| (51) International Patent Classification 6: | A61K 31/44, 47/18 |
| (11) International Publication Number: | WO 99/18959 |
| (43) International Publication Date: | 22 April 1999 (22.04.99) |
| (21) International Application Number: | PCT/US98/21972 |
| (22) International Filing Date: | 14 September 1998 (14.09.98) |
| (30) Priority Data: | 60/062,089 14 October 1997 (14.10.97) US |
| (54) Title: | PHARMACEUTICAL FORMULATION COMPRISING GLYCINE AS A STABILIZER |
| (57) Abstract: | The present invention provides pharmaceutical formulations suitable for intravenous injection comprising a lyophilized anti-ulcerative agent reconstituted in isotonic solutions suitable for intravenous administration, such as 5 % dextrose or 0.9 % sodium chloride. The solutions are brought to a pH of between about 9 and about 12, preferably between about pH 10 and 11, by a glycine–sodium hydroxide buffer. Such formulations are chemically and physically stable, and do not significantly change color, for at least between about 6 and about 12 hours at room temperature, and are stable to color change for from between about 24 and 48 hours if kept at 5 °C. |
# FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

<table>
<thead>
<tr>
<th>AL</th>
<th>Albania</th>
<th>ES</th>
<th>Spain</th>
<th>LS</th>
<th>Lesotho</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM</td>
<td>Armenia</td>
<td>FI</td>
<td>Finland</td>
<td>LT</td>
<td>Lithuania</td>
</tr>
<tr>
<td>AT</td>
<td>Austria</td>
<td>FR</td>
<td>France</td>
<td>LU</td>
<td>Luxembourg</td>
</tr>
<tr>
<td>AU</td>
<td>Australia</td>
<td>GA</td>
<td>Gabon</td>
<td>LV</td>
<td>Latvia</td>
</tr>
<tr>
<td>AZ</td>
<td>Azerbaijan</td>
<td>GB</td>
<td>United Kingdom</td>
<td>MC</td>
<td>Monaco</td>
</tr>
<tr>
<td>BA</td>
<td>Bosnia and Herzegovina</td>
<td>GE</td>
<td>Georgia</td>
<td>MD</td>
<td>Republic of Moldova</td>
</tr>
<tr>
<td>BB</td>
<td>Barbados</td>
<td>GH</td>
<td>Ghana</td>
<td>MG</td>
<td>Madagascar</td>
</tr>
<tr>
<td>BE</td>
<td>Belgium</td>
<td>GN</td>
<td>Guinea</td>
<td>MK</td>
<td>The former Yugoslav Republic of Macedonia</td>
</tr>
<tr>
<td>BF</td>
<td>Burkina Faso</td>
<td>GR</td>
<td>Greece</td>
<td>ML</td>
<td>Mali</td>
</tr>
<tr>
<td>BG</td>
<td>Bulgaria</td>
<td>HU</td>
<td>Hungary</td>
<td>MN</td>
<td>Mongolia</td>
</tr>
<tr>
<td>BJ</td>
<td>Benin</td>
<td>IE</td>
<td>Ireland</td>
<td>MR</td>
<td>Mauritania</td>
</tr>
<tr>
<td>BR</td>
<td>Brazil</td>
<td>IL</td>
<td>Israel</td>
<td>MW</td>
<td>Malawi</td>
</tr>
<tr>
<td>BY</td>
<td>Belarus</td>
<td>IS</td>
<td>Iceland</td>
<td>MX</td>
<td>Mexico</td>
</tr>
<tr>
<td>CA</td>
<td>Canada</td>
<td>IT</td>
<td>Italy</td>
<td>NR</td>
<td>Niger</td>
</tr>
<tr>
<td>CF</td>
<td>Central African Republic</td>
<td>JP</td>
<td>Japan</td>
<td>NL</td>
<td>Netherlands</td>
</tr>
<tr>
<td>CG</td>
<td>Congo</td>
<td>KE</td>
<td>Kenya</td>
<td>NO</td>
<td>Norway</td>
</tr>
<tr>
<td>CH</td>
<td>Switzerland</td>
<td>KG</td>
<td>Kyrgyzstan</td>
<td>NZ</td>
<td>New Zealand</td>
</tr>
<tr>
<td>CI</td>
<td>Côte d’Ivoire</td>
<td>KP</td>
<td>Democratic People’s Republic of Korea</td>
<td>PL</td>
<td>Poland</td>
</tr>
<tr>
<td>CM</td>
<td>Cameroon</td>
<td>KR</td>
<td>Republic of Korea</td>
<td>PT</td>
<td>Portugal</td>
</tr>
<tr>
<td>CN</td>
<td>China</td>
<td>KZ</td>
<td>Kazakhstan</td>
<td>RO</td>
<td>Romania</td>
</tr>
<tr>
<td>CU</td>
<td>Cuba</td>
<td>LC</td>
<td>Saint Lucia</td>
<td>RU</td>
<td>Russian Federation</td>
</tr>
<tr>
<td>CZ</td>
<td>Czech Republic</td>
<td>LI</td>
<td>Liechtenstein</td>
<td>SD</td>
<td>Sudan</td>
</tr>
<tr>
<td>DE</td>
<td>Germany</td>
<td>LK</td>
<td>Sri Lanka</td>
<td>SE</td>
<td>Sweden</td>
</tr>
<tr>
<td>DK</td>
<td>Denemark</td>
<td>LR</td>
<td>Liberia</td>
<td>SG</td>
<td>Singapore</td>
</tr>
<tr>
<td>EE</td>
<td>Estonia</td>
<td>SI</td>
<td>Slovenia</td>
<td>SK</td>
<td>Slovakia</td>
</tr>
<tr>
<td>SN</td>
<td>Senegal</td>
<td>SZ</td>
<td>Swaziland</td>
<td>TD</td>
<td>Chad</td>
</tr>
<tr>
<td>TG</td>
<td>Togo</td>
<td>TR</td>
<td>Turkey</td>
<td>TT</td>
<td>Trinidad and Tobago</td>
</tr>
<tr>
<td>TJ</td>
<td>Tajikistan</td>
<td>UG</td>
<td>Uganda</td>
<td>US</td>
<td>United States of America</td>
</tr>
<tr>
<td>TM</td>
<td>Turkmenistan</td>
<td>VN</td>
<td>Viet Nam</td>
<td>UZ</td>
<td>Uzbekistan</td>
</tr>
<tr>
<td>UA</td>
<td>Ukraine</td>
<td>YU</td>
<td>Yugoslavia</td>
<td>ZW</td>
<td>Zimbabwe</td>
</tr>
</tbody>
</table>
PHARMACEUTICAL FORMULATION COMPRISING GLYCINE AS A STABILIZER

FIELD OF THE INVENTION

The present invention relates to the preparation of pharmaceutical formulations with anti-ulcerative properties, and in particular, formulations that are reconstituted for intravenous administration.

BACKGROUND OF THE INVENTION

Souda et al., U.S. Patent No. 5,045,552, incorporated by reference herein, describes compounds that inhibit an H⁺/K⁺-ATPase present in the stomach. These compounds are useful for treatment of peptic ulcers and other disorders associated with secretion of gastric acid, such as heartburn and gastroesophageal reflux. For example, one such compound has the following structure:

![Chemical Structure](image)
and includes pharmaceutically acceptable salts of the compound. This compound is referred to herein as Compound 1.

It is desirable when preparing reconstituted solutions of such anti-ulcerative compounds that are suitable for intravenous administration, that the solubilized compounds exhibit physical and chemical stability for at least between about 6 and about 12 hours at room temperature. It has been found by the present inventors that anti-ulcerative compounds such as Compound 1 and the compounds described by general formula I below discolor when they are reconstituted, i.e., dissolved, in aqueous solutions, particularly in solutions suitable for intravenous administration, e.g., 5% dextrose or 0.9% saline. Such solutions quickly turn yellow to yellow-brown.

The compounds of the present invention have been determined to be more potent H⁺/K⁺-ATPase inhibitors than omeprazole sodium. However, in order to provide clinically useful pharmaceutical formulations of the compounds disclosed herein for intravenous administration, it is first necessary to provide formulations for lyophilization and intravenous administration that do not degrade physically, chemically and/or demonstrate a change in color.

**BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 is a graph showing the changes in absorption spectrum of compound 1 at a concentration of 4 mg/ml in 0.9 % saline at pH 10 as a function of time after dissolution, with storage at room temperature (25 °C) in the dark.
Figure 2 is a graph showing the changes in absorption spectrum of compound 1 at a concentration of 4 mg/ml in 0.9 % saline/50 mM glycine-NaOH buffer at pH 10 as a function of time after dissolution, with storage at room temperature (25 °C) in the dark.

Figure 3 is a graph showing the change in the absorption spectrum of compound 1, at a concentration of 4 mg/ml, in a solution which contain 5, 10, 25, and 50 mM glycine-NaOH buffer, indicating color change.

Figure 4 is a graph showing the change in the absorbance at 400, 450, 500, 550, 600, and 600 nm of compound 1, at a concentration of 2 mg/ml in 0.9% saline, at room temperature (25 °C) in the light, as a function of time.

Figure 5 is a graph showing the change in the absorbance at 400, 450, 500, 550, 600, and 600 nm of compound 1, at a concentration of 2 mg/ml in 0.9% saline, at room temperature (25 °C) in the dark, as a function of time.

Figure 6 is a graph showing the change in the absorbance at 400, 450, 500, 550, 600, and 600 nm of compound 1, at a concentration of 2 mg/ml in 0.9% saline, at 10 °C in the dark, as a function of time.

Figure 7 is a graph showing the change in the absorbance at 400, 450, 500, 550, 600, and 600 nm of compound 1, at a concentration of 2 mg/ml in 0.9% saline and 10 mM glycine-NaOH buffer, at room temperature (25 °C) in the light, as a function of time.

Figure 8 is a graph showing the change in the absorbance at 400, 450, 500, 550, 600, and 600 nm of compound 1, at a concentration of 2 mg/ml in 0.9% saline and 10 mM glycine-NaOH buffer, at room temperature (25 °C) in the dark, as a function of time.
Figure 9 is a graph showing the change in the absorbance at 400, 450, 500, 550, 600, and 600 nm of compound 1, at a concentration of 2 mg/ml in 0.9% saline and 10 mM glycine-NaOH buffer, at 10 °C in the dark, as a function of time.

**DETAILED DESCRIPTION OF THE INVENTION**

All patents, patent applications, and publications cited in this application are incorporated by reference in their entirety. In the case of a conflict of disclosure, the present specification is controlling.

It has now been surprisingly and unexpectedly discovered that if lyophilized compounds of general formula I below are reconstituted in isotonic solutions suitable for intravenous administration, such as 5% dextrose or 0.9% sodium chloride, that have been brought to a pH of between about 9 and about 12, preferably between about pH 10 and 11, by a glycine-sodium hydroxide buffer, such formulations are chemically and physically stable, and do not significantly change color, for at least between about 6 and about 12 hours at room temperature. It was also discovered that the compounds dissolved in such isotonic solutions are stable to color change for from between about 24 and 48 hours if kept at 5 °C. It has also been discovered that the use of glycine buffers with a pH of between about 9 and about 12, preferably between about pH 10 and 11, is beneficial in preparing lyophilized samples of the compounds of the invention.

Thus, the present invention provides pharmaceutical formulations suitable for intravenous injection comprising an anti-ulcerative agent having the following general formula:
where $R^1$ and $R^2$ are, independently, hydrogen, lower alkyl, lower alkoxy, halogenated lower alkyl, lower alkoxy carbonyl or carboxyl group or a halogen atom;

$$X \text{ is } O, S \text{ or } \begin{array}{c} \text{--N--} \\ \text{R}^3 \end{array} \quad \text{(in which } R^3 \text{ stands for a hydrogen atom or a lower alkyl, phenyl, benzyl or lower alkoxy carbonyl group); and}$$

$Z$ is selected from:

- (1) a group of the formula:

$$-O(CH_2)_p-O-R^4$$

where $p$ is an integer of 1 to 3 and $R^4$ is a hydrogen atom or a lower alkyl, aryl or aralkyl group;

- (2) a group of the general formula:
\[ -O(CH_2)_q - R^5 \]

where \( q \) is an integer of 1 to 3 and \( R^5 \) is a halogen atom or an alkoxy carbonyl, aryl or heteroaryl group;

(3) a group of the general formula:

\[ -O(CH_2)_r - O(CH_2)_s - R^6 \]

where \( r \) and \( s \) each independently are an integer of 1 to 5 and \( R^6 \) is a hydrogen atom or a lower alkyl group;

(4) a group of the formula:
(5) a group of the formula: 

\[ \text{O} \]

(6) a group of the formula: 

\[ \text{N} \]

(7) a group of the general formula: 

\[ \text{H} \]

\[ \text{S} \]

\[ \text{t} \]

\[ \text{A} \]

where \( t \) is an integer of 0 to 2 and \( A \) is a lower alkyl, alkoxy carbonylmethyl, pyridyl or furyl group, or a group of the general formula: 

\[ \text{N} \]

\[ \text{P} \]
where R is selected from the group consisting of: \(-\text{NH}-\), \(-\text{O}-\) or \(-\text{S}-\); or a group of the general formula:

\[
-(\text{CH}_2)_w-
\]

wherein \(R^7\) is hydrogen or lower alkyl and \(w\) is from 0 to 3;

(8) a group of the general formula:

\[
\begin{array}{c}
\text{R}^8 \\
N-(\text{CH}_2) \\
\text{R}^8
\end{array}
\]

where \(R^8\) is an acetoxy or lower alkyl group; and

(9) a group of the general formula: \(-\text{OR}^9\)

where \(R^9\) is a hydrogen atom or a lower alkyl or aryl group;

\(n\) is an integer of 0 to 2; \(m\) is an integer of 2 to 10, and

\(J\) and \(K\) are independently hydrogen or lower alkyl, with the proviso that when
Z is a group falling under the above category (9), R^9 is a lower alkyl group and m stands for an integer of 3 to 10, and pharmaceutically acceptable salts thereof.

The pharmaceutical formulations also contain a glycine-sodium hydroxide buffer system, and an agent that imparts tonicity to the formulation (a “tonicity agent”). Such agents are well-known in the art, and include sodium chloride, dextrose, mannitol, glycerin, sucrose and lactose. Isotonic solutions posses the same osmotic pressure as blood plasma, and so can be intravenously infused into a subject without changing the osmotic pressure of the subject’s blood plasma.

The definitions for R^1, R^2, X, n, J, K, Z and m are used consistently throughout the specification that follows and in the appended claims.

In the definition of the compounds of general formula (I), the lower alkyl group defined above with respect to R^1, R^2, R^3, R^4, R^5, A, R^7, R^8, J, and K in compound (I) of the present invention may be straight-chain or branched alkyl groups having 1 to 6 carbon atoms. Examples include methyl, ethyl, n-propyl, n-butyl, isopropyl, isobutyl, 1-methylpropyl, tert-butyl, n-pentyl, 1-ethylpropyl, isoamyl, n-hexyl groups, and the like, among which methyl and ethyl groups are most preferred.

The lower alkoxy group and the lower alkoxy moiety of the lower alkoxy carbonyl group defined above with respect to R^1 and R^2 may be an alkoxy group derived from the above-defined and exemplified lower alkyl group. Methoxy and ethoxy groups are most preferred alkoxy groups.

The halogen atom defined above includes chlorine, bromine, iodine or fluorine. The aryl group defined above with respect to R^4 and R^5 may be, e.g., phenyl, tolyl, xylyl, naphthyl or the like which may be substituted with a lower alkoxy or hydroxyl group,
a halogen atom or the like.

Examples of the arylalkyl defined above with respect to R<sup>4</sup> include benzyl and phenethyl groups.

Examples of the heteroaryl group defined above with respect to R<sup>5</sup> include pyridyl, furyl, and thienyl groups.

In the definition of Z in general formula (I), groups (1), (2), (3), (4), (5) and (9) are preferred; group (9) is the most preferred. R<sup>1</sup> and R<sup>2</sup> are preferably both hydrogen; another preferred configuration of R<sup>1</sup> and R<sup>2</sup> is when R<sup>1</sup> is lower alkyl, e.g., methyl, and R<sup>2</sup> is hydrogen. X is preferably –NR<sup>3</sup> where R<sup>3</sup> is hydrogen. A preferred value for n is 1. The preferred substituents for J and K are both hydrogen or, where J is lower alkyl, e.g. methyl, K is preferably hydrogen, and when J is hydrogen K is preferably lower alkyl, e.g. methyl.

Thus, J or K are independently preferably hydrogen or methyl, most preferably J is methyl and K is hydrogen.

A first preferred class of compounds included in the pharmaceutical formulations of the present invention fall within the compounds of general formula (I) are represented by the following formula (A):

![Formula (A)]
where \( R^1 \) and \( R^2 \) are independently selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, halogenated lower alkyl, lower alkoxy carbonyl, a carboxyl group, and halogen; \( R^9 \) is selected from the group consisting of hydrogen, lower alkyl, and aryl; \( J \) is selected from the group consisting of hydrogen or lower alkyl; \( m \) is an integer from 2 to 10; and pharmaceutically acceptable salts thereof. In formula A, it is preferred that \( R^1 \) and \( R^2 \) are both hydrogen; also preferred is when \( R^1 \) is 5-lower alkoxy, 5-lower alkyl or 5-halogenated lower alkyl and \( R^2 \) is hydrogen. Preferred substituents at \( J \) are hydrogen or methyl; preferred values of \( m \) are from 3 to 10, the most preferred being 3; and the preferred \( R^9 \) substituents are lower alkyl or aryl. Most preferred at \( R^9 \) is methyl.

In one group of preferred compounds of formula A, \( R^1 \) and \( R^2 \) are both hydrogen, \( J \) is methyl, \( m \) is 3 and \( R^9 \) is methyl.

In a second group of preferred compounds falling within formula A, \( R^1 \) and \( R^2 \) are both hydrogen, \( J \) is hydrogen, \( m \) is 3 and \( R^9 \) is methyl.

In a third group of preferred compounds falling within formula A, \( R^1 \) and \( R^2 \) are both hydrogen, \( J \) is methyl, \( m \) is 2 and \( R^9 \) is benzyl.

A second class of compounds falling within general formula (I) for inclusion in the pharmaceutical formulations of the present invention are represented by formula (B), as follows:
where \( R^1 \) and \( R^2 \) are independently selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, halogenated lower alkyl, lower alkoxy carbonyl, a carboxyl group, and halogen; \( R^4 \) is selected from the group consisting of hydrogen, lower alkyl, aryl, and aralkyl; \( J \) is selected from the group consisting of hydrogen or lower alkyl; \( m \) is an integer from 2 to 10; \( p \) is an integer from 1 to 3; and pharmaceutically acceptable salts thereof.

In compounds of formula (B), the preferred substituents for \( R^1 \) and \( R^2 \) are both hydrogen; also preferred are compounds where \( R^1 \) is 5-lower alkoxy, 5-lower alkyl or 5-halogenated lower alkyl and \( R^2 \) is hydrogen. Preferred values of \( m \) are 2 or 3; preferred values of \( p \) are 2 or 3; and the preferred substituents at \( R^4 \) are methyl or benzyl. Most preferred are compounds of formula (B) where \( R^1 \) is 5-methyl, \( R^2 \) is hydrogen, \( J \) is methyl, \( m \) is 2, \( p \) is 2 and \( R^4 \) is methyl.

Examples of the pharmaceutically acceptable salts include salts of inorganic
acids, such as hydrochloride, hydrobromide, sulfate and phosphate; those with organic acids, such as acetate, maleate, tartrate, methanesulfonate, benzenesulfonate, and toluenesulfonate; and those with amino acids such as arginine, aspartic acid and glutamic acid.

Some of the compounds according to the present invention can form a salt with a metal such as Na, K, Ca or Mg. These metal salts are also included among the pharmaceutically acceptable salts of the present invention. For example, compounds represented by the general formula (I), wherein $X$ is a group of \[ \text{---}N--- \] and $R^3$ is a hydrogen atom, or compounds represented by the general formula (I), where $Z$ is a group of category (7) and $B$ is an NH group, can be present as a metal salt.

The compounds of the present invention also can take the form of hydrates, prodrugs, or stereoisomers. It will be appreciated by those of ordinary skill in the art that variations and obvious modifications can be made to the presently claimed invention, said variations and modifications being within the scope of the claimed invention.

Methods for the preparation of the compounds of the stabilized formulations of the invention are disclosed in Souda et al., U.S. Patent 5,045,552.

The present invention also provides methods for the stabilization of compounds of general formula I above, both in the course of preparing lyophilized samples for reconstitution, and in reconstituted formulations suitable for intravenous administration. Prior to the present invention, the utility of glycine as a color stabilizer for solutions of the compounds of the invention was not known in the art, either in the context of preparing solutions for lyophilization, or for preparing solutions for intravenous administration.
To prepare lyophilized samples for reconstitution, a desired quantity of a compound of the invention is dissolved in a sufficient amount of an aqueous solution (i.e., an amount of solution in which the compound will completely dissolve) which also comprises a glycine-sodium hydroxide buffer such that the pH of the solution is between about 9 and 12, preferably between about pH 10 and about 11. The concentration of glycine in the solution is between about 1 and 300 mM, preferably between about 10 and about 150 mM. The concentration of compound in such solutions is generally from between about 1 mg/ml and 50 mg/ml. The solution is then lyophilized in a sealable container, such as a vial, and the container is sealed such that exchange of air between the inside of the sealable container and the external environment of the container is not possible. The container will typically contain between about 1 and 100 mg of compound, preferably between about 20 and 60 mg of compound, and most preferably about 40 mg of compound.

According to the present invention, reconstituted solutions for intravenous administration can be prepared by initially dissolving an amount of a desired lyophilized compound (plus any other solutes, such as glycine-NaOH buffer, which were lyophilized with the compound) in a sufficient amount of a sterile, aqueous solution to completely dissolve the lyophilized compound. Such initially dissolved solutions contain the original glycine-NaOH buffer system, substantially undiluted, and have a pH of from between about 10 and about 11.5. Under these conditions, as determined by the present inventors, the anti-ulcerative compounds of the invention are chemically and physically stable.

In order to deliver the compounds of the present invention intravenously, they may be dissolved in sterile solutions suitable for intravenous administration, such as normal saline (0.9% saline) or 5% dextrose. Such solutions typically have a pH of between about 4
and about 5, respectively. When the residual glycine-NaOH buffer system is diluted in the solution suitable for intravenous administration, for example a 50-fold dilution of 2 ml of a 20 mg/ml initial solution of anti-ulcerative compound, the pH of the resulting solution falls below the pH 9 to 12 range in which the anti-ulcerative compounds are most stable. Thus, according to the present invention, additional glycine-NaOH can be added to or included in the ultimate solution to be intravenously administered. The concentration of glycine-NaOH buffer in the final solution for intravenous administration should be between about 1 mM and 300 mM, preferably between about 10 mM and 150 mM, more preferably between 10 and 50 mM and most preferably between about 10 mM and 25 mM. The pH of the resulting solution should be alkaline, preferably between about pH 9 and 12, most preferably between pH 10 and 11.

The present invention is illustrated by the following examples, which are intended merely to illustrate the invention and not to limit its scope.

**EXAMPLE 1: pH Studies**

The chemical and physical stability of compound 1 at 8 mg/ml in a water for injection (WFI), adjusted with dilute (6 N) NaOH to pH 9.5, 10, 11, and 11.5, was evaluated at room temperature, 5 °C, and -20 °C. Chemical stability was monitored by evaluating the residual potency and impurity levels over 48 hours by HPLC. Physical stability was evaluated by measuring the rate of color formation at 405 nm and by visual observations.

The order of chemical and physical stability is pH 11.5 > pH 11 > pH 10.5 > pH 10 > pH 9.5 at 5 °C and room temperature. That is, chemical and physical stability of compound 1 is highest at pH 11.5, and decreases with pH; this effect is found at room
temperature and at reduced temperatures. Solutions at pH 9.5 began to assume a yellow color within 30 minutes; the color intensified rapidly. At room temperature, solutions at pH 10.5 were marginally stable at 24 hours with regard to chemical and physical stability; however, at cold temperatures (5°C), pH 10.5 was found to be adequate for 24 hours stability.

At pH 11 or greater and in cold temperatures, solutions of compound 1 appear to be adequately stable for the manufacture and handling in preparation for freeze drying. It was concluded that pH levels below 10.5 should be avoided.

**EXAMPLE 2: Preliminary Buffer Evaluation**

It is desirable that the pH of solutions of compound 1 and other compounds of the invention in 5% dextrose or normal saline remain in a range near about pH 10 to provide for an acceptable use period in a clinical setting. Phosphate and glycine buffer systems were tested. Phosphate was found to be an effective buffer in the desired pH range, but, as indicated below, precipitated during freeze-drying of samples containing it; glycine-NaOH was an effective buffer and had a stabilizing effect on color change and may affect turbidity when evaluated with compound 1.

Solutions of compound 1 in 50 mM phosphate buffer behaved similarly with regard to color formation as unbuffered compound 1 solutions (i.e., color formation was not inhibited). In 100 mM glycine/NaOH at pH values above 10, discoloration was substantially slower. Freeze-drying of compound 1 solutions in phosphate and glycine buffers yielded white, well-formed plugs. Reconstitution of the phosphate-containing plugs produced hazy solutions, i.e., precipitation. Based on this propensity to precipitate, phosphate was disqualified as a buffer.
for the compounds of the invention.

EXAMPLE 3: Glycine Concentration and Temperature Studies

Compound 1 at 8 mg/ml in glycine at 0 mM, 100 mM, and 150 mM were evaluated at pH 10.5 to 11 at room temperature, 5 °C, and -20 °C. Chemical stability was monitored by measuring the residual potency and impurity levels over 48 hours. Physical stability was evaluated by measuring the rate of color formation at 405 nm and by visual observations. The results for color formation are shown in Tables 1, 2, and 3, below. A, B, and C contain 7.5 mg/ml glycine, equal to 100 mM glycine. D and E have 11.25 mg/ml glycine, equal to 150 mM glycine. F is the control without glycine. The pH of the solution is indicated in parentheses; the values in the tables are the absorbance at 405 nm.

**TABLE 1: COLOR INFORMATION**
**ROOM TEMPERATURE (25 °C) SAMPLES**
**(ABSORBANCE AT 405 nm)**

<table>
<thead>
<tr>
<th></th>
<th>A(11.0)</th>
<th>B(10.76)</th>
<th>C(10.5)</th>
<th>D(11.0)</th>
<th>E(10.5)</th>
<th>F(10.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hours</td>
<td>0.009</td>
<td>0.010</td>
<td>0.011</td>
<td>0.008</td>
<td>0.011</td>
<td>0.012</td>
</tr>
<tr>
<td>6 hours</td>
<td>0.034</td>
<td>0.048</td>
<td>0.066</td>
<td>0.032</td>
<td>0.056</td>
<td>0.188</td>
</tr>
<tr>
<td>12 hours</td>
<td>0.053</td>
<td>0.076</td>
<td>0.107</td>
<td>0.047</td>
<td>0.089</td>
<td>0.349</td>
</tr>
<tr>
<td>24 hours</td>
<td>0.101</td>
<td>0.145</td>
<td>0.200</td>
<td>0.091</td>
<td>0.162</td>
<td>0.838</td>
</tr>
<tr>
<td>48 hours</td>
<td>0.163</td>
<td>0.245</td>
<td>0.333</td>
<td>0.152</td>
<td>0.269</td>
<td>2.396</td>
</tr>
</tbody>
</table>

**TABLE 2: REFRIGERATED SAMPLES (5 °C)**

<table>
<thead>
<tr>
<th></th>
<th>A(11.0)</th>
<th>B(10.76)</th>
<th>C(10.5)</th>
<th>D(11.0)</th>
<th>E(10.5)</th>
<th>F(10.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hours</td>
<td>0.009</td>
<td>0.010</td>
<td>0.011</td>
<td>0.008</td>
<td>0.011</td>
<td>0.012</td>
</tr>
<tr>
<td>12 hours</td>
<td>0.015</td>
<td>0.016</td>
<td>0.020</td>
<td>0.012</td>
<td>0.017</td>
<td>0.052</td>
</tr>
</tbody>
</table>
TABLE 3: FROZEN SAMPLES (-20 °C)

<table>
<thead>
<tr>
<th></th>
<th>A(11.0)</th>
<th>B(10.76)</th>
<th>C(10.5)</th>
<th>D(11.0)</th>
<th>E(10.5)</th>
<th>F(10.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>0.009</td>
<td>0.010</td>
<td>0.011</td>
<td>0.008</td>
<td>0.011</td>
<td>0.012</td>
</tr>
<tr>
<td>24 hours</td>
<td>0.011</td>
<td>0.012</td>
<td>0.0175</td>
<td>0.010</td>
<td>0.012</td>
<td>0.022</td>
</tr>
<tr>
<td>48 hours</td>
<td>0.010</td>
<td>0.013</td>
<td>0.015</td>
<td>0.010</td>
<td>0.014</td>
<td>0.027</td>
</tr>
</tbody>
</table>

No substantial difference in chemical stability was noted between 0 mM, 100 mM, and 150 mM glycine formulations. Solutions with greater glycine concentrations discolored more slowly. Solutions devoid of glycine discolored very quickly regardless of temperature conditions. At 5 °C, pH 10.5 to 11 solutions can be held for 24 hours without measurable increases in impurity levels. At room temperature, there is a <0.5% increase in impurities for the pH 11 solution, but at pH 10.5, >1 % impurities were measured at 24 hours. Color formation at 5 °C is significantly retarded compared to room temperature. Cold temperatures, i.e., those at or near 5 °C, are also preferred for the manufacture of compound 1 and the other compounds of the invention.

EXAMPLE 4: Reduced Glycine Concentration Experiments

The color change in a 4 mg/ml solution of compound 1 in 0.9% saline at pH 10, with and without 50 mM glycine-NaOH buffer, was evaluated by measurement of absorption at 405 nm as a function of time. 200 mg of compound 1 was dissolved in 50 ml of 0.9% saline, and was stored at room temperature, i.e., 25 °C, in the dark. Absorption
measurements were taken at the zero time point, and at 2, 4, 6, and 8 hours after dissolution. As can be seen from Figures 1 and 2, compound 1 discolored at a much greater rate in the glycine-free solution than in the solution that contained 50 mM glycine.

The glycine concentration-dependence of compound 1 discoloration was evaluated at 5 hours after dissolution. Compound 1 was dissolved at concentration of 4 mg/ml in 0.9% saline solution at pH 10 containing 5, 10, 25, and 50 mM glycine-NaOH buffer. As can be seen from Figure 3, at 5 hours post-dissolution, there was little difference in absorbance spectrum between the solutions, although there was a noticeably higher absorbance for the 5 mM glycine-NaOH containing solution.

EXAMPLE 5: Effect of Storage Conditions

The effect of exposure to light and temperature was evaluated as a function of time for 0.9% saline solutions containing 2 mg/ml compound 1, with or without 10 mM glycine-NaOH buffer, was evaluated by monitoring absorbance at 400, 450, 500, 600, and 650 nm. As can be seen from Figures 4 to 6, in solutions without glycine-NaOH buffer, increasing storage temperatures caused an increase in undesirable color development. The experiments also reveal that exposure to light has no detrimental effect on color development in solutions containing compound 1. These results are also found with solutions of compound 1 that do contain 10 mM glycine-NaOH buffer. However, as can be seen from Figures 7 to 9, the presence of glycine-NaOH buffer decreases absorption at all wavelengths, temperatures, and lighting conditions, i.e., glycine-NaOH buffer reduces color development in solutions of compound 1.
IN THE CLAIMS:

1. An aqueous pharmaceutical formulation suitable for intravenous injection comprising:

   an anti-ulcerative compound having the following formula:

   \[
   \begin{align*}
   &R^1, R^2, X, J, K, O \quad (O)n \quad S \quad CH_2 \\
   &-O-(CH_2)_{m} - Z
   \end{align*}
   \]

   wherein \( R^1 \) and \( R^2 \) are independently selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, halogenated lower alkyl, lower alkoxy carbonyl, a carboxyl group, and halogen;

   \( X \) is a member selected from the group consisting of \(-O-, -S-\) or

   \[
   \begin{align*}
   &N \quad R^3
   \end{align*}
   \]

   where \( R^3 \) is a member selected from the group consisting of hydrogen, lower alkyl, phenyl, benzyl, and lower alkoxy carbonyl; and

   \( Z \) is selected from the group consisting of:

   (1) a group of the formula:

   \[
   -O(CH_2)_p - O - R^4
   \]

   where \( p \) is an integer of 1 to 3 and \( R^4 \) is a hydrogen atom or a lower alkyl, aryl or aralkyl group;

   (2) a group of the general formula:
where \( q \) is an integer of 1 to 3 and \( R^5 \) is a halogen atom or an alkoxy carbonyl, aryl or heteroaryl group;

(3) a group of the general formula:

\[-\text{O(CH}_2\text{)}_q-\text{O(CH}_2\text{)}_s-R^6\]

where \( r \) and \( s \) each independently are an integer of 1 to 5 and \( R^6 \) is a hydrogen atom or a lower alkyl group;

(4) a group of the formula:

\[
\begin{array}{c}
\text{O} \\
\text{N} \\
\text{O}
\end{array}
\]

(5) a group of the formula:

\[
\begin{array}{c}
\text{N} \\
\text{O}
\end{array}
\]
(6) a group of the formula: \[\text{N-S-O} \]

(7) a group of the general formula: \[\text{O}^t \quad \text{S-A} \]

where \( t \) is an integer of 0 to 2 and A is a lower alkyl, alkoxy carbonylmethyl, pyridyl or furyl

group, or a group of the general formula: \[\text{P} \]

where \( P \) is selected from the group consisting of: \(-\text{NH-}, -\text{O-} \) or \(-\text{S-})\). or a group of the general formula:

\[\text{(CH}_2)_w \]

wherein \(R^7\) is hydrogen or lower alkyl and \(w\) is from 0 to 3;
(8) a group of the general formula: $\text{N-}(\text{CH}_2)^\text{R^8}$ where R^8 is an acetoxy or lower alkyl group; and

(9) a group of the general formula: $-\text{OR^9}$ where R^9 is a hydrogen atom or a lower alkyl or aryl group;

n is an integer of 0 to 2; m is an integer of 2 to 10, and

J and K are independently hydrogen or lower alkyl, with the proviso that when Z is a group falling under the above category (9), R^9 is a lower alkyl group and m stands for an integer of 3 to 10, and pharmaceutically acceptable salts thereof; and glycine, in a pharmaceutically acceptable carrier.

2. An aqueous pharmaceutical formulation of claim 1 suitable for intravenous injection comprising:

an anti-ulcerative compound having the following formula:
wherein R¹ and R² are independently selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, halogenated lower alkyl, lower alkoxy carbonyl, a carboxyl group, and halogen;

wherein R³ is selected from the group consisting of hydrogen, lower alkyl, and aryl;

wherein J is selected from the group consisting of hydrogen or lower alkyl;

wherein m is an integer from 2 to 10;

and pharmaceutically acceptable salts thereof;

glycine, sodium hydroxide; and

a tonicity agent.

3. An aqueous pharmaceutical formulation of claim 1 suitable for intravenous injection comprising:

an anti-ulcerative compound having the following formula:

![Chemical Structure]

wherein R¹ and R² are independently selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, halogenated lower alkyl, lower alkoxy carbonyl, a carboxyl group, and halogen;
wherein R^4 is selected from the group consisting of hydrogen, lower alkyl, aryl, and aralkyl;
wherein J is selected from the group consisting of hydrogen or lower alkyl;
wherein m is an integer from 2 to 10;
wherein p is an integer from 1 to 3;
and pharmaceutically acceptable salts thereof;
glycine, sodium hydroxide; and
a tonicity agent.

4. The aqueous pharmaceutical formulation suitable for intravenous injection of claim 1 wherein said tonicity agent is selected from the group consisting of sodium chloride, glycerin, mannitol, sucrose, lactose, and dextrose.

5. The aqueous pharmaceutical formulation suitable for intravenous injection of claim 2 wherein said tonicity agent is selected from the group consisting of sodium chloride and dextrose.

6. The aqueous pharmaceutical formulation suitable for intravenous injection of claim 3 wherein said tonicity agent is selected from the group consisting of sodium chloride and dextrose.

7. The aqueous pharmaceutical formulation suitable for intravenous injection of claim 1 wherein said compound is

![Chemical Structure](attachment:image.png)
8. The aqueous pharmaceutical formulation suitable for intravenous injection of claim 7 wherein said toxicity agent is selected from the group consisting of sodium chloride and dextrose.

9. The aqueous pharmaceutical formulation suitable for intravenous injection of claim 8 wherein said toxicity agent is sodium chloride and said sodium chloride is present in said formulation at a concentration of about 0.9% by weight.

10. The aqueous pharmaceutical formulation suitable for intravenous injection of claim 8 wherein said toxicity agent is dextrose and said dextrose is present in said formulation at a concentration of about 5% by weight.

11. The aqueous pharmaceutical formulation suitable for intravenous injection of claim 1 wherein said formulation has an alkaline pH, and wherein said glycine in said formulation is present at a concentration of between about 1 mM and 300 mM.

12. The aqueous pharmaceutical formulation suitable for intravenous injection of claim 4 wherein said formulation has a pH of between about 9 and about 12, and wherein said glycine in said formulation is present at a concentration of between about 10 mM and 300 mM.

13. The aqueous pharmaceutical formulation suitable for intravenous injection of claim 8 wherein said formulation has a pH of between about 9 and 12, and wherein said glycine in said formulation is present at a concentration of between about 10 mM and 300 mM.

14. A method for stabilizing anti-ulcerative formulations suitable for intravenous injection which comprises:

    providing a compound of the formula
wherein $R^1$ and $R^2$ are independently selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, halogenated lower alkyl, lower alkoxy carbonyl, a carboxyl group, and halogen;

$X$ is a member selected from the group consisting of $-\text{O}, -\text{S}$ or $\frac{1}{\text{R}^3}$,

where $R^3$ is a member selected from the group consisting of hydrogen, lower alkyl, phenyl, benzyl, and lower alkoxy carbonyl; and

$Z$ is selected from the group consisting of:

(1) a group of the formula:

\[-\text{O}(\text{CH}_2)_p -\text{O} - \text{R}^4\]

where $p$ is an integer of 1 to 3 and $\text{R}^4$ is a hydrogen atom or a lower alkyl, aryl or aralkyl group;

(2) a group of the general formula:

\[-\text{O}(\text{CH}_2)_q - \text{R}^5\]

where $q$ is an integer of 1 to 3 and $\text{R}^5$ is a halogen atom or an alkoxy carbonyl, aryl or heteroaryl group;

(3) a group of the general formula:

\[-\text{O}(\text{CH}_2)_r - \text{O}(\text{CH}_2)_s - \text{R}^6\]

where $r$ and $s$ each independently are an integer of 1 to 5 and $\text{R}^6$ is a hydrogen atom or a
lower alkyl group;

(4) a group of the formula:

(5) a group of the formula:

(6) a group of the formula:
(7) a group of the general formula: 
\[
\frac{(O)^t}{S} \quad \frac{}{A}
\]

where \( t \) is an integer of 0 to 2 and \( A \) is a lower alkyl, alkoxy carbonylmethyl, pyridyl or furyl group, or a group of the general formula:

where \( P \) is selected from the group consisting of: \(-\text{NH}-\), \(-\text{O}-\) or \(-\text{S}-\) or a group of the general formula:

\[
\frac{}{(\text{CH}_2)_w} \quad \frac{R^7}{N}
\]

wherein \( R^7 \) is hydrogen or lower alkyl and \( w \) is from 0 to 3;

(8) a group of the general formula: 
\[
\frac{N}{R^8} \quad \frac{(\text{CH}_2)}{R^8}
\]

where \( R^8 \) is an acetoxy or lower alkyl group; and
(9) a group of the general formula: \(-OR^g\)

where \(R^g\) is a hydrogen atom or a lower alkyl or aryl group;

\(n\) is an integer of 0 to 2; \(m\) is an integer of 2 to 10, and

\(J\) and \(K\) are independently hydrogen or lower alkyl, with the proviso that when

\(Z\) is a group falling under the above category (9), \(R^g\) is a lower alkyl group and \(m\) stands for

an integer of 3 to 10, and pharmaceutically acceptable salts thereof;

providing a solution suitable for intravenous injection which has a pH of

between about 10 and 11 and which comprises glycine; and

admixing said compound and said solution until said compound is dissolved in

said solution.

15. The method of claim 14 wherein said solution contains a solute

selected from the group consisting of dextrose and sodium chloride.

16. The method of claim 14 wherein said glycine is present in said solution

at a concentration of between about 10 and about 300 mM.

17. The method of claim 14 wherein said compound is

\[
\begin{align*}
\text{O} & \quad \text{CH}_3 \\
\text{NH} & \quad \text{CH}_2 \\
\text{S} & \quad \text{NH} \\
\text{O} & \quad (\text{CH}_2)_3 \quad \text{OCH}_3
\end{align*}
\]

18. The method of claim 17 wherein said solution contains a solute
selected from the group consisting of dextrose and sodium chloride.

19. The method of claim 18 wherein said glycine is present in said solution at a concentration of between about 10 and about 300 mM.

20. The method of claim 19 wherein said solution contains a solute selected from the group consisting of dextrose and sodium chloride, and wherein said solution is isotonic with blood plasma.

21. The formulation of claim 1, which comprises a tonicity agent.

22. The formulation of claim 1, which comprises sodium hydroxide.

23. The method of claim 11, wherein said alkaline pH is between about 9 and about 12.
Changes in absorption spectrum of E3810 in saline (4mg/mL) as a function of time after the dissolution.

*Storage condition: 25°C, dark place

Changes in absorption spectrum of E3810 in 50 mM Gly-NaOH buffer (pH10) containing 0.9% NaCl (4mg/mL) as a function of time after the dissolution.

*Storage condition: 25°C, dark place
Absorption spectrum of E3810 in various solutions (4mg/mL) 5 hours after dissolution
*Storage condition: 25°C, dark place

- 50 mM Gly-NaOH buffer (pH10) containing 0.9% NaCl
- 25 mM Gly-NaOH buffer (pH10) containing 0.9% NaCl
- 10 mM Gly-NaOH buffer (pH10) containing 0.9% NaCl
- 5 mM Gly-NaOH buffer (pH10) containing 0.9% NaCl
Effects of storage condition on the change in absorbance of E3810 at various wavelengths in saline (2mg/mL).
Figure 7
Storage condition: 25°C, light place
Absorbance

Figure 8
Storage condition: 25°C, dark place
Absorbance

Figure 9
Storage condition: 10°C, dark place
Absorbance

Effects of storage condition on the change in absorbance
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/44 A61K47/18

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
IPC 6 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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>DATABASE WPI&lt;br&gt;Section Ch, Week 9335&lt;br&gt;Derwent Publications Ltd., London, GB;&lt;br&gt;Class 802, AN 93-278196&lt;br&gt;XPO02090491&lt;br&gt;&amp; JP 05 194225 A (YOSHITOMI PHARM IND KK), 3 August 1993&lt;br&gt;see abstract&lt;br&gt;&amp; JP 00 594225 A</td>
<td>1-23</td>
</tr>
<tr>
<td>Y</td>
<td>PATENT ABSTRACTS OF JAPAN&lt;br&gt;vol. 005, no. 132 (C-068), 22 August 1981&lt;br&gt;&amp; JP 56 065816 A (GREEN CROSS CORP:THE), 3 June 1981&lt;br&gt;see abstract</td>
<td>1-23</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of box C.

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 published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or with which the publication date of another citation or other special reason (as specified) are to be established
- "O" document referring to 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
- "A" document member of the same patent family

Date of the actual completion of the international search
20 January 1999

Date of mailing of the international search report
29/01/1999

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2340, Tx. 31 651 apo nl, Fax: (+31-70) 340-3016

Authorized officer
Seegert, K
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
</table>
| Y        | DE 30 00 743 A (GREEN CROSS CORP)  
8 January 1981  
see claim 5 | 1-23 |
| A        | EP 0 082 481 A (SCHERING CORP)  
29 June 1983  
see page 1 - page 2 | 1-23 |
| A        | WO 95 28951 A (GENETICS INST)  
2 November 1995  
see page 2, line 9 - line 29 | 1-23 |
| A        | EP 0 268 956 A (EISAI CO LTD) 1 June 1988  
cited in the application  
see page 17, line 32 - line 34; claims | 1-23 |
<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member(s)</th>
<th>Publication date</th>
</tr>
</thead>
<tbody>
<tr>
<td>DE 3000743 A</td>
<td>08-01-1981</td>
<td>JP 1354019 C</td>
<td>24-12-1986</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 56010110 A</td>
<td>02-02-1981</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 61021528 B</td>
<td>27-05-1986</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CH 642261 A</td>
<td>13-04-1984</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FR 2460672 A</td>
<td>30-01-1981</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GB 2051573 A,B</td>
<td>21-01-1981</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HK 66785 A</td>
<td>13-09-1985</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 4265888 A</td>
<td>05-05-1981</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 558966 B</td>
<td>19-02-1987</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 9168882 A</td>
<td>30-06-1983</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 1207665 A</td>
<td>15-07-1986</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DK 565882 A,B</td>
<td>24-06-1983</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FI 824366 A,B</td>
<td>24-06-1983</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HK 94487 A</td>
<td>18-12-1987</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IE 54468 B</td>
<td>25-10-1989</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 1833311 C</td>
<td>29-03-1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 58146504 A</td>
<td>01-09-1983</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OA 7282 A</td>
<td>30-11-1984</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PT 76013 B</td>
<td>11-03-1986</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 4496537 A</td>
<td>29-01-1985</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZA 8208580 A</td>
<td>26-10-1983</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZA 8209344 A</td>
<td>26-10-1983</td>
</tr>
</tbody>
</table>

| WO 9528951 A                           | 02-11-1995     | AU 684620 B             | 18-12-1997      |
|                                        |                | AU 1934495 A            | 16-11-1995      |
|                                        |                | CA 2182199 A            | 02-11-1995      |
|                                        |                | EP 0758901 A            | 26-02-1997      |
|                                        |                | JP 9512529 T            | 16-12-1997      |

|                                        |                | AT 168111 T             | 15-07-1998      |
|                                        |                | AU 594836 B             | 15-03-1990      |
|                                        |                | AU 8113887 A            | 19-05-1988      |
|                                        |                | CA 1265138 A            | 30-01-1990      |
|                                        |                | DE 3752163 D            | 12-03-1998      |
|                                        |                | DE 3752167 T            | 25-06-1998      |
|                                        |                | DE 3752201 D            | 13-08-1998      |
|                                        |                | DE 3752201 T            | 17-12-1998      |
|                                        |                | DE 3789536 D            | 11-05-1994      |
|                                        |                | DE 3789536 T            | 18-08-1994      |
|                                        |                | DK 131593 A             | 24-11-1993      |
|                                        |                | DK 144295 A             | 19-12-1995      |
|                                        |                | DK 575887 A             | 14-05-1988      |
|                                        |                | EP 0475456 A            | 18-03-1992      |
|                                        |                | EP 0654471 A            | 24-05-1995      |
|                                        |                | EP 0786461 A            | 30-07-1997      |
|                                        |                | ES 2061471 T            | 16-12-1994      |
|                                        |                | ES 2112260 T            | 01-04-1998      |
|                                        |                | ES 2118457 T            | 16-09-1998      |
|                                        |                | FI 874079 A,B           | 14-05-1988      |
|                                        |                | GR 3026762 T            | 31-07-1998      |
|                                        |                | HK 194296 A             | 01-11-1996      |
|                                        |                | JP 254467 B             | 16-10-1996      |
|                                        |                | JP 5247035 A            | 24-09-1993      |
|                                        |                | JP 2576843 B            | 29-01-1997      |
|                                        |                | JP 7291967 A            | 07-11-1995      |
**INTERNATIONAL SEARCH REPORT**

Information on patent family members

<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member(s)</th>
<th>Publication date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>JP 1953321 C</td>
<td>28-07-1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 6074272 B</td>
<td>21-09-1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 5840910 A</td>
<td>24-11-1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 5045552 A</td>
<td>03-09-1991</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CN 1018923 B</td>
<td>04-11-1992</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DD 290192 A</td>
<td>23-05-1991</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SU 1760535 A</td>
<td>07-12-1992</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RU 2035461 C</td>
<td>20-05-1995</td>
</tr>
</tbody>
</table>