, intravenous, intramuscular, etc.

For compositions adapted for oral administration for systemic use, the dosage is 0.1 mg to 3 g per dose, such as 0.1 mg to 1000 mg, preferably 1 mg to 600 mg, more preferably 2 mg to 400 mg, administered 1-10 times daily, such as 1-4 times daily, for 1 day to 12 months depending on the infectious disease to be treated. Dosages for compositions to be administered by inhalation fall within these same ranges.

For parenteral administration, in particular intravenous administration, a dose of about 0.1 to about 2000 mg per day is convenient. For intravenous administration a dose of about 0.1 to about 2000 mg per day administered for 1 day to 12 months is convenient.

For percutaneous and topical administration, the dosage is 0.1 mg to 5 g per dose, administered 1 to 10 times daily for 1 day to 12 months. For rectal administration, the dosage is normally 0.1 to 2000 mg per dose, administered 1 to 10 times daily for 1 day to 12 months.

The above-mentioned steady state serum concentrations and dosages will give rise to the desired clinical effects and, at the same time, avoid the severe side effects normally associated with the chemosensitising compounds described herein. Some of the chemosensitising compounds described herein, in particular the chemosensitising compounds of the general formula IIIb, may however be administered in higher amounts, thereby giving rise to steady state serum concentrations above the levels indicated above. This is due to the fact that these chemosensitising compounds are expected not to exhibit severe side effects, even when administered in higher amounts.

The invention is further illustrated by the below, non-limiting, examples.

MATERIALS AND METHODS

Bacteria

Bacteria were sub-cultured on Mueller-Hinton agar plates and incubated over night at 37°C. Pre-warmed Mueller-Hinton broth was inoculated with colonies from the plate and incubated for approximately three hours to achieve a log phase culture. The log phase culture of bacteria was diluted with fresh pre-warmed medium and adjusted to a defined Optical Density (OD) at 600 nm in order to give a final assay concentration of 1×10^4 bacteria/ ml medium.

The DR cells were approximately 10 to 100 times more resistant compared to sensitive cell lines and maintained a stable DR phenotype when grown in drug-free medium. The only

exception was the *Enterococcus faecalis* F84 isolate. This strain was cultivated in the presence of vancomycin at 4 µg/ml in order to retain the MIC value at 4 µ/ml.

Strains were obtained from Statens Seruminstitut, Denmark, the Technical University of Denmark, and the Department of Clinical Microbiology, Soenderborg Hospital, Denmark.

Isolates

E. coli, LN 3164. Acr AB-TolC MDR *in vitro*-selected mutant. Resistant to tetracycline, beta-lactams, flouroquinolones, chloramphenicol and aminoglycosides.

E. coli 331 ME. *In vivo*-selected multidrug resistant clinical isolates of *E. coli*. Strains were isolated from a patient with severe cystitis/urosepsis. Resistant to tetracycline, beta-lactams, flouroquinolones, chloramphenicol and aminoglycosides.

P. aeruginosa 432b. Clinical multidrug resistant isolate. Resistant to tetracycline, beta-lactams, flouroquinolones, and aminoglycosides. Beta-lactamase producing, changes in Penicillin Binding Proteins and changes in Outer Membrane Proteins.

Staphylococcus aureus E45 MRSA. Clinical isolate. Resistant to methicillin. Susceptible to teicoplanin chloramphenicol, fosfomycin, netilmicin and vancomycin.

Staphylococcus aureus O11. Clinical isolate resistant to penicillin. Susceptible to methicillin, tetracycline, beta-lactams, flouroquinolones, chloramphenicol and aminoglycosides.

Enterococcus faecalis, F84. A multidrug resistant clinical isolate. Resistant to ampicillin, ciprofloxacin, gentamicin, and decreased resistance to vancomycin. Expressing change in the cell wall precurcer target as a major resistance mechanism (VanA gene expression).

Enterococcus faecalis, F86. Susceptible clinical isolate. No development of resistance.

Drugs

Drugs were dissolved in small amounts of water or 1% DMSO (final culture concentration of DMSO less than 0.05% DMSO) before dilution with medium. Solutions were freshly prepared for each experiment.

Chlorpromazine, promazine, promethazine, prochlorperazine, trifluoperazine, fluphenazine, thioridazine, chlorpotixene, *trans*-clopenthixol, *cis*- and *trans*-flupenthixol were obtained

from H. Lundbeck (Copenhagen, Denmark) and British Pharmacopoeia Commission Laboratory, Middlesex, United Kingdom. 7-hydroxychlorpromazine, 7,8 dihydroxychlorpromazine, desmethylchlorpromazine hydrochloride, trifluopromazine, chlorpromazine sulfoxide, 1-chlorpromazine, phenothiazine, 2-chloro-10-(2-dimethylaminoethyl)-, hydrochloride were obtained from the National Institute of Mental Health (USA). Thiomethylpromazine was obtained from Statens Seruminstitut (Copenhagen Denmark). Perphenazine was obtained from Sigma (Copenhagen Denmark). Fucidic acid was obtained from Leo Pharma AS (Copenhagen Denmark), Aztreonam was obtained from Bristol Meyers (Bromma, Sweden). Vancomycin was obtained from Alpharma AS (Copenhagen Denmark). Ciprofloxacin was obtained from 1A Pharma (Copenhagen, Denmark), and gentamicin was obtained from Schering-Plough Europe (Brussels, Belgium).

Effect of drugs on microbial cell growth and DR

Cell growth was tested using the MIC susceptibility tests by use of the microdilution broth method in accordance to the NCCLS Guidelines (NCCLS Guidelines, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard, Sixth Edition, Volume 23; Number 2). A 100% Mueller-Hinton broth and a bacterial concentration of 1×10^4 /ml were used.

A log phase culture of bacteria was diluted with fresh pre-warmed medium and adjusted to a defined OD at 600 nm in order to give a final concentration of 1×10^4 bacteria/ml medium in each well. Each chemosensitising compound was added to the bacterial culture in the wells in two-fold dilutions in order to give final concentrations ranging from 8 to 500 µg/ml. Trays were incubated at 37°C for 16 h. Minimal Inhibitory Concentration, (MIC) is defined as the lowest inhibitory concentration showing no visible growth according to the NCCLS Guidelines.

The effects of the chemosensitising compounds on DR were studied by the microtiter assay described above by exposing cells to 0-64 µg/ml anti-infective drug in the absence or presence of chemosensitising compound. Each experiment was repeated in tripleduplicate. MIC values represent the mean values of two separate experiments.

A log phase culture of bacteria was diluted with fresh pre-warmed medium and adjusted to a defined OD at 600 nm in order to give a final concentration of 1×10^4 bacteria/ml medium in each well. A chemosensitising compound was added to the bacterial culture in the wells in order to give final concentrations at ¼ of the chemosensitising compound's MIC value. Anti-infective agent was added to the bacterial culture in the wells in two-fold dilutions in order to give final concentrations ranging from 0 to 64 µg/ml. Trays were

incubated at 37°C for 16 h. The DR ratio was defined as the ratio between the MIC value for anti-infective agent alone divided by the MIC for the anti-infective agent in the presence of the chemosensitising compound. This ratio represents the increase in apparent potency of the anti-infective agent caused by the chemosensitising compound, and may be expressed as

$$DR\ ratio = (MIC_{anti\text{-}infective\ agent})/(MIC_{anti\text{-}infective\ agent\ +\ chemosensitising\ compound})$$

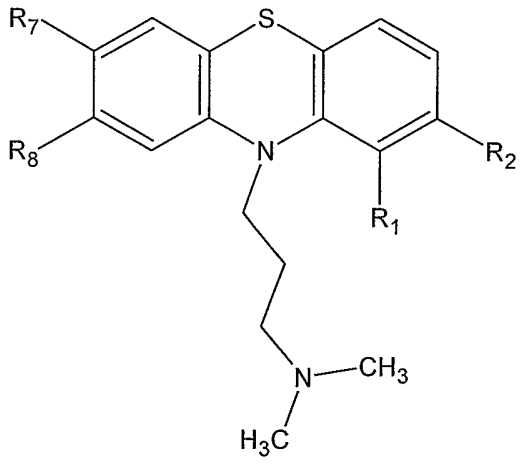
EXAMPLES

Example 1 - Effect of modifying promazine

Table 1 shows the structures, MIC values and DR Ratios for a series of promazine derivatives having different R_1, R_2, R_7 and R_8 substitutents.

The employed anti-infective agent was ciprofloxacin and the bacterial strain was *E. coli*, LN 3164.

The chemosensitising compounds (promazine and derivatives thereof) had the following general structure:



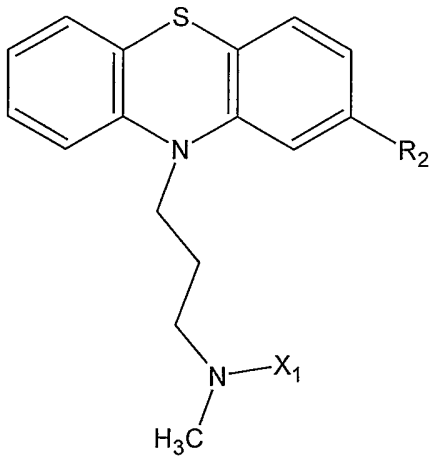
The obtained results are compiled in Table 1 below:

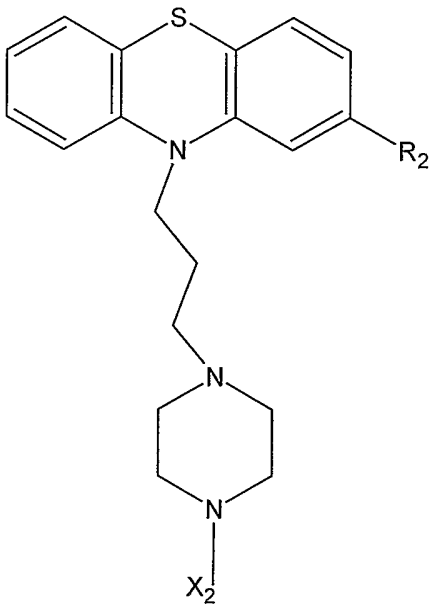
R_1	R_2	R_7	R_8	Name	MIC (μg/ml)	DR ratio
H	H	H	H	Promazine	64	2
Cl	H	H	H	1-chlorpromazine	64	2
H	Cl	H	H	chlorpromazine	32	4
H	Cl	OH	H	7-hydroxychlorpromazine	125	1
H	Cl	OH	OH	7,8-dihydroxychlorpromazine	125	0.5
H	$S\text{-}CH_3$	H	H	Thiomethylpromazine	32	4
H	CF_3	H	H	Trifluopromazine	32	8

Table 1

As can be seen, the unsubstituted chemosensitising compound (promazine) inhibited cell growth and sensitised drug resistant *E. coli* cells to ciprofloxacin by 100% (DR ratio = 2). However, introduction of a chlorine atom at position 1 or 2 increased the potency against drug resistance. In particular, introducing the chlorine atom at position 2 had the greatest effect and sensitised the drug resistant cells by 220%. Similarly, introducing a CF_3 group at position 2 also increased potency against cell growth and drug resistance. Oxidation of the ring sulfur atom to produce chlorpromazine sulfoxide had the same activity against drug resistance as did chlorpromazine.

In order to determine the influence of the amino side chain, chemosensitising compounds having the following general structures were assayed as described above:





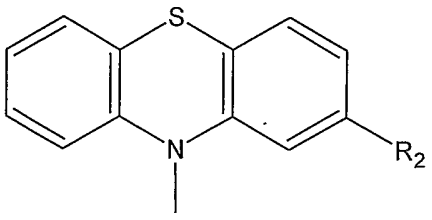
The obtained results are compiled in Table 2 below:

R_2	X_1	X_2	Name	MIC (µg/ml)	DR ratio
Cl	H	-	Desmethylchlorpromazine	32	2
Cl	CH_3	-	Chlorpromazine	32	4
CF_3	CH_3	-	Trifluopromazine	32	8
Cl	-	CH_2-CH_2-OH	Perphenazine	125	4
Cl	-	CH_3	Prochlorperazine	64	4
CF_3	-	CH_2-CH_2-OH	Fluphenazine	32	8
CF_3	-	CH_3	Trifluoperazine	16	16

Table 2

Table 2 above shows that phenothiazines containing tertiary amines (e.g. chlorpromazine) and secondary amines (desmethylchlorpromazine) possess similar activity in inhibiting cell growth (MICs of 32 µg/ml). However, phenothiazines containing tertiary amines, such as chlorpromazine, were more potent antagonists of DR than those with secondary amines, such as desmethylchlorpromazine, producing a 1.6 fold increase in anti-DR activity. Other changes in the type of amino group also affected anti DR activity. For example, piperazinyl derivatives increased potency against DR. Accordingly, the DR ratios for Trifluoperazine and Fluphenazine compounds were greater than that of Trifluopromazine, a compound with an identical ring subsitution pattern, but possessing an aliphatic side chain. Similarly Perphenazine and Prochlorperazine were more potent DR antagonists than chlorpromazine. This series of experiments also points out the importance of the CF_3 substitution at position 2 for anti DR activity. For example the DR ration for Trifluoperazine was greater than that for Prochlorperazine. These phenothiazines have identical structures except that the former has a CF_3 group instead of a chlorine atom at position 2. A similar relationship is seen by comparing fluphenazine to perphenazine. Finally a *para* methyl substitution on the piperazinyl ring appeared to be more potent than a *para* ethanol substitution, as can be seen by comparing the DR ratios for prochlorperazine to perphenazine or trifluoperazine to fluphenazine.

In order to determine the influence of the length of the amino-containing side chain, chemosensitising compounds having the following general structures were assayed as described above:



The obtained results are compiled in Table 3 below:

R_2	X	Name	MIC (µg/ml)	DR ratio
Cl	CH_2-CH_2-$N(CH_3)_2$	2-chloro-10-dimethylamino-ethyl)phenothiazine	64	2
Cl	$(CH_2)_3$-$N(CH_3)_2$	Chlorpromazine	32	4
Cl	CH_2-$CH(CH_3)$-$N(CH_3)_2$	Promethazine	64	8
H	CH_2-CH_2-$N(CH_3)_2$	Promazine	64	2

Table 3

Table 3 above shows the effect on cell growth and DR of a series of dimethylamino-phenothiazines in which the length of the amino-containing side chain was varied. As can be seen, moving from a two to three carbon alkyl bridge increased the anti DR effects of these chemosensitising compounds. For example, Chlorpromazine had a greater DR ratio than that of 2-chloro-10-(2-dimethylaminoethyl)phenothiazine. Promethazine, which has an isopropyl side chain, was a more potent DR inhibitor compared to promazine, which has a straight three-carbon alkyl chain.

Example 2 - Effect of stereochemistry

In order to investigate the influence of the cis and trans stereochemistry a series of thioxanthenes were assayed as described above. Table 4 shows the MIC values and DR Ratios for the tested thioxanthenes.

Name	MIC (µg/ml)	DR ratio
trans-flupenthixol	32	64
cis-flupenthixol	32	32
trans-clopenthixol	16	128

Table 4

The above results demonstrate that stereoisomeric configurations are required for optimum activity against DR. For example, *trans*-flupenthixol is a more potent anti-DR agent than the *cis*-form of the compound and *trans*-clopenthixol was the most potent anti-DR agent. Thus, the orientation of the side chain amine in relation to the tricyclic nucleus appears to be an important determinant for anti-DR activity.

Example 3 - Effect of hydrophobicity

To determine if the differences in anti DR potency in chemosensitising compounds with side chain alterations were also due to changes in overall hydrophobicity, the octanol:buffer partition coefficients for each of the drugs in Tables 1, 2, 3 and 4 were compared to their DR ratios. No statistically significant correlation was found between hydrophobicity and anti DR activity ($p>0.5$) (Data not shown).

Example 4 - Synergistic effects of thioxanthene derivatives

The synergistic effect of *trans*-flupenthixol, *cis*-flupenthixol and *trans*-clopenthixol on a variety of anti-infective agents was examined against a panel of susceptible and resistant bacterial isolates representing different species and resistance mechanisms.

The synergistic effect was studied by checkerboard combination studies exposing cells to 0-64 µg/ml anti-infective agent in the absence or presence of *trans*-flupenthixol, *cis*-flupenthixol or *trans*-clopenthixol. Each experiment was repeated in tripleduplicate. MIC values represent the mean values of two separate experiments,

A log phase culture of bacteria was diluted with fresh pre-warmed medium and adjusted to a defined OD at 600 nm in order to give a final concentration of 1×10^4 bacteria/ml medium in each well. Each chemosensitising compound was added to the bacterial culture in the wells in two-fold dilutions in order to give final concentrations ranging from 0 to 8 µg/ml. Anti-infective agent was added to the bacterial culture in the wells in two-fold dilutions in order to give final concentrations ranging from 0 to 64 µg/ml Trays were incubated at 37°C for 16 h. Wells were assessed visually for growth. The Fractional Inhibitory Concentration (FIC) was calculated for each anti-infective agent alone and in combination with *trans*-flupenthixol, *cis*-flupenthixol or *trans*-clopenthixol. The following formulae were used to calculate the FIC index:

$$FIC = FIC_{\text{chemosensitising compound}} + FIC_{\text{anti-infective agent}}$$

where:

$$FIC_{\text{chemosensitising compound}} = (MIC_{\text{chemosensitising compound + anti-infective agent}})/(MIC_{\text{chemosensitising compound}})$$

$$FIC_{\text{anti-infective agent}} = (MIC_{\text{anti-infective agent + chemosensitising compound}}/(MIC_{\text{anti-infective agent}})$$

Synergy was defined as a FIC index of <0.5. The calculated FIC indices are shown in Table 5 below:

Isolate	Anti-infective agent	Resistance*	*cis*-flupenthixol	*trans*-flupenthixol	*trans*-clopenthixol
E. faecalis F84	Vanomycin	MDR	0.25	0.13	0.06
	Dicloxacillin		0.13	0.06	0.03
E. faecalis F86	Dicloxacillin	S	0.50	0.50	0.25
S. aureus E45	Dicloxacillin	R	0.25	0.13	0.06
	Fucidic acid	R	0.26	0.13	0.06
S. aureus O11	Dicloxacillin	S	0.50	0.50	0.25
E. coli LN 3164	Ciprofloxacin	MDR	0.25	0.13	0.063
	Gentamicin		0.25	0.063	0.063
	Piperacillin		0.25	0.13	0.063
E. coli 331ME	Ciprofloxacin	MDR	0.013	0.006	0.003
	Gentamicin		0.06	0.02	0.008
	Piperacillin		0.25	0.13	0.063
P. aeruginosa	Ciprofloxacin	R	0.06	0.03	0.008
	Gentamicin	R	0.13	0.06	0.032
	Piperacillin	R	0.25	0.13	0.063

Table 5. *R=Isolate resistant to anti-infective agent; S=isolate susceptible to anti-infective agent; MDR= multidrug resistant isolate.

The FIC indices for *trans*-clopenthixol and *cis*- and *trans*–flupenthixol show that these compounds are strongly synergistic in promoting the bacteriostatic effects of the anti-infective agents in the drug resistant cells. Most of the FIC indices for the chemosensitising compounds assayed on drug-resistant cells were <<0.5. *Trans*-clopenthixol was the most potent of all the tested chemosensitising compounds, i.e. the *trans* form was more potent compared to the *cis* form. Thus the clinical use of e.g. *trans*-clopenthixol or *trans*–flupenthixol in combination with an anti-infective agent would likely shift the MIC of this anti-infective agent for the DR cells to well-below the clinically achievable concentration, showing effective concentrations at <500 ng/ml (the effective concentrations of the chemosensitising compounds are in the range of 0.32 µg/ml to 4µg/ml). The anti DR effect was most potent in resistant cells. However a remarkable antibiotic enhancing effect was shown also in the susceptible cells strongly indicating that the anti DR effect of these chemosensitising compounds is not restricted to cells overexpressing efflux pumps and the anti DR mechanism is not restricted to this target. FIC indices for antibiotic susceptible cells ranged from 0.25 to 0.5.

Furthermore, the results demonstrate that *cis*- and *trans*-flupenthixol as well as *trans*-clopenthixol enhanced the anti-infective activity of the beta-lactam antibioticum piperacillin against *P.aeruginosa* cells expressing beta-lactamase able to inhibit piperacillin (Giwercman B et al, 1992 and 1990) suggesting that the anti DR mechanism of the compounds is not restricted to inhibition of MDR efflux pumps. The antiproliferative effects of the chemosensitising compounds were approximately equipotent modest in both the sensitive and resistant isolates. MIC values ranged from 16 to 64 µg /ml (data not shown).

Example 5. Synergistic effects of phenothiazine derivatives

The synergistic effect of the anti-infective agent ciprofloxacin was examined against a ciprofloxacin resistant clinical bacterial isolate of *Enterococcus faecalis*.
The synergistic effect was studied by checkerboard combination studies exposing cells to 0-8 µg/ml anti-infective agent in the absence or presence of chlorpromazine, promazine or perphenazine. Each experiment was repeated in duplicate. MIC values represent the mean values of two separate experiments.

A log phase culture of bacteria was diluted with fresh pre-warmed medium and adjusted to a defined OD at 600 nm in order to give a final concentration of $1 \times 10^{4-5}$ bacteria/ml medium in each well. Each chemosensitising compound was added to the bacterial culture in the wells in two-fold dilutions in order to give final concentrations ranging from 0 to 16 µg/ml. Anti-infective agent was added to the bacterial culture in the wells in two-fold dilutions in order to give final concentrations ranging from 0 to 16 µg/ml Trays were incubated at 37°C for 16 h. Wells were assessed visually for growth. The Fractional Inhibitory Concentration (FIC) was calculated for the anti-infective agent alone and in combination with chlorpromazine, promazine or perphenazine. The following formulae were used to calculate the FIC index:

$$FIC = FIC_{\text{chemosensitising compound}} + FIC_{\text{anti-infective agent}}$$

where:

$$FIC_{\text{chemosensitising compound}} = (MIC_{\text{chemosensitising compound + anti-infective agent}})/(MIC_{\text{chemosensitising compound}})$$

$$FIC_{\text{anti-infective agent}} = (MIC_{\text{anti-infective agent + chemosensitising compound}}/(MIC_{\text{anti-infective agent}})$$

Synergy was defined as a FIC index of <0.5. The calculated FIC indices are shown in Table 6 below:

The FIC indices for chlorpromazine, promazine or perphenazine show that these compounds are synergistic in promoting the bacteriostatic effects of the anti-infective agents in the drug resistant cells. All of the FIC indices for the chemosensitising compounds assayed on drug-resistant cells were <0.5. Chlorpromazine, promazine or perphenazine was equal regarding the ability of enhancing the effect of ciprofloxacin. The lowest effective concentrations of the enhancing compounds were between 1 and 2 µg/ml. Thus the clinical use of e.g. chlorpromazine, promazine or perphenazine in combination with an anti-infective agent would likely shift the MIC of this anti-infective agent for the DR cells to well-below the clinically achievable concentration, showing effective concentrations at <2 µg/ml

Isolate	Anti-infective agent	chlorpromazine + cip* FIC	promazine + cip FIC	perphenazine + cip FIC
E. faecalis 21416A	Ciprofloxacin MIC 4 µg/ml	0.25	0.25	0.25

Table 6. *: cip= ciprofloxacin

Example 6 - Development of insensitivity to the chemosensitising compounds

One potential limitation to the combination of an anti-infective agent with inhibitors of resistance mechanism(s) is the possibility of the microorganism develops mutations which render it insensitive to the inhibitor. Such a situation has been observed for e.g. bacteria, virus, fungi and yeast.

The effect of the inhibitors on the rate of emergence of *in vitro*-selected single-step ciprofloxacin resistance on the clinical isolate of *S. aureus* O11 was determined.

Spontaneous mutants were obtained 24 h after plating *S. aureus* cells on LB agar plates containing ciprofloxacin at a concentration of 1 µg/ml (two times the MIC) in the absence or presence of *trans*-clopenthixol at 1 µg/ml. The frequency of mutant selection was determined to be 3×10^{-8} by comparing the number of colonies that grew on plates containing the anti-infective agent with the number of colonies obtained upon plating appropriate dilutions in the absence of anti-infective agents.

The probably most important aspect, when assessing the use of the inhibitors in the clinic, is the effect of these inhibitors on the emergence of resistant mutants. Importantly, and as shown in Table 7, the tested inhibitor decreased the frequency of spontaneous emergence of ciprofloxacin resistance by 100-fold or more. This dramatic effect could not be attributed to a toxic effect of the inhibitor since the same concentration of inhibitor, which was at least 10-fold less than its MIC for *S. aureus*, affected neither the colony-forming ability nor the colony size of *S. aureus* cells plated in the absence of ciprofloxacin. In conclusion, the trans-clopenthixol inhibited the emergence of ciprofloxacin resistance in *S. aureus.*

Inhibitor (1 µg/ml)	Frequency on emergence of resistance
None	3×10^{-8}
trans-clopenthixol	$<1 \times 10^{-10}$

Table 7. Frequency of emergence of *in vitro*-selected variants of *S. aureus* resistant to 1 µg of ciprofloxacin per ml (two times the MIC for the *S. aureus* strain) in either the absence or the presence of inhibitors.

Example 7. Enhancing effect of Trans-clopenthixol in a mouse peritonitis model

Bacteria.

A clinical isolate of Enterococcus faecalis BG-029 from human urine was used. This strain was resistant to ciprofloxacin; MIC, 4 µg/ml. The MIC of Trans-clopenthixol was 6 µg/ml

MICs.

The MICs were determined by the microdilution test according to the NCCLS Guidelines.

Animals.

Female NMRI mice (age, approximately 6 to 8 weeks; weight, 30 ± 2 g) were used for the mouse pneumonia peritonitis model (as described below).

Antibiotics.

Ciprofloxacin was obtained from Bayer A/S, Lyngby, Denmark, as a solution for infusion; 2 mg/ml. Trans-clopenthixol was obtained as a powder reference substance from British Pharmacopoeia Commission Laboratory, Middlesex, United Kingdom.

Pharmacokinetic studies of Trans-clopenthixol with mice.

Pharmacokinetic studies were done with NMRI mice. Concentrations in sera were determined after the administration of a single dose of 0.3 mg per mouse. The drug was administered by subcutaneous injection in the neck region in a volume of 0.2 ml per dose. At 1, 2, 4, 6, and 24 hours after the injection blood samples were obtained from the mice in groups of three. After collection, the blood was centrifuged and the serum was stored at −80°C until it was analyzed by High Pressure Liquid Chromatography.

Mouse peritonitis model.

Bacterial suspensions were prepared from fresh overnight cultures (made from frozen stock cultures) on 5% blood agar plates as described above. The inoculum for the mouse peritonitis model was prepared immediately before use and was adjusted at 540 nm of giving a density of approximately 10^7 CFU/ml. The size of the inoculum was determined by viability counting on 5% blood agar.

Neutropenia was introduced by pretreating the mice with cyclophosphamide (6 mg daily for three days) .The mice were injected intraperitoneally with 0.5 ml of the enterococcal suspension, resulting in bacteremia within 1 h of inoculation. Antibiotic therapy was initiated 1 h after inoculation. Ciprofloxacin and Trans-clopenthixol was administered subcutaneously in the neck region in a volume of 0.1 ml per dose. Five mice were in each treatment group. Inoculated untreated control mice were included in all trials. (Method reference: Erlandsdottir et al; Antimicrob Agents Chemother. 2001 Apr;45(4):1078-85)

Table 8. Treatment regimes of infected mice.

Group	**Treatment**
1. Control	None
2. Ciprofloxacin alone	12,5 mg ciprofloxacin per mouse
3. Trans-clopenthixol alone	5 mg per kg mouse
4. Trans-clopenthixol and ciprofloxacin	5 mg per kg mouse of trans-clopenthixol immediately followed by 12,5 mg ciprofloxacin per mouse

The effects of the various treatment regimens were determined during 6 h of treatment by evaluation of bacterial counts in the peritoneal fluid. After the mice were killed, peritoneal washes were performed by injecting 2 ml of sterile saline intraperitoneally, followed by massage of the abdomen and then opening of the peritoneum to collect the fluid. Peritoneal fluids were immediately diluted 10-fold in saline, from which 20 µl was plated

onto 5% blood agar plates in spots, with subsequent counting of colonies after incubation overnight at 35°C. The lowest detection levels for bacterial counts in blood and peritoneal fluid were 50 and 250 CFU/ml, respectively.

The bactericidal efficacies of the treatment regimens in the mouse models were calculated by subtracting the results for each treated mouse from the mean results for control mice at the end of therapy (6 h). A *P* value of <0.05 was considered significant. All statistical comparisons were two-tailed.

Results:

Strong Enhancing activity of trans-clopenthixol in mouse peritoneum.

The bactericidal activity of ciprofloxacin and Trans-clopenthixol, alone or in combination, in mouse peritoneum is shown in Figure 3. As seen, ciprofloxacin alone in sub-therapeutic dose had no effect on the infection and the resistant bacteria are not eradicated from the mouse peritoneum. But when the mice were treated with ciprofloxacin and trans-clopenthixol in combination the bacteria was eradicated ($p < 0.05$). Trans-clopenthixol (TC) alone did not affect the bacteria in accordance with the sub-therapeutic dose given. (Serum values of TC in mice are less than 300 ng/ml. This is far below the MIC value at 6 µg TC/ml, as seen in Figure 4).

Example 8 - Synergistic effects of thioxanthene derivatives on fungals

The synergistic effect of Trans-clopenthixol was studied by checkerboard combination studies exposing cells to 0-256 µg/ml anti-infective agent in the absence or presence of *trans*-clopenthixol (0 to 8 µg/ml in two-fold dilutions). Each experiment was repeated in tripleduplicate. MIC values represent the mean values of two separate experiments.

Fungal strain:

Clinical isolate of a fluconazole resistant Candida albicans from a patient with candidemia.

Antifungal agent:

Fluconazole (Pfizer, Ballerup, Denmark)

The isolates were subcultured for 24 h on Sabouraud glucose agar before susceptibility testing.

Broth microdilution tests were performed according to NCCLS document M27-A (Ref: National Commitee for Clinical Laboratory Standards. (1997). *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts: Approved Standard M27-A*. NCCLS, Wayne, PA.)

Microtitre plates were read spectrophotometrically at 530 nm, after mixing the wells by pipetting to resuspend yeast sediments. The MIC was defined as the lowest drug dilution resulting in 80% growth inhibition for fluconazole. The following tentative breakpoints were applied: fluconazole susceptible (S), MIC ≥ 8 µg/ml; susceptible dose-dependent (SDD), 8 µg/ml < MIC < 64 µg/ml; and resistant (R), MIC ≤ 64 µg/ml

The Fractional Inhibitory Concentration (FIC) was calculated for the anti-infective agent alone and in combination with *trans*-clopenthixol. The following formulae were used to calculate the FIC index:

$$FIC = FIC_{\text{chemosensitising compound}} + FIC_{\text{anti-infective agent}}$$

where:

$$FIC_{\text{chemosensitising compound}} = (MIC_{\text{chemosensitising compound + anti-infective agent}})/(MIC_{\text{chemosensitising compound}})$$

$$FIC_{\text{anti-infective agent}} = (MIC_{\text{anti-infective agent + chemosensitising compound}}/(MIC_{\text{anti-infective agent}})$$

Synergy was defined as a FIC index of <0.5. The calculated FIC index is shown in Table 9 below

Table 9

Fungal Strain	MIC µg/ml Fluconazole (FL)	MIC µg/ml Trans-clopenthixol (TC)	MIC µg/ml FL + TC (3 µg/ml)	FIC index
Candida albicans	128	32	4	0,16

The FIC index for *trans*-clopenthixol shows that this compound is strongly synergistic in promoting the antifungal effect of the anti-fungal agents in the drug resistant cells. As seen the FIC index for the chemosensitising compounds assayed on drug-resistant cells were <0.5. Thus the clinical use of e.g. *trans*-clopenthixol in combination with an anti-fungal agent would likely shift the MIC of this anti-fungal agent for the DR cells to well-below the clinically achievable concentration, showing effective concentrations at ≤3 µg/ml.

Example 9 - Enhancing effects of thioxanthene derivatives on anti-viral compounds

The enhancing effect of *trans*-clopenthixol on anti-viral agents was studied by checkerboard combination studies exposing HIV infected cells to 0-3 µM anti-viral agent in

the absence or presence of *trans*-clopenthixol in concentrations from 0 to 6 µM. Each experiment was repeated in tripleduplicate. MIC values represent the mean values of two separate experiments.

Methods:

Viruses and cells.

The HIV-1 strain HTLV-IIIB were propagated in H9 cells at 37°C, 5% CO2 using RPMI 1640 with 10% heat-inactivated foetal calf serum (FCS) and antibiotics (growth medium). Culture supernatant was filtered (0.45 nm), aliquotted, and stored at -80°C until use. The HIV-1 strain was obtained from NIH AIDS Research and Reference Program.

Compounds.

Antiviral drug: AZT, (3'-Azido-3'-deoxythymidine), Glaxo Wellcome.

Enhancing compound: Trans-clopenthixol was obtained as a powder reference substance from British Pharmacopoeia Commission Laboratory, Middlesex, United Kingdom.

Inhibition of HIV-1 replication.

Compounds were examined for possible antiviral activity against strain IIIB of HIV-1 using MT4 cells as target cells. MT4 cells were incubated with virus (0.005 MOI) and growth medium containing the test dilutions of compound(s) for six days in parallel with virus-infected and uninfected control cultures without compound added. Expression of HIV in the cultures was indirectly quantified using the MTT assay as previously described. Compounds mediating less than 30% reduction of HIV expression were considered without biological activity. Compounds were tested in parallel for cytotoxic effect in uninfected MT4 cultures containing the test dilutions of compound as described above. Cultures for test of both antiviral activity and cytotoxic effect were set up in tripleduplicates, 200 ml per culture in micro titre plates.

A 30% inhibition of cell growth relative to control cultures was considered significant. The 50% inhibitory concentration was determined by interpolation from the plots of percent inhibition versus concentration of compound.

EC50 is defined as the effective concentration that inhibits 50% of viral production, 50% of viral infectivity, or 50% of the virus-induced cytopathic effect.

CC50 is defined as the inhibitory concentration that reduces cellular growth or viability of uninfected cells by 50%.

Results

As seen in Table 10, the combination of *Trans*-clopenthixol and AZT resulted in a 10 time enhancement of the antiviral effect of AZT and thus may be sufficient to inhibit resistant viral strains. *Trans*-clopenthixol alone had no antiviral or cytotoxic effect at the concentrations used

Table 10: Enhancing effect of Trans-clopenthixol (TC) on an antiviral compound AZT (A). Concentrations in µM. (see text)

EC50 A	CC50 A	EC50 TC	CC50 TC	EC50 A+TC (1 µM)	CC50 A+TC (1 µM)
0.05	>3	>3	>3	0.01	> 3

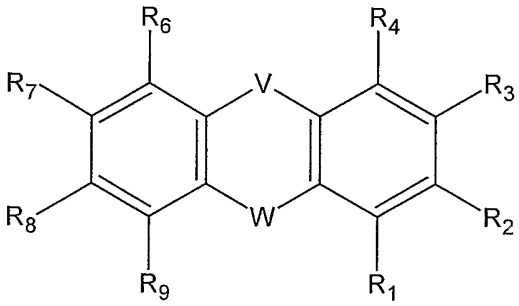
EC50 is defined as the effective concentration that inhibits 50% of viral production, 50% of viral infectivity, or 50% of the virus-induced cytopathic effect

CC50 is defined as the inhibitory concentration that reduces cellular growth or viability of uninfected cells by 50%.

Viral test Method **Reference**: Petersen L, Jørgensen PT, Nielsen C, Hansen TH, Nielsen J, Pedersen EB. Synthesis and Evaluation of Double-Prodrugs against HIV. Conjugation of D4T with 6-Benzyl-1-(ethoxymethyl)-5-isopropyluracil (MKC-442, Emivirine) Type Reverse Transcriptase Inhibitors via the SATE Prodrug Approach. J. Med. Chem. 2005, 48, 1211-1220.

CLAIMS

1. Use of a compound of the general formula (I)



(I)

wherein

V is selected from the group consisting of S, SO_2, SO, O and NH;

W is $N\text{-}(CHX)_n\text{-}N(R_{10})(R_{11})$ or W is $C{=}CH\text{-}(CHX)_m\text{-}N(R_{10})(R_{11})$;

n is an integer in the range of from 2 to 6;

m is an integer in the range of from 1 to 5;

each X is individually selected from the group consisting of hydrogen, halogen, hydroxy, amino, nitro, optionally substituted C_{1-6}-alkyl and optionally substituted C_{1-6}-alkoxy;

R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8 and R_9 are each individually selected from the group consisting of hydrogen, halogen, hydroxy, amino, nitro, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted C_{2-6}-alkynyl and optionally substituted C_{1-6}-alkoxy, optionally substituted C_{2-6}-alkenyloxy, carboxy, optionally substituted C_{1-6}-alkoxycarbonyl, optionally substituted C_{1-6}-alkylcarbonyl, fomyl, optionally substituted C_{1-6}-alkylsulphonylamino, optionally substituted aryl, optionally substituted aryloxycarbonyl, optionally substituted aryloxy, optionally substituted arylcarbonyl, optionally substituted arylamino, arylsulphonylamino, optionally substituted heteroaryl, optionally substituted heteroaryloxycarbonyl,

optionally substituted heteroaryloxy, optionally substituted heteroarylcarbonyl, optionally substituted heteroarylamino, heteroarylsulphonylamino, optionally substituted heterocyclyl, optionally substituted heterocyclyloxycarbonyl, optionally substituted heterocyclyloxy, optionally substituted heterocyclylcarbonyl, optionally substituted heterocyclylamino, heterocyclylsulphonylamino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)aminocarbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, amino-C_{1-6}-alkyl-carbonylamino, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-carbonylamino, amino-C_{1-6}-alkyl-amino, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-amino, cyano, guanidino, carbamido, C_{1-6}-alkanoyloxy, C_{1-6}-alkylsulphonyl, C_{1-6}-alkylsulphinyl, C_{1-6}-alkylsulphonyloxy, aminosulfonyl, mono- and di(C_{1-6}-alkyl)aminosulfonyl, and optionally substituted C_{1-6}-alkylthio; and

R_{10} and R_{11} are each independently selected from the group consisting of hydrogen, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted C_{2-6}-alkynyl, optionally substituted C_{1-6}-alkoxycarbonyl, optionally substituted C_{1-6}-alkylcarbonyl, optionally substituted aryl, optionally substituted aryloxycarbonyl, optionally substituted arylcarbonyl, optionally substituted heteroaryl, optionally substituted heteroaryloxycarbonyl, optionally substituted heteroarylcarbonyl, aminocarbonyl, mono- and di(C_{1-6}-alkyl)aminocarbonyl; or R_{10} and R_{11} together with the nitrogen atom to which they are attached form an optionally substituted nitrogen-containing heteroaryl or optionally substituted heterocyclyl;

or a metabolite or salt thereof for the manufacture of a medicament for the treatment or prophylaxis of an infectious disease in combination with an anti-infective agent.

2. Use according to claim 1, wherein R_2 is selected from the group consisting of F, Cl, Br, I, CH_2Y, CHY_2 and CY_3, wherein Y is a halogen atom.

3. Use according to claim 2, wherein R_2 is selected from the group consisting of F, Cl, CF_3 and CCl_3.

4. Use according to claim 3, wherein R_2 is Cl or CF_3.

5. Use according to any of the preceding claims, wherein R_1, R_3, R_4, R_5, R_6, R_7, R_8 and R_9 are each individually selected from the group consisting of hydrogen, optionally substituted C_{1-6}-alkyl and optionally substituted C_{1-6}-alkoxy.

6. Use according to claim 5, wherein R_1, R_3, R_4, R_5, R_6, R_7, R_8 and R_9 are hydrogen.

7. Use according to any of the preceding claims, wherein V is S or SO.

8. Use according to claim 7, wherein V is S.

9. Use according to any of the preceding claims, wherein W is N-$(CHX)_n$-$N(R_{10})(R_{11})$ and X, n, R_{10} and R_{11} are as defined in claim 1.

10. Use according to claim 9, wherein W is N-$(CH_2)_3$-$N(R_{10})(R_{11})$ or N-CH_2-$CH(CH_3)$-$N(R_{10})(R_{11})$ and R_{10} and R_{11} are as defined in claim 1.

11. Use according to claim 10, wherein W is N-$(CH_2)_3$-$N(R_{10})(R_{11})$ and R_{10} and R_{11} are as defined in claim 1.

12. Use according to claim 11, wherein R_{10} and R_{11} are each individually selected from the group consisting of hydrogen and optionally substituted C_{1-6}-alkyl.

13. Use according to claim 12, wherein R_{10} and R_{11} are both CH_3.

14. Use according to any of claims 1-11, wherein R_{10} and R_{11}, together with the nitrogen atom to which they are attached, form an optionally substituted heterocyclyl.

15. Use according to claim 14, wherein said optionally substituted heterocyclyl is selected from the group consisting of optionally substituted 2-pyrrolinyl, optionally substituted 3-pyrrolinyl, optionally substituted pyrrolidinyl, optionally substituted 2-imidazolinyl, optionally substituted imidazolidinyl, optionally substituted 2-pyrazolinyl, optionally substituted 3-pyrazolinyl, optionally substituted pyrazolidinyl, optionally substituted piperidinyl, optionally substituted morpholinyl, optionally substituted thiomorpholinyl and optionally substituted piperazinyl,

16. Use according to claim 15, wherein said optionally substituted heterocyclyl is an optionally substituted piperidinyl or an optionally substituted piperazinyl.

17. Use according to claim 16, wherein said optionally substituted heterocyclyl is an optionally substituted piperazinyl.

18. Use according to claim 17, wherein said piperazinyl is substituted in the *para* position.

19. Use according to claim 17 or 18, wherein said piperazinyl is substituted with an optionally substituted C_{1-6}-alkyl group.

20. Use according to claim 19, wherein said optionally substituted C_{1-6}-alkyl is selected from the group consisting of $-CH_3$, $-CH_2OH$, $-CH_2-CH_3$ and $-CH_2-CH_2OH$.

21. Use according to claim 20, wherein said optionally substituted C_{1-6}-alkyl is $-CH_3$ or $-CH_2-CH_2OH$.

22. Use according to claim 21, wherein said optionally substituted C_{1-6}-alkyl is $-CH_2-CH_2OH$.

23. Use according to any of claims 19-22, wherein said optionally substituted C_{1-6}-alkyl is in the *para* position.

24. Use according to claim 17, wherein said piperazinyl is unsubstituted.

25. Use according to any of the preceding claim, wherein said compound is selected from the group consisting of promazine, 1-chlorpromazine, chlorpromazine, 7-hydroxychlorpromazine, 7,8-dihydroxychlorpromazine, thiomethlypromazine, trifluorpromazine, desmethylclorpromazine, perphenazine, prochlorperazine and 2-chloro-10-(2-dimethylaminoethyl)phenothiazine.

26. Use according to any of claims 1-8, wherein W is $C=CH-(CHX)_m-N(R_{10})(R_{11})$ and X, m, R_{10} and R_{11} are as defined in claim 1.

27. Use according to claim 26, wherein m is 2 or 3.

28. Use according to claim 27, wherein m is 2.

29. Use according to claim 28, wherein W is $C=CH-(CH_2)_2-N(R_{10})(R_{11})$ and R_{10} and R_{11} are as defined in claim 1.

30. Use according to claim 29, wherein R_{10} and R_{11} are each individually selected from the group consisting of hydrogen and optionally substituted C_{1-6}-alkyl.

31. Use according to claim 30, wherein R_{10} and R_{11} are both CH_3.

32. Use according to any of claims 26-29, wherein R_{10} and R_{11}, together with the nitrogen atom to which they are attached, form an optionally substituted heterocyclyl.

33. Use according to claim 32, wherein said optionally substituted heterocyclyl is selected from the group consisting of optionally substituted 2-pyrrolinyl, optionally substituted 3-pyrrolinyl, optionally substituted pyrrolidinyl, optionally substituted 2-imidazolinyl, optionally substituted imidazolidinyl, optionally substituted 2-pyrazolinyl, optionally substituted 3-pyrazolinyl, optionally substituted pyrazolidinyl, optionally substituted piperidinyl, optionally substituted morpholinyl, optionally substituted thiomorpholinyl and optionally substituted piperazinyl,

34. Use according to claim 33, wherein said optionally substituted heterocyclyl is an optionally substituted piperidinyl or an optionally substituted piperazinyl.

35. Use according to claim 34, wherein said optionally substituted heterocyclyl is an optionally substituted piperazinyl.

36. Use according to claim 35, wherein said piperazinyl is substituted in the *para* position.

37. Use according to claim 35 or 36, wherein said piperazinyl is substituted with an optionally substituted C_{1-6}-alkyl group.

38. Use according to claim 37, wherein said optionally substituted C_{1-6}-alkyl is selected from the group consisting of $-CH_3$, $-CH_2OH$, $-CH_2-CH_3$ and $-CH_2-CH_2OH$.

39. Use according to claim 38, wherein said optionally substituted C_{1-6}-alkyl is $-CH_3$ or $-CH_2-CH_2OH$.

40. Use according to claim 39, wherein said optionally substituted C_{1-6}-alkyl is $-CH_2-CH_2OH$.

41. Use according to any of claims 37-40, wherein said optionally substituted C_{1-6}-alkyl is in the *para* position.

42. Use according to claim 35, wherein said piperazinyl is unsubstituted.

43. Use according to any of claims 26-42, wherein said $C=CH-(CHX)_m-N(R_{10})(R_{11})$ group is in the *trans* configuration.

44. Use according to any of claims 1-8 or 26-43, wherein said compound is selected from the group consisting of *trans*-flupenthixol, *cis*-flupenthixol, *trans*-clopenthixol and *cis*-clopenthixol.

45. Use according to claim 44, wherein said compound is *trans*-flupenthixol or *trans*-clopenthixol.

46. Use according to claim 45, wherein said compound is *trans*-clopenthixol.

47. Use according to any of the preceding claims, wherein said compound is administered in a clinically relevant amount.

48. Use according to claim 47, wherein said compound is administered in a clinically relevant amount giving rise to a steady state serum concentration of less than 8.0 mg/l.

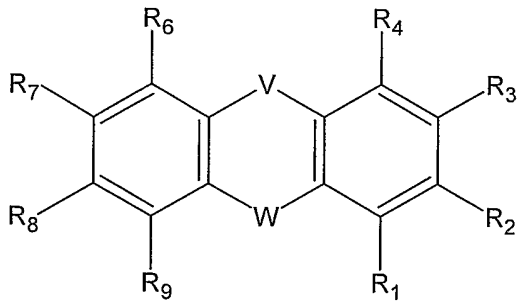
49. Use according to claim 48, wherein said steady state serum concentration is in the interval of from 0.01 μg/l to less than 8.0 mg/l.

50. Use according to any of the preceding claims, wherein said infectious disease is caused by an infectious agent.

51. Use according to claim 50, wherein said infectious agent is drug resistant.

52. Use according to claim 50, wherein said infectious agent is multidrug resistant.

53. A method for treating or preventing an infectious disease in a subject, said method comprising administering to said subject a compound of the general formula (I)



(I)

wherein

V is selected from the group consisting of S, SO_2, SO, O and NH;

W is N-$(CHX)_n$-$N(R_{10})(R_{11})$ or W is C=CH-$(CHX)_m$-$N(R_{10})(R_{11})$;

n is an integer in the range of from 2 to 6;

m is an integer in the range of from 1 to 5;

each X is individually selected from the group consisting of hydrogen, halogen, hydroxy, amino, nitro, optionally substituted C_{1-6}-alkyl and optionally substituted C_{1-6}-alkoxy;

R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8 and R_9 are each individually selected from the group consisting of hydrogen, halogen, hydroxy, amino, nitro, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted C_{2-6}-alkynyl and optionally substituted C_{1-6}-alkoxy, optionally substituted C_{2-6}-alkenyloxy, carboxy, optionally substituted C_{1-6}-alkoxycarbonyl, optionally substituted C_{1-6}-alkylcarbonyl, fomyl, optionally substituted C_{1-6}-alkylsulphonylamino, optionally substituted aryl, optionally substituted aryloxycarbonyl, optionally substituted aryloxy, optionally substituted arylcarbonyl, optionally substituted arylamino, arylsulphonylamino, optionally substituted heteroaryl, optionally substituted heteroaryloxycarbonyl, optionally substituted heteroaryloxy, optionally substituted heteroarylcarbonyl, optionally substituted heteroarylamino, heteroarylsulphonylamino, optionally substituted heterocyclyl, optionally substituted heterocyclyloxycarbonyl, optionally substituted heterocyclyloxy, optionally substituted heterocyclylcarbonyl, optionally substituted heterocyclylamino, heterocyclylsulphonylamino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)aminocarbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, amino-C_{1-6}-alkyl-carbonylamino, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-carbonylamino, amino-C_{1-6}-alkyl-amino, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-amino, cyano, guanidino, carbamido, C_{1-6}-alkanoyloxy, C_{1-6}-alkylsulphonyl, C_{1-6}-alkylsulphinyl, C_{1-6}-alkylsulphonyloxy, aminosulfonyl, mono- and di(C_{1-6}-alkyl)aminosulfonyl, and optionally substituted C_{1-6}-alkylthio; and

R_{10} and R_{11} are each independently selected from the group consisting of hydrogen, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted C_{2-6}-alkynyl, optionally substituted C_{1-6}-alkoxycarbonyl, optionally

substituted C_{1-6}-alkylcarbonyl, optionally substituted aryl, optionally substituted aryloxycarbonyl, optionally substituted arylcarbonyl, optionally substituted heteroaryl, optionally substituted heteroaryloxycarbonyl, optionally substituted heteroarylcarbonyl, aminocarbonyl, mono- and di(C_{1-6}-alkyl)aminocarbonyl; or R_{10} and R_{11} together with the nitrogen atom to which they are attached form an optionally substituted nitrogen-containing heteroaryl or optionally substituted heterocyclyl;

or a metabolite or salt thereof in combination with an anti-infective agent.

54. The method according to claim 53, wherein said compound is as defined in any of claims 2-46.

55. The method according to claim 53 or 54, wherein said compound is administered as defined in any of claims 47-49.

56. The method according to any of claims 53-55, wherein said infectious disease is caused by an infectious agent.

57. The method according to claim 56, wherein said infectious agent is as defined in claim 51 or 52.

58. A pharmaceutical composition comprising a compound as defined in any of claims 1-46 and at least one pharmaceutically acceptable carrier or excipient.

59. The pharmaceutical composition according to claim 58, wherein said composition further comprises an anti-infective agent.

60. The pharmaceutical composition according to claim 58 or 59, wherein said composition is in a unit dosage form.

61. The pharmaceutical composition according to claim 60, wherein said composition is in the form of a tablet.

62. The pharmaceutical composition according to claim 60, wherein said composition is in the form of a sterile solution.

63. A kit comprising a first dosage unit comprising a compound as defined in claims 1 to 45 and a further dosage unit comprising an antimicrobial agent.

FIG.1

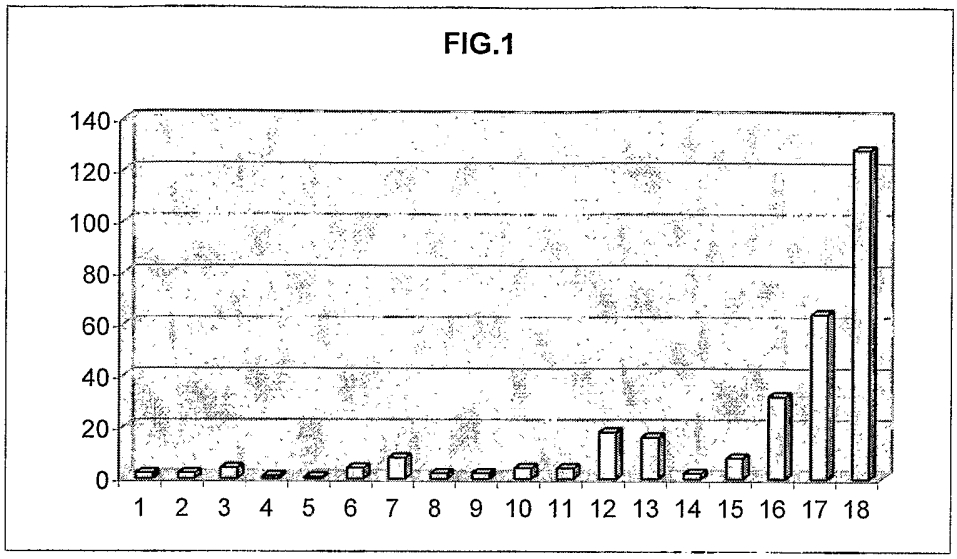


Fig. 1

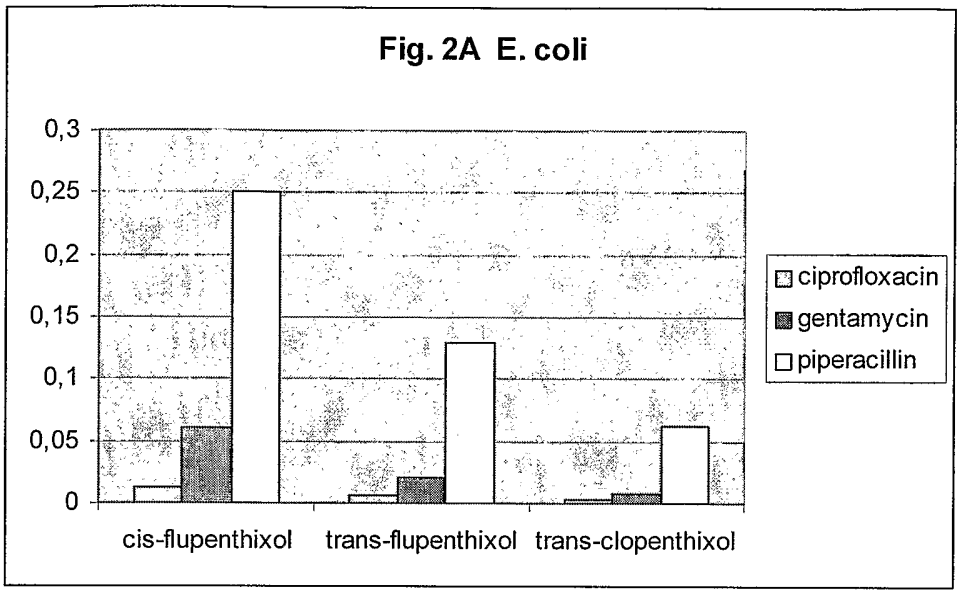


Fig. 2A E. coli
0,3
0,25
0,2
0,15
0,1
0,05
0
cis-flupenthixol
trans-flupenthixol
trans-clopenthixol
ciprofloxacin
gentamycin
piperacillin

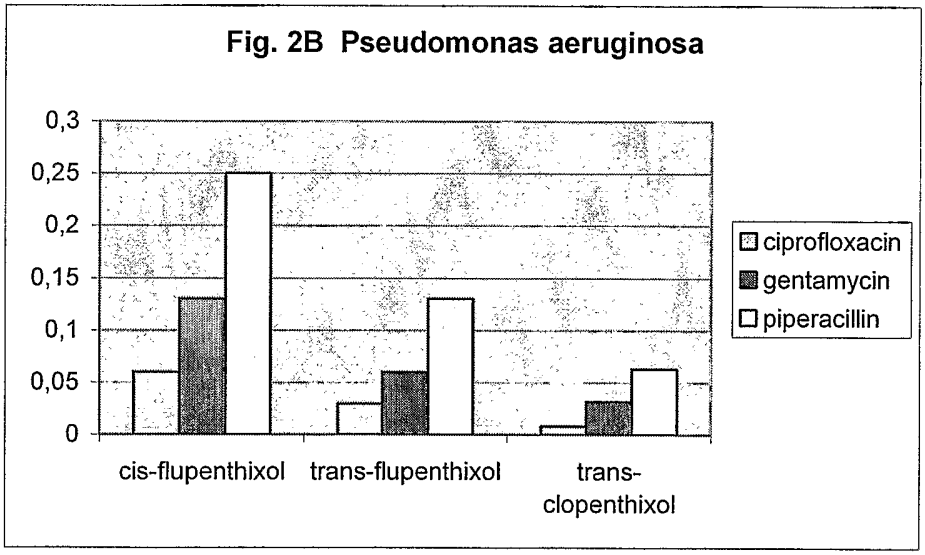
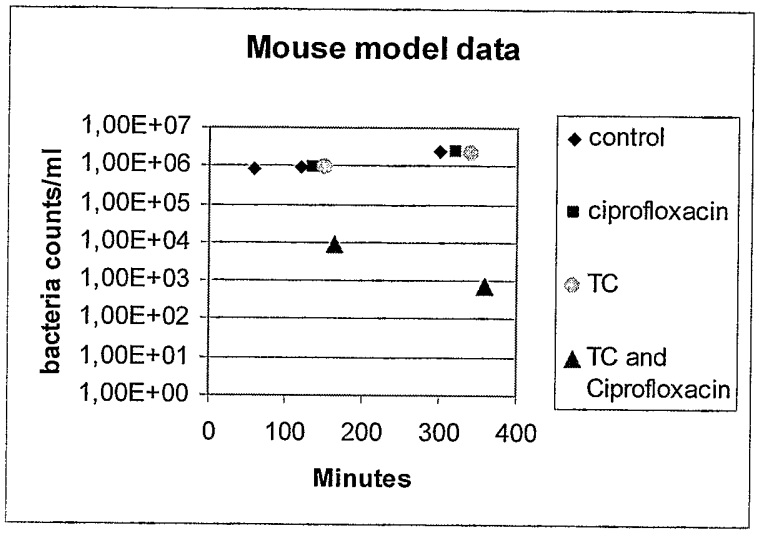


Fig. 2B Pseudomonas aeruginosa
0,3
0,25
0,2
0,15
0,1
0,05
0
cis-flupenthixol
trans-flupenthixol
trans-clopenthixol
ciprofloxacin
gentamycin
piperacillin

Fig. 2

Figure 3



TC: trans-clopenthixol

Fig. 3

Figure 4

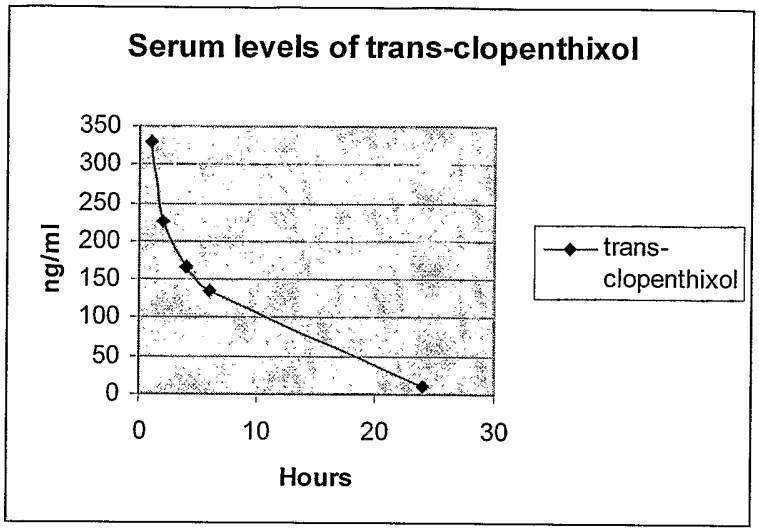


Fig. 4

INTERNATIONAL SEARCH REPORT

International Application No
PCT/DK2005/000294

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K45/06 A61P31/00 A61P33/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MOLNAR J ET AL: "SYNERGISTIC EFFECT OF PROMETHAZINE WITH GENTAMICIN IN FREQUENTLY RECURRING PYELONEPHRITIS" INTERNATIONAL UROLOGY AND NEPHROLOGY, vol. 22, no. 5, 1990, pages 405-412, XP008048691 ISSN: 0301-1623 page 410	1-25, 47-63
X	EP 0 338 532 A (MERRELL DOW PHARMACEUTICALS INC) 25 October 1989 (1989-10-25) page 2, line 33 - page 3 page 5, line 10 - line 22	1-63

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 August 2005	08/08/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Leherte, C

Form PCT/ISA/210 (second sheet) (January 2004)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/089810 A (UNITED STATES ARMY MEDICAL RESEARCH AND MATERIEL COMMAND) 14 November 2002 (2002-11-14) claims 1-6	1-25, 47-63
X	WO 03/062388 A (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 31 July 2003 (2003-07-31) paragraph '0095! claims 31,37,38	1-25, 47-63
X	EP 0 123 157 A (EGYT GYOGYSZERVEGYESZETI GYAR; EGIS GYOGYSZERGYAR) 31 October 1984 (1984-10-31) page 3	1-25, 47-63
X	KRISTIANSEN JETTE ELISABETH ET AL: "The potential management of resistant infections with non-antibiotics" JOURNAL OF ANTIMICROBIAL CHEMOTHERAPY, vol. 40, no. 3, September 1997 (1997-09), pages 319-327, XP008048674 ISSN: 0305-7453 page 321, column 1, paragraph 2 - page 322, column 1, paragraph 2 page 325, column 1, paragraph 3 - column 2	1-63
X	AFELTRA J ET AL: "Antifungal activity of nonantifungal drugs." EUROPEAN JOURNAL OF CLINICAL MICROBIOLOGY & INFECTIOUS DISEASES : OFFICIAL PUBLICATION OF THE EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY. JUL 2003, vol. 22, no. 7, July 2003 (2003-07), pages 397-407, XP008048583 ISSN: 0934-9723 page 398, column 1, paragraph 3 - column 2, paragraph 1	1-25, 47-63
X	COUVARIS M ET AL: "COMPARATIVE STUDY OF THE EFFECTS OF SOME INDUCERS WITH OR WITHOUT BINDING PROPERTIES ON BIOAVAILABILITY OF ISOXAZOLYLPENICILLINS IN RATS" EUROPEAN JOURNAL OF DRUG METABOLISM AND PHARMACOKINETICS, vol. 11, no. 3, 1986, pages 187-194, XP008048575 ISSN: 0378-7966 page 190, column 1, paragraph 5 - page 192, column 1 tables 1-3 figures 1-3	1-25, 47-63

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CROWLE ALFRED J ET AL: "Chlorpromazine: A drug potentially useful for treating mycobacterial infections" CHEMOTHERAPY, vol. 38, no. 6, 1992, pages 410-419, XP008048689 ISSN: 0009-3157 page 416, column 2 - page 418, column 2	1-25, 47-63
X	ORDWAY D ET AL: "CLINICAL CONCENTRATIONS OF THIORIDAZINE KILL INTRACELLULAR MULTIDRUG-RESISTANT MYCOBACTERIUM TUBERCULOSIS" ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON, DC, US, vol. 47, no. 3, March 2003 (2003-03), pages 917-922, XP008044806 ISSN: 0066-4804 abstract page 918, column 1, paragraph 2 page 921, column 1, paragraph 2	1-25, 47-63
X	KAATZ G W ET AL: "PHENOTHIAZINES AND THIOXANTHENES INHIBIT MULTIDRUG EFFLUX PUMP ACTIVITY IN STAPHYLOCOCCUS AUREUS" ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON, DC, US, vol. 47, no. 2, February 2003 (2003-02), pages 719-726, XP008044805 ISSN: 0066-4804 cited in the application abstract	1-63
X	KRISTIANSEN M M ET AL: "PHENOTHIAZINES ALTER RESISTANCE OF METHICILLIN-RESISTANT STRAINS OF STAPHYLOCOCCUS AUREUS (MRSA) TO OXACILLIN IN VITRO" INTERNATIONAL JOURNAL OF ANTIMICROBIAL AGENTS, AMSTERDAM, NL, vol. 22, no. 3, 1 September 2003 (2003-09-01), pages 250-253, XP008044880 ISSN: 0924-8579 page 252, column 1, paragraph 3 - page 253, column 1	1-25, 47-63

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AMARAL LEONARD ET AL: "Activity of phenothiazines against antibiotic-resistant Mycobacterium tuberculosis: A review supporting further studies that may elucidate the potential use of thioridazine as anti-tuberculosis therapy" JOURNAL OF ANTIMICROBIAL CHEMOTHERAPY, vol. 47, no. 5, May 2001 (2001-05), pages 505-511, XP008048568 ISSN: 0305-7453 abstract	1-25, 47-63
X	RAJYAGURU J M ET AL: "Enhancement of Burkholderia cepacia antimicrobial susceptibility by cationic compounds." THE JOURNAL OF ANTIMICROBIAL CHEMOTHERAPY. SEP 1997, vol. 40, no. 3, September 1997 (1997-09), pages 345-351, XP008048598 ISSN: 0305-7453 abstract page 347, column 2, paragraph 2	1-25, 47-63
X	HEWLETT INDIRA ET AL: "Inhibition of HIV infection of H9 cells by chlorpromazine derivatives" JOURNAL OF ACQUIRED IMMUNE DEFICIENCY SYNDROMES AND HUMAN RETROVIROLOGY, vol. 15, no. 1, 1997, pages 16-20, XP008048577 ISSN: 1077-9450 page 20, column 1, paragraph 4	1-25, 47-63
X	KENNY M T ET AL: "Beta-Lactam antibiotic modulation of murine neutrophil cytokinesis" IMMUNOPHARMACOLOGY AND IMMUNOTOXICOLOGY, vol. 14, no. 4, 1992, pages 797-811, XP008048576 ISSN: 0892-3973 page 808, paragraph 4 table 7	1-25, 47-63
X	KRISTIANSEN J E ET AL: "Synergy between a non-neuroleptic thioxanthene stereo-isomer and penicillin in vivo" APMIS 1988 DENMARK, vol. 96, no. 12, 1988, pages 1079-1084, XP008050461 ISSN: 0903-4641 page 1083, paragraph 2 - paragraph 3	1-8, 26-63

-/--

INTERNATIONAL SEARCH REPORT

International Application No
PCT/DK2005/000294

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 2005/046694 A (KOEBENHAVNS UNIVERSITET; SYDDANSK UNIVERSITET; SOENDERJYLLANDS AMT; CH) 26 May 2005 (2005-05-26) the whole document	1-25, 47-63

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK2005/000294

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 53-57 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [X] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

1-6, 9-24, 26-43, 47-63 (all partially), 7, 8, 25, 44-46

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[X] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-24, 47-63 (all partially), 25

Use of a compound of the general formula (I), as represented in claim 1, wherein V is selected from the group consisting of S, SO2 and SO and W is N-(CHX)-N(R10)(R11), in combination with an anti-infective agent for the manufacture of a medicament for the treatment or prophylaxis of an infectious disease,

---

2. claims: 1-6, 9-24, 47-63 (all partially)

Use of a compound of the general formula (I), as represented in claim 1, wherein V is O and W is N-(CHX)-N(R10)(R11), in combination with an anti-infective agent for the manufacture of a medicament for the treatment or prophylaxis of an infectious disease,

---

3. claims: 1-6, 9-24, 47-63 (all partially)

Use of a compound of the general formula (I), as represented in claim 1, wherein V is NH and W is N-(CHX)-N(R10)(R11), in combination with an anti-infective agent for the manufacture of a medicament for the treatment or prophylaxis of an infectious disease,

---

4. claims: 1-8, 26-43, 47-63 (all partially), 44-46

Use of a compound of the general formula (I), as represented in claim 1, wherein V is selected from the group consisting of S, SO2 and SO and W is C=CH-(CHX)m-N(R10)(R11), in combination with an anti-infective agent for the manufacture of a medicament for the treatment or prophylaxis of an infectious disease,

---

5. claims: 1-6, 26-43, 47-63 (all partially)

Use of a compound of the general formula (I), as represented in claim 1, wherein V is O and W is C=CH-(CHX)m-N(R10)(R11), in combination with an anti-infective agent for the manufacture of a medicament for the treatment or prophylaxis of an infectious disease,

---

6. claims: 1-6, 26-43, 47-63 (all partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Use of a compound of the general formula (I), as represented in claim 1, wherein V is NH and W is C=CH-(CHX)m-N(R10)(R11), in combination with an anti-infective agent for the manufacture of a medicament for the treatment or prophylaxis of an infectious disease.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/DK2005/000294

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0338532 A	25-10-1989	AU 625071 B2	02-07-1992
		AU 3302389 A	26-10-1989
		DE 338532 T1	03-05-1990
		DK 190189 A	21-10-1989
		EP 0338532 A2	25-10-1989
		JP 1311026 A	15-12-1989
		NZ 228748 A	26-07-1991
		US 5373005 A	13-12-1994
		ZA 8902724 A	27-12-1989
WO 02089810 A	14-11-2002	WO 02089810 A1	14-11-2002
WO 03062388 A	31-07-2003	WO 03062388 A2	31-07-2003
		US 2003229082 A1	11-12-2003
EP 0123157 A	31-10-1984	HU 190078 B	28-08-1986
		AT 27912 T	15-07-1987
		AU 564187 B2	06-08-1987
		AU 2606884 A	27-09-1984
		CA 1212325 A1	07-10-1986
		DE 3464331 D1	30-07-1987
		EP 0123157 A1	31-10-1984
		ES 8507346 A1	16-12-1985
		JP 1754366 C	23-04-1993
		JP 4041127 B	07-07-1992
		JP 59210023 A	28-11-1984
WO 2005046694 A	26-05-2005	WO 2005046694 A1	26-05-2005