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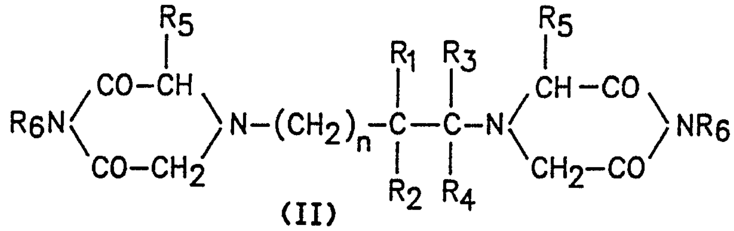

(II)

(57) Abstract

Compounds of formula (II), wherein n is 0, 1 or 2, R_1, R_2, R_3 and R_4 are each separately selected from hydrogen, unsubstituted acyclic aliphatic hydrocarbon groups having a maximum of six carbon atoms and C_{1-6} alkyl groups substituted by a hydroxy group or by a C_{1-6} alkoxy group, or one of R_1 and R_2 and one of R_3 and R_4 is hydrogen and the others together form a trimethylene, tetramethylene or pentamethylene bridging group, R_5 is hydrogen or an unsubstituted acyclic aliphatic hydrocarbon group having a maximum of six carbon atoms, and R_6 is an acyclic aliphatic hydrocarbon group having a maximum of six carbon atoms or a group CH_2R_7 in which R_7 is a C_{1-5} alkyl group substituted by a hydroxy group or by a C_{1-6} alkoxy group, or a salt thereof formed with a physiologically acceptable inorganic or organic acid, are of use in therapy, particularly as cardioprotective agents.

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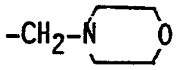
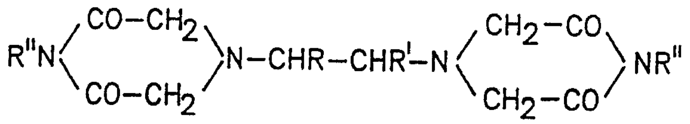
BIS-DIOXOPIPERAZINES AND THEIR USE AS PROTECTION AGENTS

This invention relates to pharmaceutical compounds and to compositions containing them, being primarily concerned with substances of use as cardioprotective agents and in certain other protective roles.

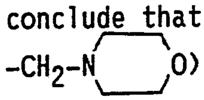
Certain bis-dioxopiperazines of formula (I) are cytotoxic and have been used in the treatment of cancer. Thus UK patent 1,234,935 describes the compounds of formula (I) having $R = CH_3$ and $R' = R'' = H$ (as the dl, d and l isomers); $R = R' = R'' = H$; $R = R' = CH_3$ and $R'' = H$ (as the meso isomer); and $R + R' = -CH_2CH_2-$ and $R'' = H$. Of these the first named compound has proved to be of most value although a further compound of formula (I) having $R = R' = H$ and $R'' =$  has also been used in treating cancer.



(I)

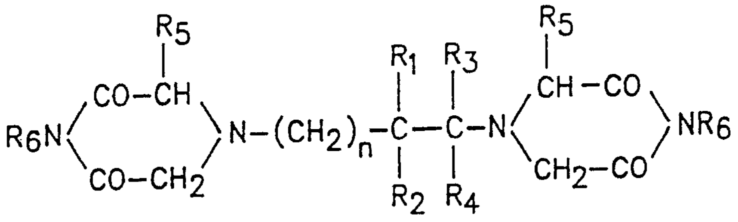
Studies have been reported by various authors on the chelating properties of these bis-dioxopiperazines and the use in treatment of lead poisoning has been proposed by Wittig and Hultsch, Int. Arch. Occup. Environ. Health, 1981, 48, 89, for the compound of formula (I) having $R = R' = H$ and $R'' = CH_3$ and by May *et al*, Agents and Actions, 1984, 15, 448 and Willes and Williams, Plzen. Lek. Sborn., 1985, 49, 113, for the compound having $R = R'' = H$ and $R' = C_2H_5$ in the dl form.

German Offenlegungschrift 2,511,891 and 2,511,892 describe the use of certain bis-dioxopiperazines of formula (I) for the treatment and prophylaxis of thrombosis and embolism. These compounds (I) include, *inter alia*, those in which R and R' are each separately selected from hydrogen and C_{1-6} alkyl or together are linked so as to form a cycloalkyl residue and R" is C_{1-6} alkyl, with the sum of the carbon atoms in R, R' and R" being greater than two.

In Research Communications in Chemical Pathology and Pharmacology, 1985, 48, 39, Herman et al report tests on the protective effect against acute daunorubicin toxicity of a range of bis-dioxopiperazines of formula (I). They conclude that although the compound bimolane (R = R' = H and R" =  $-CH_2-N\langle\;\rangle O$) and the compound having R = CH_3 and R' = R" = H (as the dl, d or l isomer) give protection against the lethal effects of daunorubicin, the remainder of the compounds tested (R = R' = R" = H; R = R' = H and R" = CH_3; R = R" = CH_3 and R' = H (l); R = R' = CH_3 and R" = H (meso); R = C_2H_5 and R' = R" = H (dl); R = CH_3, R' = C_2H_5 and R" = H (dl-erythro); and R + R' = $-CH_2-CH_2-$ and R" = H; as well as the compound in which -CHR-CHR'- is replaced by $-(CH_2)_3-$ and the ring opened bis-diacid diamide compound having R = CH_3 and R' = H) all showed either no protective activity or only minimal protective activity.

It is the case that all of the bis-dioxopiperazines identified in this paper as exhibiting a useful level of cardioprotection against daunorubicin toxicity are cytotoxic. We have now found that, despite the indications to the contrary in the paper, various bis-dioxopiperazines which are substantially non-cytotoxic are of interest as cardioprotective agents, as well as in other roles outside the area of lead poisoning. These non-cytotoxic bis-dioxopiperazines are of interest for providing protection against the cardiotoxic effects of various anthracycline drugs but particularly doxorubicin (adriamycin). In this context it is relevant that, in addition to the comments in the Herman et al Research Communications in Chemical Pathology and Pharmacology paper, it is indicated by Herman et al in Advances in Pharmacology and Chemotherapy, 1982, 19, 249 that even the cardioprotective compound ICRF 159, which is the dl isomer of the compound of formula (I) having R = CH_3 and R = R' = H, is consistently more effective in reducing high dose daunorubicin toxicity than doxorubicin toxicity.

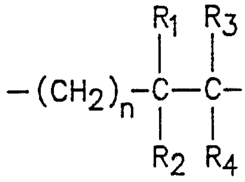
Accordingly the present invention comprises the use of a compound of formula (II):



(II)

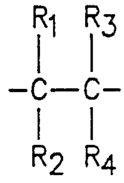
wherein n is 0, 1 or 2, R_1, R_2, R_3 and R_4 are each separately selected from hydrogen, unsubstituted acyclic aliphatic hydrocarbon groups having a maximum of six carbon atoms and C_{1-6} alkyl groups substituted by a hydroxy group or by a C_{1-6} alkoxy group, or one of R_1 and R_2 and one of R_3 and R_4 is hydrogen and the others together form a trimethylene, tetramethylene or pentamethylene bridging group, R_5 is hydrogen or an unsubstituted acyclic aliphatic hydrocarbon group having a maximum of six carbon atoms, and R_6 is an acyclic aliphatic hydrocarbon group having a maximum of six carbon atoms or a group CH_2R_7 in which R_7 is a C_{1-5} alkyl group substituted by a hydroxy group or by a C_{1-6} alkoxy group, or a salt thereof formed with a physiologically acceptable inorganic or organic acid, for the manufacture of a medicament for use as a cardioprotective agent.

In the compounds (II) of the present invention the central grouping in the molecule has the form:



$$-(CH_2)_n-\underset{R_2}{\overset{R_1}{C}}-\underset{R_4}{\overset{R_3}{C}}-$$

Although n may be 2 or more especially 1, each of R_1 to R_4 then conveniently being hydrogen, it is preferred that n is 0, in which latter case the grouping will be of the form:



$$-\underset{R_2}{\overset{R_1}{C}}-\underset{R_4}{\overset{R_3}{C}}-$$

As indicated, R_1, R_2, R_3 and R_4 may be hydrogen, an unsubstituted acyclic C_{1-6} aliphatic hydrocarbon group or a C_{1-6} alkyl group substituted by a hydroxy group or by a C_{1-6} alkoxy group. The term acyclic aliphatic hydrocarbon group is used herein to include both branched and especially straight chain groups. The group may be unsaturated or especially saturated, conveniently containing one double or triple bond in the former case. Thus, in particular, unsubstituted groups may be alkenyl, alkynyl and particularly alkyl groups, which may be straight or branched chain. The aliphatic hydrocarbon groups conveniently contain a maximum of four or especially three carbon atoms, preferred groups therefore being C_1-C_4 or C_1-C_3 alkyl groups and C_2-C_4 or C_2-C_3 alkenyl and alkynyl groups.

As regards the substituted C_{1-6} alkyl groups, these may be branched or especially straight chain alkyl groups substituted, particularly terminally, by a hydroxy group or particularly an alkoxy group. Conveniently the alkyl groups are of 1 to 3 or 1 to 4 carbon atoms, substituted ethyl and particularly substituted methyl groups being of most interest. Preferred alkoxy group substituents similarly contain 1 to 3 or 1 to 4 carbon atoms with ethoxy and particularly methoxy groups being of most interest. Conveniently the total number of carbon atoms in such an alkoxyalkyl group is from 2 to 6, particularly 2 to 4 and especially 2 or 3. Examples of specific substituted groups R_1, R_2, R_3 and R_4 are the groups hydroxymethyl, 2-hydroxyethyl and methoxymethyl. Preferably no more than two of R_1 to R_4, for example R_1 and R_3 or R_2 and R_4, and especially only one is a substituted alkyl group and when this is the case the others of R_1 to R_4 are conveniently hydrogen.

It is generally preferred, however, that where they do not constitute trimethylene, tetramethylene or pentamethylene bridging groups the groups R_1, R_2, R_3 and R_4 are selected from hydrogen and unsubstituted acyclic aliphatic hydrocarbon groups, especially from the group consisting of hydrogen, methyl, ethyl, n-propyl, isopropyl, allyl and propargyl. Preferably R_1 is hydrogen and conveniently either R_2 is also hydrogen but R_3 and R_4 are not or, more usually, R_3 is also hydrogen whilst R_2 and R_4 are either hydrogen or not, for example conveniently being selected from the whole group specified above. Also of some particular interest, however, are compounds in which one of R_1 and R_2 and one of R_3 and R_4 is hydrogen and the others together form a bridging group, especially a tetramethylene group. Examples of compounds of particular interest are those in which n is 0, R_1 and R_2 are each hydrogen and R_3 and R_4 are each methyl, or more particularly n is 0, R_1 and R_3 are each hydrogen and (a) R_2 is hydrogen and R_4 is hydrogen, methyl, ethyl, n-propyl, isopropyl, allyl or propargyl, (b) R_2 is methyl or ethyl and R_4 is methyl, ethyl, n-propyl, isopropyl, allyl or propargyl, or (c) R_2 and R_4 together form a tetramethylene bridging group.

Specific combinations of particular interest are $n = 0$, $R_1 = R_2 = H$, $R_3 = R_4 = CH_3$; $n = 0$, $R_1 = R_2 = R_3 = H$, $R_4 = CH_2OH$ or CH_2OCH_3; $n = 0$, $R_1 = R_3 = H$, $R_2 = R_4 = CH_3$; $n = 0$, $R_1 = R_3 = H$, $n = 0$, $R_1 = R_2 = R_3 = H$, $R_4 = CH_3$ or C_2H_5; particularly $n = 0$, $R_1 = R_2 = R_3 = R_4 = H$; and especially $R_2 + R_4 = CH_2CH_2CH_2CH_2$.

As regards the groups R_5 one preference is for these to be hydrogen but where this is not the case the groups may conveniently be a C_{1-4} aliphatic hydrocarbon group, particularly a C_{1-4} or C_{1-3} alkyl group or, to a lesser extent, a C_{2-4} or C_{2-3} alkenyl or alkynyl group, an ethyl or especially a methyl group being of most interest and constituting another preference. When R_5 is other than hydrogen each of R_1, R_2, R_3 and R_4 is preferably hydrogen or alternatively R_1, R_2 and R_3 are each hydrogen and R_4 is ethyl or especially methyl.

As regards the group R_6 this is preferably selected from unsubstituted aliphatic hydrocarbon groups, the detailed preferences among such groups being as described hereinbefore for the groups R_1 to R_4. Thus groups R_6 which are alkyl groups, particularly C_{1-4} or C_{1-3} alkyl groups, for example ethyl or particularly methyl are preferred, and where R_6 is unsaturated it preferably has the form CH_2R_8 where R_8 is a C_{2-5} alkenyl or alkynyl group, preferably a C_2 or C_3 group, for example CH_2R_8 being allyl or propargyl.

As regards the groups R_6 of the form CH_2R_7, these may be branched or especially straight chain alkyl groups substituted, particularly terminally, by a hydroxy group or particularly by an alkoxy group. Conveniently the groups R_7 are substituted alkyl groups of 1 to 2 or 1 to 3 carbon atoms, substituted ethyl and articularly substituted methyl groups being of most interest. Preferred alkoxy group substituents similarly contain 1 to 3 or 1 to 4 carbon atoms with ethoxy and particularly methoxy groups being of most interest. Conveniently the total number of carbon atoms in such an alkoxyalkyl group CH_2R_7 is from 3 to 6, especially 3 or 4.

Compounds of particular interest are those in which R_6 is selected from 2-hydroxyethyl, 2-methoxyethyl, particularly ethyl, n-propyl, isopropyl, and especially methyl, for example together with the combinations of R_1 to R_4 indicated as preferred.

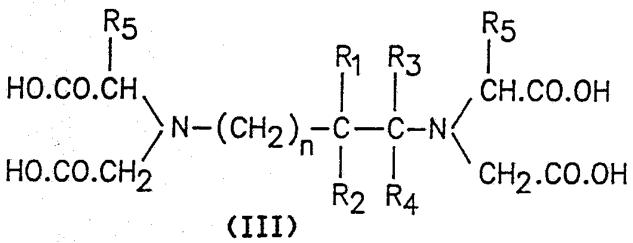
Specific preferred compounds according to the present invention are those having the specific combinations of n, R_1, R_2, R_3 and R_4 as indicated previously, together with R_5 being ethyl, particularly methyl or especially hydrogen and with R_6 being ethyl or especially methyl. Among these compounds may particularly be mentioned those in which $n = 0$, $R_1 = R_3 = H$, $R_2 = R_4 = CH_3$, $R_5 = H$, $R_6 = CH_2CH_2OH$, particularly C_2H_5 or especially CH_3; $n = 0$, $R_1 = R_2 = R_3 = R_5 = H$, $R_4 = CH_3$ or especially C_2H_5, $R_6 = CH_2CH_2OH$, particularly C_2H_5 or especially CH_3; $n = 0$, $R_1 = R_2 = R_3 = R_4 = H$, $R_5 = CH_3$, C_2H_5 or especially H, and $R_6 = CH_2CH_2OH$, particularly C_2H_5 or especially CH_3; and particularly $n = 0$, $R_1 = R_3 = R_5 = H$, $R_2 + R_4 = CH_2CH_2CH_2CH_2$; $R_6 = C_2H_5$ or especially CH_3 or CH_2CH_2OH. Enhanced water solubility will result from the presence of hydroxyalkyl groups in the molecule, for example in compounds having $R_6 = CH_2CH_2OH$ and also in compounds having $R_4 = CH_2OH$, for example in the latter instance with $n = 0$, $R_1 = R_2 = R_3 = H$, $R_5 = H$, CH_3 or C_2H_5 and $R_6 = CH_3$ or C_2H_5.

The compounds may exist in various stereochemical forms, each of which is included by the present invention. Thus, when neither of the groupings $-CR_1R_2-$ and CR_3R_4- is $-CH_2-$ the possibility of geometrical isomerism exists (the compounds being in the <u>dl</u> or <u>threo</u> configuration or alternatively in the <u>meso</u> or <u>erythro</u> configuration when n = 0). Moreover, compounds in which two of R_1 to R_4 provide a bridging group may be in the <u>cis</u> or <u>trans</u> form. It will also be appreciated that when the bridging grouping $-(CH_2)_n-CR_1R_2-CR_3R_4-$ does not have a centre of symmetry, the compounds can exist in enantiomorphic <u>d</u> and <u>l</u> forms. In addition, when R_5 is not hydrogen this will lead to the presence of two identically substituted asymmetric carbon atoms in the molecule with the possible stereochemical variations this produces. The invention includes the use of the various different isomers of the compounds. In some cases the optically active <u>d</u>- and <u>l</u>-isomers may have the advantage of significantly higher water solubility than the corresponding racemate and it may also be the case that the biological activity of the compound will differ as between the isomers. The invention does therefore extend to the use of such compounds not only as the <u>dl</u>-racemate but also in a form in which the amount of the compound in either the <u>d</u> or <u>l</u> configuration is greater than that in the <u>l</u> or <u>d</u> configuration, respectively (including amounts in that configuration present in the <u>dl</u> racemate). In particular the compound may be essentially in the form of the <u>d</u> or <u>l</u> isomer, for example being substantially free from (i.e. containing no more than 20% and conveniently no more than 10% of) the <u>dl</u> and <u>l</u> or <u>dl</u> and <u>d</u> isomers. However, where the advantage lies in enhanced solubility of the optically active isomers compared with the racemate, rather than enhanced biological activity for one isomer, any enhancement of the proportion of one isomer should have some effect.

Of the specific compounds previously indicated as being particularly preferred (as the free compound or a salt), 1,3-bis(4-methyl-3,5-dioxopiperazin-1-yl)propane and 1,2-bis(4-methyl-3,5-dioxopiperazin-1-yl)ethane do not exist in optically active forms but 1,2-bis(4-methyl-3,5-dioxopiperazin-1-yl)propane and 1,2-bis(4-methyl-3,5-dioxopiperazin-1-yl)butane and 1,2-bis(4-methyl-3,5-dioxopiperazin-1-yl)cyclohexane can do so. Compounds in which R_5 is methyl, for example the analogues of the four compounds just mentioned, can also exist in optically active forms.

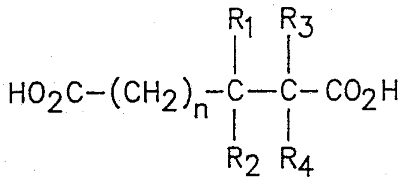
It will be appreciated that certain of the compounds of formula (II) as defined hereinbefore are novel and that the present invention extends to such compounds <u>per se</u>.

The compounds of formula (II) may conveniently be prepared by reaction of the corresponding tetra-acetic acid of formula (III) with an N-substituted formamide in which the nitrogen atom is substituted by a group R_6, this reaction being carrried out in an excess of the substituted formamide as the solvent where convenient and at an elevated temperature, preferably under nitrogen.



(III)

The tetra-acetic acids of formula (III) may conveniently be obtained from the corresponding dicarboxy acid of formula (IV).

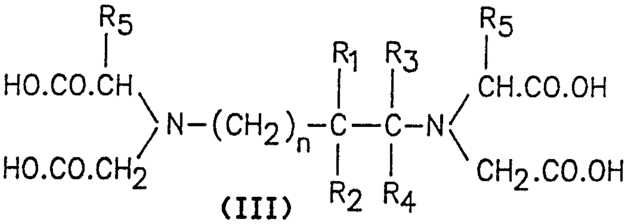


$$HO_2C-(CH_2)_n-\overset{R_1}{\underset{R_2}{C}}-\overset{R_3}{\underset{R_4}{C}}-CO_2H$$

(IV)

Examples of such procedures and/or of intermediates of use in such procedures are to be found in UK patents 966,802, 978,724, 1,234,935 and 1,374,979, and in German Offenlegungschrift 2,511,891. Moreover, intermediates of formula (III) in which R_5 is other than hydrogen are described in UK patent 723,316.

In general terms, a process for the preparation of a compound of formula (II) as defined hereinbefore and salts thereof formed with a physiologically acceptable inorganic or organic acid comprises reacting a tetra-acetic acid of formula (III)



$$(\mathrm{HO.CO.CH}(R_5))(\mathrm{HO.CO.CH_2})\mathrm{N{-}(CH_2)_n{-}C}(R_1)(R_2)\mathrm{{-}C}(R_3)(R_4)\mathrm{{-}N}(\mathrm{CH}(R_5)\mathrm{.CO.OH})(\mathrm{CH_2.CO.OH})$$

(III)

in which n has the same value as in the compound of formula (II) and R_1 to R_5 represent groups as in the compound of formula (II);

(i) as the tetra-acetic acid or as its bis anhydride by heating with an acid amide of the general formula $RCONH_6$ in which R represents hydrogen, an aliphatic residue, for example an alkyl group, having one to four carbon atoms, or an amino group NH_2; or

(ii) as the tetra-acetic acid or as its bis anhydride, tetra halide, for example the acid chloride or bromide, or tetra ester, for example one formed with a lower aliphatic alcohol (particularly C_{1-4}) or a phenol, with an amine of the general formula

$$H_2N\text{-}R_6$$

in which R_6 represents a group as in the compound of formula (II); and, where appropriate, separating the required compound (II) from other isomers thereof and/or converting the compound (II) to a physiologically acceptable addition salt thereof.

The preferred procedure is (i), particularly as described hereinbefore.

To obtain compounds (II) of the desired stereochemistry it is most convenient to use an intermediate compound having the equivalent stereochemistry, particularly as regards the preparation of geometrical isomers, for example compounds in the dl or threo form as opposed to the meso or erythro form, but conveniently also as regards the preparation of optically active compounds. Thus the tetra-acetic acid which is used conveniently has the same stereochemistry as is desired in the final product. When a d or l isomer is required rather than the dl racemate, however, an alternative to the preferred utilisation of a d or l intermediate compound is to effect a resolution of the compound (II), for example using an appropriate optically active acid to form a mixture of salts of the d and l forms of the compound (II) which are then separated.

Only certain of the compounds (II) have previously been indicated in the literature as being of use in therapy. Accordingly the present invention further comprises a compound of formula (II) where this is not the case, for use in therapy.

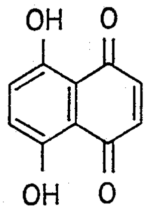
The present invention also includes pharmaceutical compositions comprising as an active component such compounds of formula (II) where this is not the case, together with a physiologically acceptable diluent or carrier. In general, compositions containing the compounds of formula (II) which are of particular interest are those in which the diluent or carrier excludes any liquid which is not sterile and pyrogen free.

As indicated, the compounds (II) may be formulated as salts formed with physiologically acceptable inorganic or organic acids and, when so formulated, it is preferred to use methane sulphonic acid, isethionic acid, tartaric acid or another solubilising acid.

The compounds of formula (II) may be formulated singly, or as a mixture of two or more compounds, for use as pharmaceuticals by a variety of methods. For instance, they may be applied as aqueous, oily (e.g. as a suspension in isopropyl myristate), or in some cases emulsified compositions for parenteral administration and therefore preferably sterile and pyrogen-free. Some of these compounds have rather low solubility in aqueous media and are therefore usually administered in the form of aqueous suspensions containing suitable surface active agents. It will be appreciated that the dosage levels used may vary over quite a wide range especially since certain of the compounds (II) are more active than others. However, without commitment to a rigid definition of dosages it may be stated that a daily dosage of active constituent (estimated as the free base), divided if necessary, of from 10 mg to 3 g is proposed for parenteral mammalian use. This dosage may conveniently be applied as a solution in 500-1000 ml of liquid for intravenous injection by slow infusion, or as a solution or suspension in about 10 ml of liquid by the intramuscular route, or in small volumes subcutaneously. (Parenteral, particularly intravenous, administration is the route preferred for use in conjunction with the anthracycline drugs so that injectable compositions are of especial interest.) More particularly, with many compounds (II) the daily dose for a 70 kg human, administered parenterally, will often be in the range from about 100 mg to about 500 mg but with the more active compounds it may be less than this (the dose being varied pro rata for humans of a different weight or other mammals). When used in conjunction with an anthracycline drug, where a single administration of the drug and the compound (II) is common, however, higher doses than this may often be employed, for example between about 500 mg and about 3 g, with doses of more than this being considered where appropriate in terms of the ratios of compound (II):anthracycline drug as discussed hereinafter.

Where appropriate, the substances may also be compounded for oral administration in dosages which may be similar but may often be somewhat higher, for example in a range from 100 mg to 1 g or even as high as 3 g for the daily dose for a 70 kg human for many compounds (II) but possibly somewhat less than this for the more active compounds. Such oral formulations may particularly take the form of tablets compounded in the presence of conventional solid carrier materials such as starch, lactose, dextrin and magnesium stearate, or of capsules or cachets. Suppositories, pessaries, aerosol and other formulations may also be employed. The compounds may be formulated in unit dosage form, i.e. in discrete portions each containing a unit dose, or a multiple or sub-multiple of a unit dose of the active ingredient.

The compounds (II) as defined hereinbefore are primarily of value as cardioprotective agents and it should be noted that their potential in such a use extends not only to use in conjunction with drugs having a cardiotoxic side effect, these often being cytotoxic agents such as the anthracycline drugs, which are of particular value in treating breast cancer, but also extends to pathological conditions where the heart is at risk. The term "anthracycline drug" is used herein to include not only natural and semi-synthetic anthracyclines such as epirubicin, idarubicin, daunorubicin and especially doxorubicin (which names are used herein to include salts of these compounds), but also synthetic anthracyclines such as mitoxantrone. Indeed, the compounds (II) are of value in providing cardioprotection against the cardiotoxic side effect of various compounds containing a moiety



the toxic effect of such compounds being believed to derive from their chelating ability.

The compounds (II) also find a secondary use in protection against other toxic effects arising from natural diseases or induction by drugs, for example by various agents which are either toxic as such or when present in the body in excess, such agents including paracetamol (p-hydroxyacetanilide).

The compounds (II) find most application in the treatment of humans although they can find veterinary use in certain other mammals such as dogs, rabbits, cattle, and horses.

When used as a cardioprotective agent in the context of a pathological condition where the heart is at risk as a result of that condition the compounds (II) are administered for a period dictated by the existence of this condition. When used in a cardioprotective role in conjunction with a drug having a cardiotoxic side effect, the period of administration will be related to that of the use of the drug which will usually be administered at normal dosage rates and by the usual regimen, often parenterally. The compounds (II) may conveniently be administered before, together with or less often after the drug, the choice depending to some extent on the particular drug in question. In the first and third usages both the compound (II) and the drug will each be formulated separately, usually in a conventional manner, for example both being formulated as described above, although the two compositions may be packaged together for ease of sequential administration to the patient. A suitable time lapse between administration of the compound (II) and the drug in either order is quite short, being no more than about 1 to 4 hours, for example 2 hours, and particularly being about 1 hour or somewhat less, depending on the drug in question.

When the compound (II) is administered together with the drug, the two may be formulated separately but it may be preferred to include the compound (II) and the drug in the same composition. Such a pharmaceutical composition may again conveniently take one of the forms described above for compositions containing only the compound (II) and may, if desired, contain more than one compound (II) and/or more than one drug. The present invention thus includes (a) a pharmaceutical composition which comprises a compound of formula (II), as defined hereinbefore, and a drug having a cardiotoxic or other toxic side effect, for example an anthracycline drug, together with a physiologically acceptable diluent or carrier, and also (b) a kit comprising in association a compound of formula (II), as defined hereinbefore, and a drug having cardiotoxic or other toxic side effect.

As indicated, the compounds (II) are of particular interest for use with doxorubicin and the present invention therefore particularly includes a pharmaceutical composition comprising a compound of formula (II) as defined hereinbefore and doxorubicin, together with a physiologically acceptable diluent or carrier.

In instances where a series of doses of the drug is administered it may not be necessary for each administration of the drug to be made concomitantly with, or at the interval given above after or before the administration of the compound (II). It may be possible to administer the compound (II) alone or together with the drug, followed by one or more repeated spaced doses of the drug alone or, more often, in view of the more rapid metabolisation of the compound (II), to administer the drug alone or together with the compound (II), followed by one or more repeated spaced doses of the compound (II) alone. If the treatment with the drug is continued over an extended period repeat doses of the compound (II) are also likely to be required and one possible regimen would involve the administration of the drug and compound (II) together on certain occasions followed by the compound (II) alone on others.

As regards the relative amounts of the compound (II) and a drug to be used, this will depend on both the particular compound (II), the drug used and the regimen of use, a good indication being provided, however, by the dosages indicated hereinbefore for the compounds (II) and the conventional doses used for the drug. However, some additional comments may be made concerning the proportions of compound or compounds (II) to anthracycline drug which are used either singly or together in a pharmaceutical composition containing both a compound or compounds (II) and an anthracycline drug. Thus, by way of guidance it may be stated that a dose ratio of between 5:1 to 20:1 or even 25:1 w/w of compound or compounds (II) to drug, especially about 10:1 w/w, is often suitable. By way of further guidance, it may be mentioned that a normal single dosage of doxorubicin is in the range of about 0.75 to 2 mg/kg, i.e. about 50 to 150 mg for a 70 kg human being, but that the use of the compounds (II) is intended to enable some increase in the dosage, for example to 4 or 5 mg/kg, if desired, in order to enhance the anti-cancer effect of the doxorubicin whilst its cardiotoxic side effects are controlled by the presence of the compound (II).

The exact dosage of an anthracycline drug such as doxorubicin which is used will depend on whether it is given with other anti-tumour agents. Thus anthracycline drugs are often given together with one or more of other such agents, for example fluorouracil and cyclophosphamide and where desired, a pharmaceutical composition containing a compound or compounds (II) and an anthracycline drug can contain other such anti-tumour agents. Moreover, it may be advantageous to administer a calcium supplement together with the compounds (II), this usually being administered separately.

When used as a protective agent against the toxic effect of paracetamol, the compounds (II) may be used protectively before occurrence of the toxicity or following occurrence of the toxicity. It may even be possible to formulate the compound (II) with paracetamol in order automatically to counter the effect of an overdose thereof. Broadly similar dosage levels may be used to those described hereinbefore although where the toxic effect is acute, as for example is usually the case following an overdose of paracetamol, higher dosages over a shorter period may be indicated.

Other forms of protection include the use of the compounds (II) in conjunction with any condition which is either "naturally occurring" or drug induced where free radical damage occurs (this may also be involved in some of the conditions described hereinbefore such as a anthracycline drug-induced damage), for example in reducing the diabetogenic effect of drugs such as alloxan which generate free hydroxyl radicals. The compounds (II) may once again be used in a broadly similar manner as when employed in cardioprotection, including formulation together with the drug, and the dosage levels used.

The present invention thus includes a method of providing protection against a toxic effect on the body, particularly a cardiotoxic effect, which comprises administering to a patient in need thereof a therapeutically effective amount of a compound (II) as defined hereinbefore.

The present invention is illustrated by the following Examples.

EXAMPLES

Example 1 : Preparation of 1,2-bis(4-methyl-3,5-dioxopiperazin-1-yl)ethane

1,2-Diaminoethane tetra-acetic acid (20 g) and N-methylformamide (50 ml) are heated together in an atmosphere of nitrogen at 160-170°C for 8 hours. The reaction mixture is cooled, filtered and the collected solid is washed with cold isopropanol and then petroleum ether (b.p. 60-80°C) to give 1,2-bis(4-methyl-3,5-dioxopiperazin-1-yl)ethane (16.5 g, 85%), m.p. 180-182°C, ν_{max} (nujol) 1675, 1720, 3350 cm^{-1}.

Example 2 : Preparation of 1,2-bis(4-methyl-3,5-dioxopiperazin-1-yl)propane

(±)-, (-)-(R) and (+)-(S)-1,2-Diaminopropane tetra-acetic acid are each reacted with N-methylformamide and the reaction mixture is worked up in a similar manner to that described in Example 1. The resultant products are respectively (±)-1,2-bis(4-methyl-3,5-dioxopiperazin-1-yl)-propane obtained in 27% yield, m.p. 156-158°C from aqueous ethanol, (-)-(R)-1,2-bis(4-methyl-3,5-dioxopiperazin-1-yl)-propane obtained in 81% yield, m.p. 132-133°C from methanol, and (+)-(S)-1,2-bis(4-methyl-3,5-dioxopiperazin-1-yl)-propane obtained in 48% yield, m.p. 132-133°C from methanol, $[\alpha]^{D}_{26} = -9.4$ in dimethylformamide. The solubilities of the two isomers in water isomers in water at 25°C are about 10 mg/ml for the (±)isomer and about 40 mg/ml for the (-)-(R) and (+)-(S) isomers.

Example 3 : Preparation of meso-2,3-bis(4-methyl-3,5-dioxopiperazin-1-yl)butane

meso-2,3-Diaminobutane tetra-acetic acid is reacted with N-methyl formamide and the reaction mixture is worked up in a similar manner to that described in Example 1 to give meso-2,3-bis(4-methyl-3,5-dioxopiperazin-1-yl)-butane in 55% yield, m.p. 262-265°C from N-methylformamide.

Example 4 : Preparation of dl-trans-1,2-bis(4-methyl-3,5-dioxopiperazin-1-yl)cyclohexane

dl-trans-1,2-Diaminocyclohexane tetra-acetic acid (20 g) (prepared essentially as described in Example 14 of U.S. Patent 3,196,153) is heated with freshly distilled methylformamide (100 ml) under nitrogen at 150-160°C for 6 hours. The reaction mixture is then evaporated under reduced pressure to remove the excess methylformamide and the residue is taken up in hot methanol (50 ml). The solid obtained on cooling is recrystallised from ethanol to give dl-trans-1,2-bis(4-methyl-3,5-dioxopiperazin-1-yl)-cyclohexane (6.2 g, 33%), m.p. 145-147°C.

Example 5 : Formulation of compounds

(A) Tablets of the following composition are prepared:

	mg/tablet
Compound of Example 1 (micronised)	250
'Avicel' (microcrystalline cellulose)*	38
polyvinylpyrrolidone	3
alginic acid	6
magnesium stearate	3

The compound of Example 1 is mixed with 'Avicel' and polyvinylpyrrolidone is added, dissolved in sufficient industrial methylated spirits (74° OP) to produce a mass suitable for granulating. The mass is granulated through a 20 mesh sieve and the resultant granules are dried at a temperature not exceeding 50°C. The dried granules are passed through a 20 mesh sieve and the alginic acid and magnesium stearate are then added and mixed with the granules. The product is compressed into tablets each weighing 300 mg on 3/8 inch flat bevelled edge divided punches.

(B) Tablets of the following composition are prepared:

	mg/tablet
Compound of Example 1 (micronised)	250
'Avicel' (microcrystalline cellulose)	134
polyvinylpyrrolidone	4
alginic acid	8
magnesium stearate	4

The tablets are prepared by essentially the same procedure as described in (A) and are compressed at a tablet weight of 400 mg on 7/16 inch flat bevelled edge punches.

* 'Avicel' is a Registered Trade Mark or Service Mark.

(C) Tablets of the following composition are prepared:

	mg/tablet
Compound of Example 1 (micronised)	250
lactose (300 mesh)	19
maize starch	15
gelatine	10
magnesium stearate	6

The tablets are prepared by mixing the compound of Example 1 with lactose and half the total quantity of maize starch required, and adding to the mass a 5% solution of gelatine in water. The product is granulated through a 16 mesh sieve, and the resultant granules are dried to constant weight at a temperature not exceeding 50°C. The dried granules are passed through a 20 mesh sieve and mixed with magnesium stearate and the remainder of the maize starch. The product is compressed at a 300 mg tablet weight on 3/8 inch flat bevelled edge divided punches.

Similar procedures may be followed with the compounds of Examples 2, 3 and 4.

Example 6 : Cardioprotective effect of *trans*-1,2-bis(4-methyl-3,5-dioxopiperazin-1-yl)cyclohexane against the toxicity of doxorubicin

The experiments were conducted as follows.

Animals

Mature male Sprague Dawley rats, 13-14 weeks old, weighing 400-500 gm were used. The animals were caged in groups of three and were kept in a constant environment. They were fed 41-B cubed diet and water *ad libitum*. The animal house was maintained in an alternating 12-hour wake-sleep cycle.

Drug administration

The cardioprotective activity of *trans*-1,2-bis(4-methyl-3,5-dioxopiperazin-1-yl)cyclohexane (hereinafter referred to as the compound) was studied in the rats. The compound was administered as a saline solution. The solution was prepared shortly before use and was administered at a constant injection volume of 1 ml per 100 gm body weight. Doxorubicin (Farmitalia, Milan, Italy) was dissolved in sterile water (2 mg/ml) before injection.

A group of 8 animals received a combined treatment of the compound and doxorubicin. The compound was administered to animals as a single intraperitoneal (i.p.) injection into the lower right quadrant of the abdomen to avoid damge to vital organs. An hour after the administration of the compound, the animals received a single intravenous (i.v.) injection of doxorubicin (4 mg/kg), via the femoral vein. A group of 10 animals received an i.p. injection of saline (1 ml/100 gm) followed by an i.v. injection of saline (0.2 ml/100 gm) one hour later. These rats acted as control animals. Nine animals received an i.p. injection of saline one hour before an i.v. administration of doxorubicin (4 mg/kg). To minimise the effects of circadian timing, doxorubicin administration was carried out between 13.30 h and 15.30 h. During intravenous drug administration and for subsequent cardiac output measurements, animals were anaesthetised with choral hydrate (300 mg/kg).

Assessment of toxicity

The protective activity of the compound against doxorubicin-induced cardiotoxicity in the rats was assessed at 8 weeks by measuring the cardiac output in animals receiving the treatment, using an external counting technique wherein a radioactive tracer, technetium ($^{99m}TcO_4$) was injected as a bolus into the femoral vein of an anaesthetised animal. The activity time curve over the heart was recorded at 0.1 second intervals for 40 seconds, using an NaI detector connected to a multi-channel analyzer (ND-62, Nuclear Data). ECG and heart rate were determined concomitantly with the cardiac output measurement, using a human ECG monitor (Hewlett Packard 7830A) coupled to a scope memory (model VK-12-2, Seltek Instrument Ltd.) and a chart recorder.

Gross post-mortem examinations were carried out on the animal that died during the study as well as those killed at the end of experiment (8 weeks).

In this study statistical differences between group means were analysed using Student's t-test.

Results

The results are summarised in the Table.

All animals showed a transient reduction in body weight in the first three weeks aftrer treatment. Animals receiving doxorubicin and pretreated with saline or the compound all showed a >10% reduction in body weight. Control animals, i.e. those receiving saline (i.p.) and saline (i.v.), showed a 3% reduction in body weight over this period; significantly less than animals receiving saline and doxorubicin ($p<0.001$).

Measurement in control animals at 8 weeks showed a mean cardiac output value of 222.4 ± 5.8 ml/min/kg and a mean heart rate of 441 ± 7 beats per minute. All control animals survived the course of study of 8 weeks. Animals receiving saline and doxorubicin showed a 60% reduction in cardiac output and a 35% reduction in heart rate. In this group of animals, one animal died just before 8 weeks. Information concerning this animal was loss due to severe post-mortem changes.

TABLE

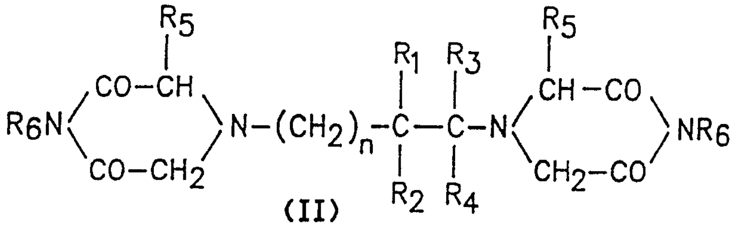
Treatment	Dose of Compound (II)	Relative Cardiac Output	Relative Heart Rate	Survival Rate	Incidence of CHF(1)
saline + saline	-	1.00±0.05	1.00±0.02	10/10	0/10
saline + doxorubicin	-	0.41±0.04	0.65±0.19	8/9(2)	0/8
Compound	100 mg/kg	0.53±0.05	0.85±0.02	8/8	0/8

(1) CHF = total incidence of congestive heart failure over the course of study of 8 weeks. Symptoms of congestive heart failure are defined by the appearance of general subcutaneous oedema, ascites and/or pleural effusion.

(2) Animal died just before 8 weeks. Information on this animal lost due to severe post-mortem changes.

CLAIMS

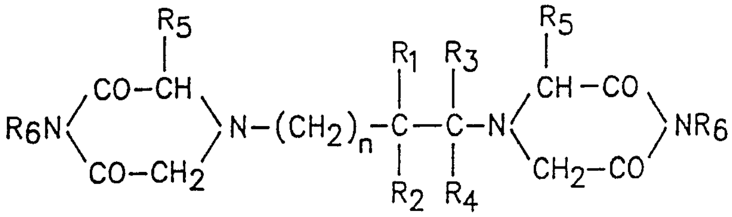
1. The use of a compound of formula (II):



(II)

wherein n is 0, 1 or 2, R_1, R_2, R_3 and R_4 are each separately selected from hydrogen, unsubstituted acyclic aliphatic hydrocarbon groups having a maximum of six carbon atoms and C_{1-6} alkyl groups substituted by a hydroxy group or by a C_{1-6} alkoxy group, or one of R_1 and R_2 and one of R_3 and R_4 is hydrogen and the others together form a trimethylene, tetramethylene or pentamethylene bridging group, R_5 is hydrogen or an unsubstituted acyclic aliphatic hydrocarbon group having a maximum of six carbon atoms, and R_6 is an acyclic aliphatic hydrocarbon group having a maximum of six carbon atoms or a group CH_2R_7 in which R_7 is a C_{1-5} alkyl group substituted by a hydroxy group or by a C_{1-6} alkoxy group, or a salt thereof formed with a physiologically acceptable inorganic or organic acid, for the manufacture of a medicament for use as a protective agent in (a) cardioprotection, (b) protection against the toxic effects of paracetamol or (c) protection against damage caused by free radicals.

2. The use of a compound of formula (II):



(II)

wherein n is 0, 1 or 2, R_1, R_2, R_3 and R_4 are each separately selected from hydrogen, unsubstituted acyclic aliphatic hydrocarbon groups having a maximum of six carbon atoms and C_{1-6} alkyl groups substituted by a hydroxy group or by a C_{1-6} alkoxy group, or one of R_1 and R_2 and one of R_3 and R_4 is hydrogen and the others together form a trimethylene, tetramethylene or pentamethylene bridging group, R_5 is hydrogen or an unsubstituted acyclic aliphatic hydrocarbon group having a maximum of six carbon atoms, and R_6 is an acyclic aliphatic hydrocarbon group having a maximum of six carbon atoms or a group CH_2R_7 in which R_7 is a C_{1-5} alkyl group substituted by a hydroxy group or by a C_{1-6} alkoxy group, or a salt thereof formed with a physiologically acceptable inorganic or organic acid, for the manufacture of a medicament for use as a cardioprotective agent.

3. The use according to Claim 1 or 2, in which R_1, R_2, R_3 and R_4 are each separately selected from hydrogen and C_{1-4} alkyl, C_{2-4} alkenyl and C_{2-4} alkynyl groups or one of R_1 and R_2 and one of R_3 and R_4 is hydrogen and the others are a trimethylene, tetramethylene or pentamethylene bridging group.

4. The use according to Claim 3, in which R_1, R_2, R_3 and R_4 are each separately selected from hydrogen, methyl, ethyl, n-propyl, isopropyl, allyl and propargyl or one of R_1 and R_2 and one of R_3 and R_4 is hydrogen and the others are tetramethylene.

5. The use according to any of Claims 1 to 4, in which n is 0.

6. The use according to Claim 1 or 2, in which n is 0, R_1 and R_2 are each hydrogen and R_3 and R_4 are each methyl, or n is 0, R_1 and R_3 are each hydrogen and (a) R_2 is hydrogen and R_4 is hydrogen, methyl, ethyl, n-propyl, isopropyl, allyl or propargyl or (b) R_2 is methyl or ethyl and R_4 is methyl, ethyl, n-propyl, isopropyl, allyl or propargyl.

7. The use according to Claim 1 or 2, in which $n = 0$, $R_1 = R_2 = H$, $R_3 = R_4 = CH_3$; $n = 0$, $R_1 = R_2 = R_3 = H$, $R_4 = CH_2OH$ or CH_2OCH_3;
$n = 0$, $R_1 = R_3 = H$, $R_2 + R_4 = CH_2CH_2CH_2CH_2$;
$n = 0$, $R_1 = R_2 = R_3 = H$, $R_4 = CH_3$ or C_2H_5; or
$n = 0$, $R_1 = R_2 = R_3 = R_4 = H$.

8. The use according to any of Claims 1 to 7, in which R_5 is hydrogen.

9. The use according to any of Claims 1 to 7, in which R_5 is methyl or ethyl.

10. The use according to any of Claims 1 to 9, in which R_6 is a C_{1-4} alkyl group, a group CH_2R_8 wherein R_8 is a C_2 or C_3 alkenyl or alkynyl group, or a group CH_2R_7 wherein R_7 is a C_1 or C_2 alkyl group substituted by a hydroxy, methoxy or ethoxy group.

11. The use according to Claim 10, in which R_6 is methyl, ethyl, n-propyl, isopropyl, 2-hydroxyethyl or 2-methoxyethyl.

12. The use according to Claim 1, in which $n = 0$, $R_1 = R_3 = H$, $R_2 = R_4 = CH_3$, $R_5 = H$, $R_6 = CH_3$, C_2H_5 or CH_2CH_2OH; $n = 0$, $R_1 = R_3 = H$, $R_2 + R_4 = CH_2CH_2CH_2CH_2$, $R_5 = H$, $R_6 = CH_3$, C_2H_5 or CH_2CH_2OH; $n = 0$, $R_1 = R_2 = R_3 = R_5 = H$, $R_4 = CH_3$ or C_2H_5, $R_6 = CH_3$, C_2H_5 or CH_2CH_2OH; or $n = 0$, $R_1 = R_2 = R_3 = R_4 = H$, $R_5 = H$, $R_6 = CH_3$, C_2H_5 or CH_2CH_2OH.

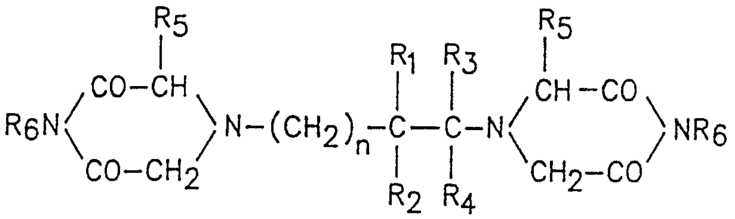
13. The use according to Claim 1 or 2, in which the compound (II) is 1,2-bis(4-methyl-3,5-dioxopiperazin-1-yl)ethane, 1,2-bis(4-methyl-3,5-dioxopiperazin-1-yl)propane, or 1,2-bis(4-methyl-3,5-dioxopiperazin-1-yl)butane.

14. The use according to Claim 1 or 2, in which the compound (II) is 1,2-bis(4-methyl-3,5-dioxopiperazin-1-yl)cyclohexane.

15. The use according to any of Claims 1 to 14, in which the compound is used in conjunction with an anthracycline drug.

16. The use according to Claim 15, in which the anthracycline drug is doxorubicin.

17. A pharmaceutical composition comprising a compound of formula (II):



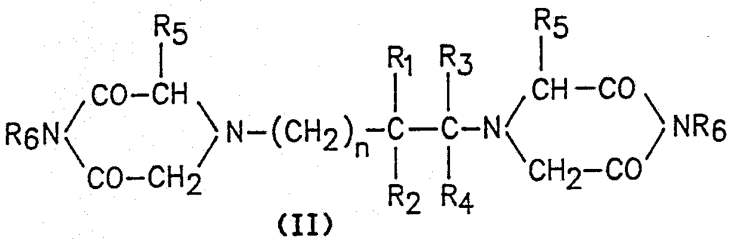
(II)

wherein n is 0, 1 or 2, R_1, R_2, R_3 and R_4 are each separately selected from hydrogen, unsubstituted acyclic aliphatic hydrocarbon groups having a maximum of six carbon atoms and C_{1-6} alkyl groups substituted by a hydroxy group or by a C_{1-6} alkoxy group, or one of R_1 and R_2 and one of R_3 and R_4 is hydrogen and the others together form a trimethylene, tetramethylene or pentamethylene bridging group, R_5 is hydrogen or an unsubstituted acyclic aliphatic hydrocarbon group having a maximum of six carbon atoms, and R_6 is an acyclic aliphatic hydrocarbon group having a maximum of six carbon atoms or a group CH_2R_7 in which R_7 is a C_{1-5} alkyl group substituted by a hydroxy group or by a C_{1-6} alkoxy group, or a salt thereof formed with a physiologically acceptable inorganic or organic acid, and an anthracycline drug, together with a physiologically acceptable diluent or carrier.

18. A pharmaceutical composition according to Claim 17, in which the anthracycline drug is doxorubicin.

19. A pharmaceutical composition according to Claim 17 or 18 in a form suitable for parenteral administration.

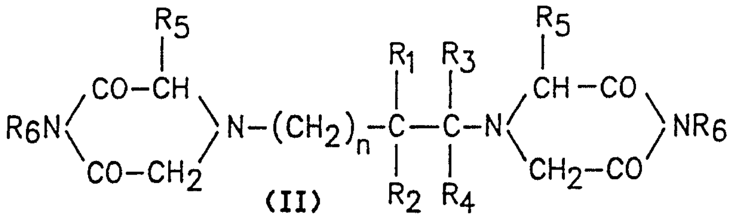
20. The use of a compound of formula (II):



(II)

wherein n is 0, 1 or 2, R_1, R_2, R_3 and R_4 are each separately selected from hydrogen, unsubstituted acyclic aliphatic hydrocarbon groups having a maximum of six carbon atoms and C_{1-6} alkyl groups substituted by a hydroxy group or by a C_{1-6} alkoxy group, or one of R_1 and R_2 and one of R_3 and R_4 is hydrogen and the others together form a trimethylene, tetramethylene or pentamethylene bridging group, R_5 is hydrogen or an unsubstituted acyclic aliphatic hydrocarbon group having a maximum of six carbon atoms, and R_6 is an acyclic aliphatic hydrocarbon group having a maximum of six carbon atoms or a group CH_2R_7 in which R_7 is a C_{1-5} alkyl group substituted by a hydroxy group or by a C_{1-6} alkoxy group, or a salt thereof formed with a physiologically acceptable inorganic or organic acid, for the manufacture of a medicament for providing protection against the toxic effects of paracetamol.

21. The use of a compound of formula (II):



(II)

wherein n is 0, 1 or 2, R_1, R_2, R_3 and R_4 are each separately selected from hydrogen, unsubstituted acyclic aliphatic hydrocarbon groups having a maximum of six carbon atoms and C_{1-6} alkyl groups substituted by a hydroxy group or by a C_{1-6} alkoxy group, or one of R_1 and R_2 and one of R_3 and R_4 is hydrogen and the others together form a trimethylene, tetramethylene or pentamethylene bridging group, R_5 is hydrogen or an unsubstituted acyclic aliphatic hydrocarbon group having a maximum of six carbon atoms, and R_6 is an acyclic aliphatic hydrocarbon group having a maximum of six carbon atoms or a group CH_2R_7 in which R_7 is a C_{1-5} alkyl group substituted by a hydroxy group or by a C_{1-6} alkoxy group, or a salt thereof formed with a physiologically acceptable inorganic or organic acid, for the manufacture of a medicament for providing protection against damage caused by free radicals.

22. The use according to Claim 20 or 21, in which the compound of formula (II) is as defined in any of Claims 3 to 13.

23. A method for the treatment of a patient in need of cardioprotection or in need of protection against the toxic effects of paracetamol or against damage caused by free radicals which comprises administering to said patient a therapeutically effective amount of a compound of formula (II) as defined in any of Claims 2 to 13.