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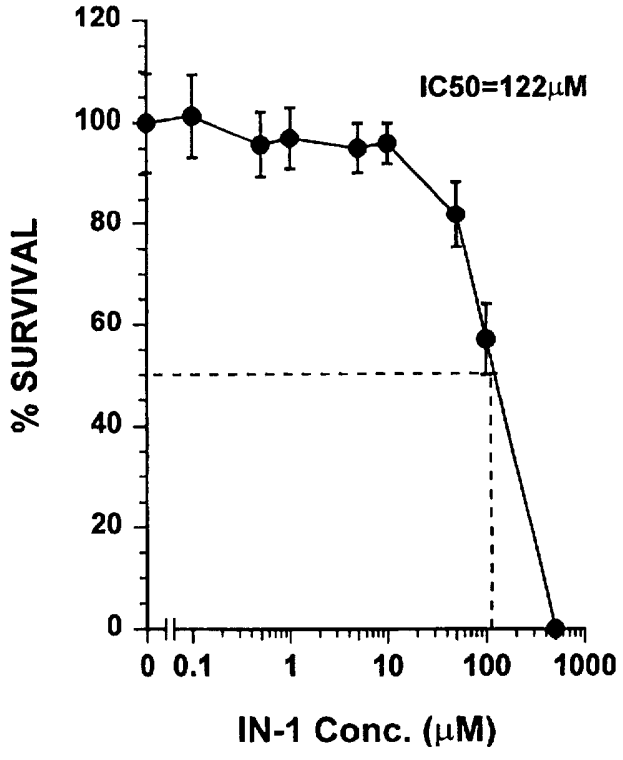
(57) Abstract

A therapeutic system comprising: (a) a compound comprising a target cell-specific portion and a portion capable of converting a substance into another substance; and (b) a molecule capable of substantially inhibiting the conversion of said substance, or a precursor of said molecule. In one particularly preferred embodiment said other substance is cytotoxic and said substance is substantially non-cytotoxic, the system further comprising said substance. In a second particularly preferred embodiment said substance, in its native state, is able to inhibit the effect of a cytotoxic agent and said other substance has less effect against said cytotoxic agent, the system further comprising (a) a cytotoxic agent and (b) said substance.



In vitro cytotoxicity of IN-1 on LS174T cells

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DRUG THERAPY

The present invention relates to drug therapy, in particular to the treatment of tumours by localisation of cytotoxic agents at the site of the tumour.

WO 88/07378 describes a two-component system, and therapeutic uses thereof, wherein a first component comprises an antibody fragment capable of binding with a tumour-associated antigen and an enzyme capable of converting a pro-drug into a cytotoxic drug, and a second component which is a pro-drug which is capable of conversion to a cytotoxic drug. This general system, which is often referred to as "antibody-directed enzyme pro-drug therapy" (ADEPT), is also described in relation to specific enzymes and pro-drugs in EP 0 302 473 and WO 91/11201.

WO 89/10140 describes a modification to the system described in WO 88/07378 wherein a further component is employed in the system. This further component accelerates the clearance of the first component from the blood when the first and second components are administered clinically. The second component is usually an antibody that binds to the antibody-enzyme conjugate and accelerates clearance. An antibody which was directed at the active site on the enzyme had the additional advantage of inactivating the enzyme. However, such an inactivating antibody has the undesirable potential to inactivate enzyme at the tumour sites, but its penetration into tumours was obviated by the addition of galactose residues to the antibody. The galactosylated antibody was rapidly removed from the blood, together with bound antibody-enzyme component, via galactose receptors in the liver. The system has been used safely and effectively in clinical trials. However, galactosylation of such an inactivating antibody which results in its rapid clearance from blood also inhibits its penetration

of normal tissue and inactivation of enzyme localised there.

WO 93/13805 describes a system comprising a compound comprising a target cell-specific portion, such as an antibody specific to tumour cell antigens, and an inactivating portion, such as an enzyme, capable of converting a substance which in its native state is able to inhibit the effect of a cytotoxic agent into a substance which has less effect against said cytotoxic agent. The prolonged action of a cytotoxic agent at tumour sites is therefore possible whilst protecting normal tissues from the effects of the cytotoxic agent.

WO 93/13806 describes a further modification of the ADEPT system comprising a three component kit of parts for use in a method of destroying target cells in a host. The first component comprises a target cell-specific portion and an enzymatically active portion capable of converting a pro-drug into a cytotoxic drug; the second component is a pro-drug convertible by said enzymatically active portion to the cytotoxic drug; and the third component comprises a portion capable of at least partly restraining the component from leaving the vascular compartment of a host when said compound is administered to the vascular compartment, and an inactivating portion capable of converting the cytotoxic drug into a less toxic substance.

Although all of the aforementioned methods are useful, it is still desirable to attempt to improve the specificity of the systems in order to limit side-effects to the patient.

An object of the invention is to provide a means for increasing specificity and to limit side-effects to the patient particularly in conjunction with the systems described in WO 88/07378 and WO 93/13805.

A first aspect of the invention provides a therapeutic system comprising: (a) a compound comprising a target cell-specific portion and a portion capable of converting a substance into another substance; and (b) a molecule capable of substantially inhibiting the conversion of said substance, or a precursor of said molecule.

In a first particularly preferred embodiment, which is related to the system described in WO 88/07378 which is incorporated herein by reference, the other substance is cytotoxic and said substance is substantially non-cytotoxic, and the system further comprises said substance.

In a second particularly preferred embodiment, which is related to the system described in WO 93/13805 which is incorporated herein by reference, said substance, in its native state, is able to inhibit the effect of a cytotoxic agent and said other substance has less effect against said cytotoxic agent, and the system further comprises (a) a cytotoxic agent and (b) said substance.

The entity which is recognised by the target cell-specific portion may be any suitable entity which is expressed by tumour cells, virally-infected cells, pathogenic microorganisms, cells introduced as part of gene therapy or normal cells of the body which one wishes to destroy for a particular reason. The entity should preferably be present or accessible to the targeting portion in significantly greater concentrations in or on cells which are to be destroyed than in any normal tissues of the host that cannot be functionally replaced by other therapeutic means. Use of a target expressed by a cancer cell would not be precluded, for example, by its equal or greater expression on an endocrine tissue or organ. In a life-saving situation the organ could be sacrificed provided its function was either not essential to life, for example in the case of the testes, or could

be supplied by hormone replacement therapy. Such considerations would apply, for instance, to the thyroid gland, parathyroids, adrenal cortex and ovaries.

The entity which is recognised will often be an antigen. Tumour-associated antigens, when they are expressed on the cell membrane or secreted into tumour extra-cellular fluid, lend themselves to the role of targets for antibodies.

The term "tumour" is to be understood as referring to all forms of neoplastic cell growth, including tumours of the lung, liver, blood cells (leukaemias), skin, pancreas, colon, prostate, uterus or breast.

The antigen-specific portion may be an entire antibody (usually, for convenience and specificity, a monoclonal antibody), a part or parts thereof (for example an Fab fragment or $F(ab')_2$) or a synthetic antibody or part thereof. A conjugate comprising only part of an antibody may be advantageous by virtue of optimizing the rate of clearance from the blood and may be less likely to undergo non-specific binding due to the Fc part. Suitable monoclonal antibodies to selected antigens may be prepared by known techniques, for example those disclosed in "Monoclonal Antibodies: A manual of techniques", H. Zola (CRC Press, 1988) and in "Monoclonal Hybridoma Antibodies: Techniques and Applications", J.G.R. Hurrell (CRC Press, 1982). All references mentioned in this specification are incorporated herein by reference. Bispecific antibodies may be prepared by cell fusion, by reassociation of monovalent fragments or by chemical cross-linking of whole antibodies, with one part of the resulting bispecific antibody being directed to the cell-specific antigen and the other to the enzyme. The bispecific antibody can be administered bound to the enzyme or it can be administered first, followed by the

enzyme. It is preferred that the bispecific antibodies are administered first, and after localization to the tumour cells, the enzyme is administered to be captured by the tumour localized antibody. Methods for preparing bispecific antibodies are disclosed in Corvalan *et al* (1987) *Cancer Immunol. Immunother.* **24**, 127-132 and 133-137 and 138-143, and Gillsland *et al* (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7719-7723.

The variable heavy (V_H) and variable light (V_L) domains of the antibody are involved in antigen recognition, a fact first recognised by early protease digestion experiments. Further confirmation was found by "humanisation" of rodent antibodies. Variable domains of rodent origin may be fused to constant domains of human origin such that the resultant antibody retains the antigenic specificity of the rodent parented antibody (Morrison *et al* (1984) *Proc. Natl. Acad. Sci. USA* **81**, 6851-6855).

That antigenic specificity is conferred by variable domains and is independent of the constant domains is known from experiments involving the bacterial expression of antibody fragments, all containing one or more variable domains. These molecules include Fab-like molecules (Better *et al* (1988) *Science* **240**, 1041); Fv molecules (Skerra *et al* (1988) *Science* **240**, 1038); single-chain Fv (ScFv) molecules where the V_H and V_L partner domains are linked via a flexible oligopeptide (Bird *et al* (1988) *Science* **242**, 423; Huston *et al* (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5879) and single domain antibodies (dAbs) comprising isolated V domains (Ward *et al* (1989) *Nature* **341**, 544). A general review of the techniques involved in the synthesis of antibody fragments which retain their specific binding sites is to be found in Winter & Milstein (1991) *Nature* **349**, 293-299.

By "ScFv molecules" we mean molecules wherein the V_H and V_L partner

domains are linked via a flexible oligopeptide.

The advantages of using antibody fragments, rather than whole antibodies, are several-fold. The smaller size of the fragments may lead to improved pharmacological properties, such as better penetration of solid tissue. Effector functions of whole antibodies, such as complement binding, are removed. Fab, Fv, ScFv and dAb antibody fragments can all be expressed in and secreted from *E. coli*, thus allowing the facile production of large amounts of the said fragments.

Whole antibodies, and $F(ab')_2$ fragments are "bivalent". By "bivalent" we mean that the said antibodies and $F(ab')_2$ fragments have two antigen combining sites. In contrast, Fab, Fv, ScFv and dAb fragments are monovalent, having only one antigen combining sites. Fragmentation of intact immunoglobulins to produce $F(ab')_2$ fragments is disclosed by Harwood *et al* (1985) *Eur. J. Cancer Clin. Oncol.* **21**, 1515-1522.

IgG class antibodies are preferred.

Alternatively, the entity which is recognised may or may not be antigenic but can be recognised and selectively bound to in some other way. For example, it may be a characteristic cell surface receptor such as the receptor for melanocyte-stimulating hormone (MSH) which is expressed in high numbers in melanoma cells. The cell-specific portion may then be a compound or part thereof which specifically binds to the entity in a non-immune sense, for example as a substrate or analogue thereof for a cell-surface enzyme or as a messenger.

Considerable work has already been carried out on antibodies and fragments thereof to tumour-associated antigens and antibodies or antibody

fragments directed at carcinoembryonic antigen (CEA) and antibodies or their fragments directed at human chorionic gonadotrophin (hCG) can be conjugated to carboxypeptidase G2 and the resulting conjugate retains both antigen binding and catalytic function. Following intravenous injection of these conjugates they localise selectively in tumours expressing CEA or hCG respectively. Other antibodies are known to localise in tumours expressing the corresponding antigen. Such tumours may be primary and metastatic colorectal cancer (CEA) and choriocarcinoma (hCG) in human patients or other forms of cancer. Although such antibody-enzyme conjugates may also localise in some normal tissues expressing the respective antigens, antigen expression is more diffuse in normal tissues. Such antibody-enzyme conjugates may be bound to cell membranes via their respective antigens or trapped by antigen secreted into the interstitial space between cells.

Examples of tumour-associated, immune cell-associated and infection reagent-related antigens are given in Table 1.

TABLE 1: Cell surface antigens for targeting

a) Tumour Associated Antigens

Antigen	Antibody	Existing uses
Carcino-embryonic Antigen	C46 (Amersham) 85A12 (Unipath)	Imaging and therapy of colon/rectum tumours.
Placental Alkaline Phosphatase	H17E2 (ICRF, Travers & Bodmer)	Imaging and therapy of testicular and ovarian cancers.

Pan Carcinoma	NR-LU-10 (NeoRx Corporation)	Imaging and therapy of various carcinomas including small cell lung cancer.
Polymorphic Epithelial Mucin (Human milk fat globule)	HMFG1 (Taylor-Papadimitriou, ICRF)	Imaging and therapy of ovarian cancer and pleural effusions.
β-human Chorionic Gonadotropin	W14	Targeting of carboxypeptidase to human xenograft choriocarcinoma in nude mice (Searle *et al* (1981) *Br. J. Cancer* **44**, 137-144).
A carbohydrate on Human Carcinomas	L6 (IgG2a)[1]	Targeting of alkaline phosphatase (Senter *et al* (1988) *PNAS USA* **85**, 4842-4846.
CD20 Antigen on B Lymphoma (normal and neoplastic)	1F5 (IgG2a)[2]	Targeting of alkaline phosphatase (Senter *et al* (1988) *PNAS USA* **85**, 4842-4846.

[1]Hellström *et al* (1986) *Cancer Res.* **46**, 3917-3923

[2]Clarke *et al* (1985) *Proc. Natl. Acad. Sci. USA* **82**, 1766-1770

Other antigens include alphafoetoprotein, Ca-125 and prostate specific antigen.

b) Immune Cell Antigens

Antigen	**Antibody**	**Existing uses**
Pan T Lymphocyte Surface Antigen (CD3)	OKT-3 (Ortho)	As anti-rejection therapy for kidney transplants.

B-lymphocyte Surface Antigen (CD22)	RFB4 (Janossy, Royal Free Hospital)	Immunotoxin therapy of B cell lymphoma.
Pan T lymphocyte Surface Antigen (CD5)	H65 (Bodmer and Knowles, ICRF; licensed to Xoma Corp., USA)	Immunotoxin treatment of acute graft versus host disease, rheumatoid arthritis.

c) Infectious Agent-Related Antigens

Antigen	Antibody	Existing uses
Mumps virus-related	Anti-mumps polyclonal antibody	Antibody conjugated to diphtheria toxin for treatment of mumps.
Hepatitis B Surface Antigen	Anti HBs Ag	Immunotoxin against hepatoma.

Other tumour selective targets and suitable binding moieties are shown in Table 2.

Table 2: Binding moieties for tumour-selective targets and tumour-associated antigens

Target	Binding moiety	Disease
Truncated EGFR	anti-EGFR mAb	Gliomas
Idiotypes	anti-id mAbs	B-cell lymphomas
EGFR (*c-erb*B1)	EGF, TGFα anti-EGFR mAb	Breast cancer
*c-erb*B2	mAbs	Breast cancer
IL-2 receptor	IL-2 anti-Tac mAb	Lymphomas and leukaemias

IL-4 receptor	IL-4	Lymphomas and leukaemias
IL-6 receptor	IL-6	Lymphomas and leukaemias
MSH (melanocyte-stimulating hormone) receptor	α-MSH	Melanomas
Transferrin receptor (TR)	Transferrin anti-TR mAb	Gliomas
gp95/gp97	mAbs	Melanomas
p-glycoprotein cells	mAbs	drug-resistant
cluster-1 antigen (N-CAM)	mAbs	Small cell lung carcinomas
cluster-w4	mAbs	Small cell lung carcinomas
cluster-5A	mAbs	Small cell lung carcinomas
cluster-6 (LeY)	mAbs	Small cell lung carcinomas
PLAP (placental alkaline phosphatase)	mAbs	Some seminomas Some ovarian; some non small cell lung cancer
CA-125	mAbs	Lung, ovarian
ESA (epithelial specific antigen)	mAbs	carcinoma
CD 19, 22, 37	mAbs	B-cell lymphomas
250 kDa	mAbs	Melanoma
proteoglycan p55	mAbs	Breast cancer
TCR-IgH fusion	mAbs	Childhood T-cell leukaemia
Blood gp A antigen (in B or O individuals)	mAbs	Gastric and colon tumours

Mucin protein core	mAbs	Breast cancer

It is preferred if the target cell-specific portion comprises an antibody or fragment or derivative thereof.

Conveniently the portion capable of converting a substance into another substance is an enzyme (or at least is a macromolecule which has catalytic activity and could, therefore, be a catalytic RNA molecule or a catalytic carbohydrate molecule or at least the catalytic portion of an enzyme).

It is likely that the portion of the compound capable of converting a substance into another substance, when it is an enzymatically active portion, will be enzymatically active in isolation from the target cell-specific portion but it is necessary only for it to be enzymatically active when (a) it is in combination with the target cell-specific portion and (b) the compound is attached to or adjacent to target cells.

The two portions of the compound of the first aspect of the invention may be linked together by any of the conventional ways of cross-linking polypeptides, such as those generally described in O'Sullivan *et al* (1979) *Anal. Biochem.* **100**, 100-108. For example, the antibody portion may be enriched with thiol groups and the enzyme portion reacted with a bifunctional agent capable of reacting with those thiol groups, for example the N-hydroxysuccinimide ester of iodoacetic acid (NHIA) or N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP). Amide and thioether bonds, for example achieved with m-maleimidobenzoyl-N-hydroxysuccinimide ester, are generally more stable *in vivo* than disulphide bonds.

It may not be necessary for a whole enzyme to be present in the

compound of the first aspect of the invention but, of course, the catalytic portion must be present.

Alternatively, the compound may be produced as a fusion compound by recombinant DNA techniques whereby a length of DNA comprises respective regions encoding the two portions of the compound of the invention either adjacent to one another or separated by a region encoding a linker peptide which does not destroy the desired properties of the compound. Conceivably, the two portions of the compound may overlap wholly or partly. The antibody component of the fusion must be represented by at least one binding site. Examples of the construction of antibody (or antibody fragment)-enzyme fusions are disclosed by Neuberger *et al* (1984) *Nature* **312**, 604.

The DNA is then expressed in a suitable host to produce a polypeptide comprising the compound of this aspect of the invention. Thus, the DNA encoding the polypeptide constituting the compound of this aspect of the invention may be used in accordance with known techniques, appropriately modified in view of the teachings contained herein, to construct an expression vector, which is then used to transform an appropriate host cell for the expression and production of the polypeptide of the invention. Such techniques include those disclosed in US Patent Nos. 4,440,859 issued 3 April 1984 to Rutter *et al*, 4,530,901 issued 23 July 1985 to Weissman, 4,582,800 issued 15 April 1986 to Crowl, 4,677,063 issued 30 June 1987 to Mark *et al*, 4,678,751 issued 7 July 1987 to Goeddel, 4,704,362 issued 3 November 1987 to Itakura *et al*, 4,710,463 issued 1 December 1987 to Murray, 4,757,006 issued 12 July 1988 to Toole, Jr. *et al*, 4,766,075 issued 23 August 1988 to Goeddel *et al* and 4,810,648 issued 7 March 1989 to Stalker, all of which are incorporated herein by reference.

The DNA encoding the polypeptide constituting the compound of this aspect of the invention may be joined to a wide variety of other DNA sequences for introduction into an appropriate host. The companion DNA will depend upon the nature of the host, the manner of the introduction of the DNA into the host, and whether episomal maintenance or integration is desired.

Generally, the DNA is inserted into an expression vector, such as a plasmid, in proper orientation and correct reading frame for expression. If necessary, the DNA may be linked to the appropriate transcriptional and translational regulatory control nucleotide sequences recognised by the desired host, although such controls are generally available in the expression vector. The vector is then introduced into the host through standard techniques. Generally, not all of the hosts will be transformed by the vector. Therefore, it will be necessary to select for transformed host cells. One selection technique involves incorporating into the expression vector a DNA sequence, with any necessary control elements, that codes for a selectable trait in the transformed cell, such as antibiotic resistance. Alternatively, the gene for such selectable trait can be on another vector, which is used to co-transform the desired host cell.

Host cells that have been transformed by the recombinant DNA of the invention are then cultured for a sufficient time and under appropriate conditions known to those skilled in the art in view of the teachings disclosed herein to permit the expression of the polypeptide, which can then be recovered.

Many expression systems are known, including bacteria (for example *E. coli* and *Bacillus subtilis*), yeasts (for example *Saccharomyces cerevisiae*), filamentous fungi (for example *Aspergillus*), plant cells, animal cells and

insect cells.

The vectors include a procaryotic replicon, such as the ColE1 *ori*, for propagation in a procaryote, even if the vector is to be used for expression in other, non-procaryotic, cell types. The vectors can also include an appropriate promoter such as a procaryotic promoter capable of directing the expression (transcription and translation) of the genes in a bacterial host cell, such as *E. coli*, transformed therewith.

A promoter is an expression control element formed by a DNA sequence that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with exemplary bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites for insertion of a DNA segment of the present invention.

Typical procaryotic vector plasmids are pUC18, pUC19, pBR322 and pBR329 available from Biorad Laboratories, (Richmond, CA, USA) and p*Trc*99A and pKK223-3 available from Pharmacia, Piscataway, NJ, USA.

A typical mammalian cell vector plasmid is pSVL available from Pharmacia, Piscataway, NJ, USA. This vector uses the SV40 late promoter to drive expression of cloned genes, the highest level of expression being found in T antigen-producing cells, such as COS-1 cells.

An example of an inducible mammalian expression vector is pMSG, also available from Pharmacia. This vector uses the glucocorticoid-inducible promoter of the mouse mammary tumour virus long terminal repeat to drive expression of the cloned gene.

Useful yeast plasmid vectors are pRS403-406 and pRS413-416 and are

generally available from Stratagene Cloning Systems, La Jolla, CA 92037, USA. Plasmids pRS403, pRS404, pRS405 and pRS406 are Yeast Integrating plasmids (YIps) and incorporate the yeast selectable markers *his3, trp1, leu2* and *ura3*. Plasmids pRS413-416 are Yeast Centromere plasmids (YCps).

A variety of methods have been developed to operatively link DNA to vectors via complementary cohesive termini. For instance, complementary homopolymer tracts can be added to the DNA segment to be inserted to the vector DNA. The vector and DNA segment are then joined by hydrogen bonding between the complementary homopolymeric tails to form recombinant DNA molecules.

Synthetic linkers containing one or more restriction sites provide an alternative method of joining the DNA segment to vectors. The DNA segment, generated by endonuclease restriction digestion as described earlier, is treated with bacteriophage T4 DNA polymerase or *E. coli* DNA polymerase I, enzymes that remove protruding, 3′-single-stranded termini with their 3′-5′-exonucleolytic activities, and fill in recessed 3′-ends with their polymerizing activities.

The combination of these activities therefore generates blunt-ended DNA segments. The blunt-ended segments are then incubated with a large molar excess of linker molecules in the presence of an enzyme that is able to catalyze the ligation of blunt-ended DNA molecules, such as bacteriophage T4 DNA ligase. Thus, the products of the reaction are DNA segments carrying polymeric linker sequences at their ends. These DNA segments are then cleaved with the appropriate restriction enzyme and ligated to an expression vector that has been cleaved with an enzyme that produces termini compatible with those of the DNA segment.

Synthetic linkers containing a variety of restriction endonuclease sites are commercially available from a number of sources including International Biotechnologies Inc, New Haven, CN, USA.

A desirable way to modify the DNA encoding the polypeptide of this aspect of the invention is to use the polymerase chain reaction as disclosed by Saiki *et al* (1988) *Science* **239**, 487-491.

In this method the DNA to be enzymatically amplified is flanked by two specific oligonucleotide primers which themselves become incorporated into the amplified DNA. The said specific primers may contain restriction endonuclease recognition sites which can be used for cloning into expression vectors using methods known in the art.

Exemplary genera of yeast contemplated to be useful in the practice of the present invention are *Pichia, Saccharomyces, Kluyveromyces, Candida, Torulopsis, Hansenula, Schizosaccharomyces, Citeromyces, Pachysolen, Debaromyces, Metschunikowia, Rhodosporidium, Leucosporidium, Botryoascus, Sporidiobolus, Endomycopsis*, and the like. Preferred genera are those selected from the group consisting of *Pichia, Saccharomyces, Kluyveromyces, Yarrowia* and *Hansenula*. Examples of *Saccharomyces* are *Saccharomyces cerevisiae*, *Saccharomyces italicus* and *Saccharomyces rouxii*. Examples of *Kluyveromyces* are *Kluyveromyces fragilis* and *Kluyveromyces lactis*. Examples of *Hansenula* are *Hansenula polymorpha, Hansenula anomala* and *Hansenula capsulata*. *Yarrowia lipolytica* is an example of a suitable *Yarrowia* species.

Methods for the transformation of *S. cerevisiae* are taught generally in EP 251 744, EP 258 067 and WO 90/01063, all of which are incorporated herein by reference.

Suitable promoters for *S. cerevisiae* include those associated with the *PGK1* gene, *GAL1* or *GAL10* genes, *CYC1*, *PHO5*, *TRP1*, *ADH1*, *ADH2*, the genes for glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, triose phosphate isomerase, phosphoglucose isomerase, glucokinase, α-mating factor pheromone, a-mating factor pheromone, the *PRB1* promoter, the *GUT2* promoter, and hybrid promoters involving hybrids of parts of 5′ regulatory regions with parts of 5′ regulatory regions of other promoters or with upstream activation sites (eg the promoter of EP-A-258 067).

The transcription termination signal is preferably the 3′ flanking sequence of a eukaryotic gene which contains proper signals for transcription termination and polyadenylation. Suitable 3′ flanking sequences may, for example, be those of the gene naturally linked to the expression control sequence used, ie may correspond to the promoter. Alternatively, they may be different in which case the termination signal of the *S. cerevisiae* *AHD1* gene is preferred.

By "precursor of a molecule capable of substantially inhibiting the conversion of said substance" we include any molecule which, when introduced into a host, such as a patient to be treated, will generate the said molecule capable of substantially inhibiting the conversion of said substance. Example of molecules capable of substantially inhibiting the conversion of said substance, and precursor of said molecule are given below.

In the first particularly preferred embodiment of the invention, the said substance which is substantially non-cytotoxic is conveniently a pro-drug and the other substance which is cytotoxic is conveniently a cytotoxic drug. Plainly, in this embodiment the portion capable of converting a

substance into another substance includes a portion capable of converting a pro-drug into a cytotoxic drug. Many pro-drugs, cytotoxic drugs and enzymes for converting the pro-drug into the cytotoxic drug are known (for example, in WO 88/07378; WO 91/11201; and EP 0 302 473 all incorporated herein by reference). Thus, it is preferred if the enzyme and pro-drug are chosen from the following combinations:

Alkaline phosphatase useful for converting phosphate-containing pro-drugs into free drugs, aryl sulphatase useful for converting sulphate-containing pro-drugs into free drugs, cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anticancer drug 5-fluorouracil, proteases such as Serratia protease, thermolysin, subtilisin, carboxy-peptidases and cathepsins that are useful for converting peptide-containing pro-drugs into free drugs, D-alanylcarboxypeptidases, useful for converting pro-drugs that contain D-amino acid substituents, carbohydrate-enzymes such as β-galactosidase and neuraminidase useful for converting glycosylated pro-drugs into free drugs, β-lactamase useful for converting drugs derivatized with β-lactams into free drugs and penicillin amidases useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups into free drugs.

Other enzymes and pro-drugs include hydrolases, amidases, sulphatases, lipases, glucuronidases, phosphatases and carboxypeptidases, and pro-drugs be prepared from any of the various classes of anti-tumour compounds for example alkylating agents (nitrogen mustards) including cyclophosphamide, bisulphan, chlorambucil and nitrosoureas; intercalating agents including adriamycin and dactinomycin; spindle poisons such as vinca alkaloids; and anti-metabolites including anti-folates, anti-purines, anti-pyrimidines or hydroxyurea.

Also included are cyanogenic pro-drugs such as amygdalin which produce cyanide upon action with a carbohydrate cleaving enzyme.

It is particularly preferred if the portion capable of converting a pro-drug into a cytotoxic drug is a carboxypeptidase, especially carboxypeptidase G2. It is also preferred that the pro-drug is a nitrogen mustard glutamate, more preferably a benzoic acid nitrogen mustard glutamate as described in WO 88/07378. It is also preferred that the pro-drug is a nitrogen mustard glutamate derived from phenol or phenylenediamine mustard as described in WO 94/02450 (inventors P.J. Burke, R.J. Dowell, A.B. Mauger and C.J. Springer). It is also preferred that the pro-drug is of the self-immolative type as described in WO 95/02420 (inventors C.J. Springer and R. Marais).

It will be appreciated that, advantageously, the first particularly preferred embodiment can be used in conjunction with the clearance system described in WO 89/10140 or in conjunction with the restraining system of WO 93/13806, both incorporated herein by reference.

In the second particularly preferred embodiment of the invention the portion capable of converting a substance, which in its native state, is able to inhibit the effect of a cytotoxic agent to said other substance which has less effect against said cytotoxic agent is an inactivating portion.

By "inactivating" we include that the portion itself is able to inactivate the said substance, for example by converting it into an inactive form.

Preferably, the inactivating portion is an enzymatically active portion.

Substances which "inhibit" the effect of a cytotoxic agent are those which

diminish to a useful extent the ability of the cytotoxic agent to destroy target cells. Preferably, the said ability is reduced to substantially zero. Similarly, the inactivating portion will reduce such inhibition to a useful extent and will preferably reduce it to substantially zero.

The inhibitor-inactivating protein is preferably an enzyme capable of metabolising the said inhibitor to an inactive form.

The substance which in its native state is able to inhibit the effect of a cytotoxic agent may be any sufficiently non-toxic substance which may be converted into a substance which has less effect on said cytotoxic agent. A suitable compound is folinic acid. Folinic acid reverses the biological effect of the cytotoxic agent trimetrexate, for example, which acts on the enzyme dihydrofolate reductase. Folinic acid is deglutamated and rendered inactive against trimetrexate by the enzyme carboxypeptidase G2 and other deglutamating enzymes.

The same principle may be applied to other anti-cytotoxic agent substances. For example, thymidine blocks the effect of a cytotoxic agent, such as CB3717 and ICI D1694 (Jodrell *et al* 1991, *BJC* **64**, 833-8; Jones *et al* (1986) *J. Med. Chem.* **29**, 468-472), which acts on the enzyme thymidylate synthetase. Hence a thymidine degrading enzyme (such as dihydrothymine dehydrogenase, Shiotani & Weber 1981 *J. Biol. Chem.* **256**, 219-224) or thymidine kinase (Shiotani *et al* (1989) *Cancer Res.* **49**, 1090-1094) may be used as the inactivating portion of the compound of the invention to render the thymidine ineffective against the cytotoxic agent.

Similar considerations relate to other agents which interfere with the normal processes of nucleotide incorporation into DNA or RNA since

these are potentially reversible by the normal metabolite which in turn can be degraded by an appropriate enzyme targeted to tumour sites.

For instance, it has been shown that the cytotoxic effects of the widely used cytotoxic 5-fluorouracil (available from Roche Products Inc) can be at least partly attenuated by uridine (Groeningen *et al* (1989) *J. Natl. Cancer Inst.* **81**, 157-162). It follows that conjugation of an antitumour antibody with a uridine degrading enzyme can be used in conjunction with 5-fluorouracil and uridine. Such a combination would be particularly relevant in colorectal and breast carcinoma for which 5-fluorouracil is one of the most effective cytotoxic agents. Such a combination of agents may be further combined with folinic acid which augments the cytotoxicity of 5-fluorouracil or additionally with thymidine and a thymidine inactivating enzyme.

The inactivating portion of the compound will be chosen by reference to the anti-cytotoxic agent substance.

Enzymes other than carboxypeptidase G2 and its equivalents can be used. They should be specific for the targeted metabolite but may be of human or non-human origin.

It may not be necessary to use a conventional enzyme. Antibodies with catalytic capacity have been developed (Tramontano *et al Science* **234**, 1566-1570) and are known as 'abzymes' or catalytic antibodies. These have the potential advantage of being able to be humanized to reduce their immunogenicity.

Enzymes derived from human lymphocytes and able to degrade thymidine have been disclosed. (Schiotani *et al* (1989) *Cancer Res.* **49**, 1090-1094).

A dihydrothymine dehydrogenase and thymidine kinase can be used in the system of the type herein disclosed for use in conjunction with inhibitors of thymidine synthetase.

Thymidine degrading and phosphorylating enzymes can be used as an additional element in anti-folate therapy as herein disclosed by blocking the thymidine salvage pathway. They can also be used in conjunction with uridine catalysing enzymes used with the cytotoxic drug 5-fluorouracil.

The bacterial enzymes carboxypeptidase G1 and G2 (CPG1 and CPG2) degrade folates including methotrexate by cleavage of the terminal glutamic acid. The actions of the two enzymes are thought to be the same. The following description of preferred aspects of the invention refers to CPG2 but is equally applicable to CPG1 and to any other enzymes acting on the same substrates, and to abzymes acting on the same substrates.

The isolation, purification and some of the properties of carboxypeptidase G2 from *Pseudomonas sp.* strain RS-16 have been disclosed by Sherwood *et al* (1984) *Eur. J. Biochem.* **148**, 447-453. The cloning of the gene encoding the said carboxypeptidase G2, its nucleotide sequence and its expression in *E. coli* have been disclosed by Minton *et al* (1984) *Gene* **31**, 31-38 and Minton *et al* (1983) *J. Bacteriol.* **156**, 1222-1227. CP2G2 is available from the Division of Biotechnology, Centre for Applied Microbiological Research, Porton Down, Salisbury, UK. Carboxypeptidase G1 (CPG1) is disclosed by Chabner *et al* (1972) *Cancer Res.* **32**, 2114-2119.

Thus, in this preferred embodiment it is particularly preferred if the portion capable of converting a substance, which in its native state, is able

to inhibit the effect of a cytotoxic agent to said other substance which has less effect against said cytotoxic agent is a carboxypeptidase such as carboxypeptidase G2. It is also preferred if the said substance is folinic acid and if the said cytotoxic agent is trimetrexate.

In the first aspect of the invention (and in both particularly preferred embodiments) by "a molecule capable of substantially inhibiting the conversion of said substance" we mean a molecule which, when present with the compound comprising a target cell-specific portion and a portion capable of converting a substance into another substance, prevents to a useful extent the said conversion. The extent of inhibition is preferably $>5\%$, more preferably $>10\%$, still more preferably $>50\%$ and most preferably $>90\%$.

Preferably, when the portion capable of converting a substance into another substance is an enzyme or other macromolecule with catalytic activity the said molecule binds to the active site of the enzyme or other macromolecule.

By "active site" we include any site on the enzyme or other macromolecule which influences the catalytic activity whether or not the site is the site of catalysis.

In further preference, the said molecule binds to the active site of the enzyme or other macromolecule and in still further preference the said molecule is not exposed on the surface of the enzyme or other macromolecule.

Preferably, the molecule is a relatively small molecule and it is further preferred if the molecule has a relative molecular mass of less than 10000,

more preferably less than 5000 and most preferably less than 1000.

When the portion capable of converting a substance to another substance is an enzyme or other macromolecule with catalytic activity it is particularly preferred if the molecule capable of substantially inhibiting the conversion of the substance is a substantially irreversible inhibitor. By "substantially irreversible inhibitor" we include an inhibitor which, once bound to an enzyme or other macromolecule with catalytic activity, substantially inhibits the catalytic activity and is unlikely to become unbound.

It is particularly preferred if the k_{cat} of said enzyme, or other macromolecule with catalytic activity, with respect to the molecule is $<10s^{-1}$, preferably $<1s^{-1}$, more preferably $<0.1s^{-1}$, still more preferably $<0.01s^{-1}$ and most preferably substantially $0s^{-1}$.

It is also particularly preferred if the K_i of said enzyme, or other macromolecule with catalytic activity, is $<100\mu M$, preferably $<1\mu M$, more preferably $<1nM$ and still more preferably substantially zero.

It is preferred, particularly in relation to the first particularly preferred embodiment, if the molecule is not an antibody. It is also preferred if the molecule is not an antibody fragment derivable from an antibody by proteolytic digestion, such as a Fab fragment or $F(ab')_2$ fragment.

It is particularly preferred if the molecule capable of substantially inhibiting the conversion of said substance selectively inhibits the portion capable of converting a substance into another substance. In particular, it is preferred if said molecule does not inhibit an enzyme activity which is normally present in a host, such as a patient to be treated and, more

preferably said molecule does not inhibit an enzyme activity which is normally present in the vascular space of a host, such as a patient to be treated.

It is preferred if the molecule capable of substantially inhibiting the conversion of said substance is non-proteinaceous.

If the molecule is a peptide it is preferred that it comprises less than 50 amino acid residues, more preferably less than 25 amino acid residues and most preferably less than 10 amino acid residues.

It is further preferred if the said molecule is relatively stable to degradation in the host, such as a body of a patient. By "relatively stable to degradation" we mean that the molecule has a useful lifetime in the host before it is destroyed by, or removed from, the host. It is particularly preferred if the compound is relatively stable to degradation when present in plasma.

A particularly preferred molecule capable of substantially inhibiting the conversion of said substance is a molecule which is soluble in aqueous solutions suitable for pharmaceutical administration. Conveniently the aqueous solution is suitable for intravenous or intramuscular administration.

It is preferred that said molecule is substantially non-toxic, at least at the level that is administered to a host, such as a patient to be treated.

It is also preferred if the compound does not bind to carriers in the blood such as albumin, haemoglobin and the like.

It is also preferred if the molecule is substantially incapable of entering a cell in the body of a host, such as a patient to be treated.

Of course, the molecule capable of substantially inhibiting the conversion of a substance into another substance is selected by reference to the portion capable of converting said substance into said other substance.

For example, if the portion capable of converting said substance into said other substance is carboxypeptidase G2 (CPG2) then said molecule is an inhibitor of CPG2.

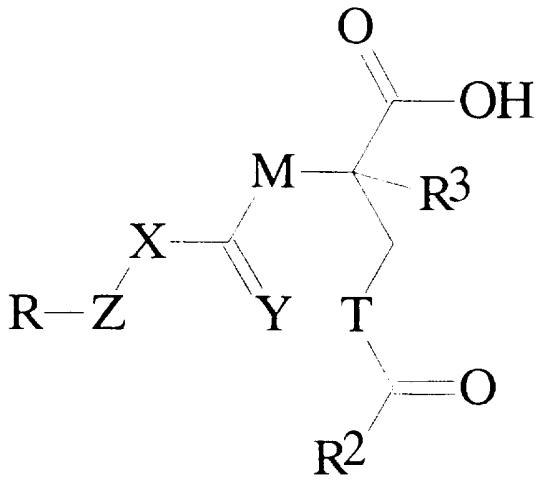
Studies with benzoic acid drugs and glutamate pro-drugs of low relative molecular mass < 1000 indicate that they penetrate tumours less well than normal tissues, probably because tumours are poorly vascularised (P. Antonin, PhD thesis, 1991, University of London). Only one normal tissue, brain, had a lower uptake of a pro-drug than tumour. To avoid activation of a pro-drug at any site other than in tumours it is desirable to inactivate residual enzyme in normal tissues as well as in blood. Since an antibody-enzyme component localises in the tumours to a higher concentration than in other tissues it follows that a small amount of inactivating agent, sufficient to inactivate enzyme in normal tissues, will only inactivate a small proportion of enzyme in the tumour, leaving sufficient enzyme there to activate a subsequently administered pro-drug.

Moreover, since a low molecular weight enzyme inhibitor may bind stoichiometrically to the active site of the enzyme, the total mass of the inhibitor necessary to inactivate the enzyme will be very much less than that of the antibody-enzyme component.

The first enzyme system to be used for this approach to cancer therapy

was carboxypeptidase G2 (CPG2), which cleaves the terminal glutamate from molecules which resemble folates with a benzene ring attached to a glutamate. We have designed and made molecules to inactivate carboxypeptidase G2 (CPG2).

We have found that suitable inhibitors of CPG2 include compounds with the general formula:



wherein R is selected from alkyl, haloalkyl, cycloalkyl, aryl, substituted aryl (1 to 5 substituents selected from halogen, alkyl, alkoxy, NH_2, OH, NR_2, COOH, CN, $CONH_2$), heteroaryl (5 or 6 membered ring containing 1 to 3 heteroatoms selected from N or S), OH, alkoxy, H. halogen, NH_2, O-sugar, O-amino acid, N-sugar, N-amino acid, aminopyridine N-oxide (aminopy N^+O^-), alkenyl, phenyl, nitro, nitroso, carbohydrate, aminoacid, lipid, pteridine derivative; R^2 is selected from OH, aminopyridine, aminopyridine N-oxide and amide; R^3 is H or CH_3; M is selected from NH, CH_2, O and S; T is selected from NH, CH_2, O, S and PO; X is selected from S, Se, O, NH or is absent; Y is selected from O and S; and Z is a six-membered carbon ring, a five-membered carbon ring, a seven-membered carbon ring or a heterocyclic five-, six- or seven-membered ring or a fused ring system consisting of 1 to 3 rings (either aryl or

heteroaryl) and R is substituted on the ring or ring system.

It is more preferred if R is substituted furthest from X on ring Z. It is less preferred if R is substituted close to X on ring Z.

Preferably, Z is a benzene ring and R is substituted at the *para* or *meta* positions, most preferably at the *para* position. Substitution at the *ortho* position is less preferred.

By "alkyl" we include and prefer C_{1-20} alkyl, both straight chain and branched. C_{1-5} alkyl is preferred.

By "haloalkyl" we include and prefer C_{1-20} haloalkyl, both straight chain or branched wherein the haloalkyl contains from one to a full number of halogen atoms (ie perhalo). C_{1-5} haloalkyl is preferred.

By "alkoxy" we include and prefer C_{1-20} alkoxy. C_{1-5} alkoxy is preferred.

It is preferred that if Z is a fused ring system each ring of the system is a five, six or seven-membered ring.

It is preferred if M is CH_2 or NH; more preferably NH.

It is preferred if T is NH or CH_2.

It is preferred if R is alkoxy; more preferably methoxy.

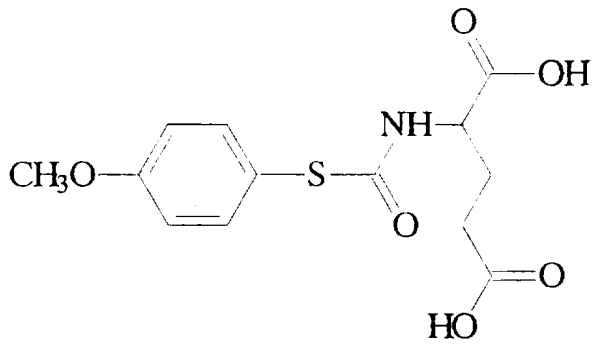
It is preferred if R^2 is OH.

It is preferred if R^3 is H.

It is preferred if X is S.

Preferably, R is methoxy, Z is a benzene ring where R is *para* to X, M is NH_2, T is CH_2, X is S, Y is O, R^2 is OH and R^3 is H. This preferred compound is called In-1 and is described in more detail in Example 1.

The structure of In-1 is shown below:



The chirality of glutamic acid or its analogue is either D or L (R or S).

By "lipid" we include any hydrocarbon chain, whether saturated or unsaturated, up to C_{15} in length. The nature of the lipid may be useful in directing the inhibitor molecule to a particular organ.

By "amino acid" we include any natural or synthetic amino acid. The nature of the amino acid will influence the solubility of the inhibitor molecule.

Suitable amino acids include α-amino acids.

By "α amino acid", we mean any compound having a group

$$R^5 - \underset{H}{\overset{R^4}{C}} - NH_2$$ where R^4 is the residual group of an amino acid,

for example hydrogen, straight or branched C_{1-6} alkyl (such as methyl, iso-propyl, 2-methylpropyl or 1-methylpropyl), hydroxyalkyl (such as $-CH_2OH$ or 1-hydroxyethyl), aralkyl (such as benzyl or 4-hydroxy-benzyl), thiolalkyl (such as $-CH_2SH$), alkylthioalkyl (such as $-CH_2CH_2SCH_3$), acyl (such as $-CH_2COOH$ or $-CH_2CH_2COOH$), amidalkyl (such as $-CH_2CO.NH_2$ or $-CH_2CH_2CO.NH_2$) or linear or cyclic, aromatic or non aromatic, nitrogen-containing heterocyclic groups such as the groups forming part of tryptophan, lysine, arginine or histidine; and R^5 is a group —C(=O)R^6 wherein R^6 is —OH, or any —O—linked or —N—linked radical, for example $-O-$alkyl, -O-alkylaminoalkyl, -O-alkoxyalkyl or $-NH-NHR^4$ wherein R^4 is straight or branched alkyl, optionally substituted by -CN or -OH, an amide group (such as $-CONH_2$) or a hydrazine group (such as $-(CH_2)_2NH(CH_2)_2OH$). Examples of alkylaminoalkyl groups include $CH_3(CH_3)NCH_2CH_2-$ and $CH_3(CH_3)NCH_2CH_2NHCH_2CH_2-$.

By "alkyl", we include branched or straight chain alkyl of up to 20 carbon atoms, preferably 1-10 carbon atoms, more preferably 1-6 or 1-4 carbon atoms.

We include all of the 20 α-amino acids commonly found in naturally-occurring proteins and their D-isomers; less common naturally-occurring α-amino acids found in proteins, such as 4-hydroxyproline, 5-hydroxylysine, desmosine, ε-N-methyllysine, 3-methylhistidine and isodesmosine and their D-isomers; naturally-occurring amino acids not found in proteins, such as β-alanine, γ-aminobutyric acid, homocysteine, homoserine, citrulline, ornithine, canavanine, djenkolic acid and β-cyanoalanine and their D-isomers; and di-, tri-, tetra-, penta-, oligo- or polypeptides based on these or other amino acids (providing that the amino acid joined to the anthracenyl ring is an α amino acid) which peptides may

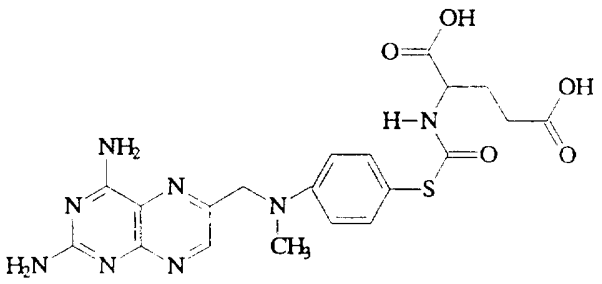
optionally include non-amino acid residues or side elements such as sugar residues. Preferably, there is only a single amino acid group.

Thus, R^4 may be: hydrogen; straight or branched chain C_{1-4} alkyl (for example methyl, isopropyl, isobutyl or sec-butyl); aryl-C_{1-4}-alkyl (for example benzyl, β-indolylmethyl, 4-hydroxybenzyl or 4-imidazolylmethyl); C_{1-4}-alkylthio-C_{1-4}-alkyl (for example methylthioethyl); hydroxy-C_{1-4}-alkyl (for example hydroxymethyl or 1-hydroxyethyl); mercaptomethyl (for example $-CH_2SH$); C_{1-4} amide (for example $-CH_2C(O)NH_2$ or $-CH_2CH_2C(O)NH_2$); C_{1-4} alkyl carboxylate (for example $-CH_2C(O)OH$ or $-CH_2CH_2C(O)OH$); C_{1-6} alkylamine (for example $(CH_2)_4NH_2$); and imino(C_{1-6})alkyl-amine (for example $-(CH_2)_3NHC(=NH)NH_2$).

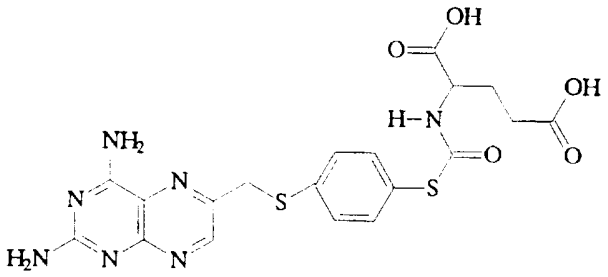
By "derivatives" of the amino acids, we include salts (acid or base addition), esters, amides, hydrazides and hydroxamic acids and other derivatives.

By "carbohydrate" we include all natural and synthetic carbohydrates especially mono- and disaccharides. Galactose and mannose are particularly preferred as they are suitable for targeting the inhibitor to hepatocytes.

Other suitable compounds are



and



Details of the synthesis and properties of some of these CPG2 inhibitors are given in Example 1. Particularly preferred inhibitors are those shown in bold in Scheme 1 in Example 1.

Inhibitors for use with other enzymes (as listed) include:

a) **Carboxypeptidase A** (Haenseler *et al* (1992) *Biochemistry* **31**, 214-220: Hydrolyses terminal peptide linkage adjacent to free carboxyl group. Wide specificity, maximally active with aromatic side group (Figure 6. Possible inhibitors for this enzyme are also given, Figure 7.)

b) **Glucuronidase** (Mitaku *et al* (1994) *Ann. Oncol.* **5** (Suppl. 5), 76: Sugar lactones are known to be inhibitors of this enzyme, such as D-saccharic acid-1,4-lactone for β-glucuronidase.

c) **β-Lactamase** (Svensson *et al* (1993) *Bioconj. Chem.* **3**, 176-181: Clavulanic acid is a known inhibitor of this enzyme. Other structures are also known sulbactam, thienamycin and imipenem. The potent antibiotics in this family possess β-lactamase inhibitory properties and thus run into thousands of derivatives. However, the above compounds are the most potent known at present.

It will be appreciated that salts of the inhibitor molecules form part of the invention.

In a further embodiment it is preferred that the molecule capable of substantially inhibiting the conversion of said substrate is provided in the form of a precursor.

Suitably, the precursor of said molecule comprises said molecule in a form capable of releasing said molecule in a host, such as a patient. Thus, the precursor may comprise said molecule bound to an entity through a linkage, said linkage being biodegradable (and therefore cleavable within the host) or the precursor may comprise said molecule bound to an entity, whether or not through a linkage, said entity being biodegradable. In any case the molecule is released from the precursor in the host, such as in the patient to be treated.

The small molecule enzyme inactivating system can be further modified to encompass a biodegradable macromolecule. This embodiment has covalently attached molecules of inhibitor so that the macromolecule-inhibitor may not be able to inhibit the enzyme while it is attached to the macromolecule. However, on degradation at normal tissues the inhibitor is released and diffuses so as to inhibit any enzyme in the vicinity. The characteristics of the macromolecule-inhibitor can be chemically modified to match the location of distribution required for the therapy, thus avoiding inhibition at the tumour site. An example of this type of system is shown in Figure 8 with reference to human serum albumin (HSA). The conjugate can readily be made and purified. The conjugate is very water soluble and rapidly metabolised by a variety of tissues, thus biodistribution is similar to the non-specific distribution of the antibody-enzyme conjugate. This macromolecule is kept in the circulation as the size is

above the glomular filtration of the kidneys. The protein backbone is hydrolysed by lysosomal enzymes, or liver enzymes, and the resulting small molecule inhibitor diffuses into the cytoplasm and then into the extravascular region of the tissue, to inhibit any enzyme present. The other by-products are expected to be non-toxic as they are based on normal human serum albumin metabolism.

The macromolecule may comprise proteins, carbohydrates or synthetic polymers such as N2-Hydroxy propyl-methacrylamide (HMPA). The macromolecule is chosen so that it can be degraded, preferably enzymatically, into small units, the inhibitor released and allowed to penetrate the vasculature around the tissue and thereby inhibit the non-specifically targeted enzyme.

The linkage between the inhibitor and the macromolecule is preferably of the amidomethylester type, this has a half-life sufficient for our purposes (4-5 hr in plasma) or a peptide type with an amino acid sequence as a substrate for a particular degradative enzyme, such as Gly-Phe-Leu-Gly. This sequence is degraded by lysosomal enzymes, such as cathepsins.

Suitable degradable linkers include those comprising esters, non-sterically-hindered disulphides, phosphates, amides, glycosides and thioesters.

Suitable non-degradable linkers include those comprising hydrocarbons, ethers, thioethers, D-amino acids, L-sugars and sterically-hindered disulphides.

A further embodiment provides a non-degradable macromolecule comprising the inhibitor molecule, such as dextran, polylysine and polyacrylamide which would be useful for long circulation times, and to

target particular organs that need to be protected, such as bone marrow, liver and central nervous tissue, this may be done by appropriate derivatisation of the polymer such as galactosylation (liver), glucosylation (brain) and polyethyleneglycosylation (increased water solubility for bone marrow). The inhibitor molecule is attached via a cleavable linker and can be cleaved at particular locations or at a particular rate, depending on which type of linker is used. The macromolecule may reach the tumour but the rate of inhibitor released is insufficient to inhibit all the enzyme that has been targeted to the tumour site.

The use of inhibitors non-cleavably linked to a non-degradable carrier would be to inactivate any enzyme in circulation. These conjugates have advantages over clearance antibodies (such as SB43) by being cheaper to produce, and having a longer shelf life. The pharmacokinetic properties of the polymer are more readily tailored for particular targeting purposes. The synthesis of these conjugates involves ether, thioether and sterically hindered amide/ester bonds to polymers such as dextran, polylysine and alginates. Suitably, the inhibitor is attached using any one of the aforementioned linkers by methods known in the art.

The use of inhibitors non-cleavably linked to a non-degradable carrier are preferred for use in the system of the second particularly preferred embodiment.

In a preferred embodiment the precursor comprises a liposome and the molecule is released from the liposome within the host. Suitably, the inhibitor is trapped within the liposome on administration and is released within the host.

The use of liposomes as drug carriers has been described in G. Gregoriadis (ed.) Liposomes as drug carriers: recent trends and progress, John Wiley and Sons, Chichester, UK, 1988 and references therein. A variety of methods of making liposomes are available including those described by Lichtenberg and Barenholz (1988) *Meth. Biochem. Anal.* **33**, 337-462; Szoka and Papahadjopoulos (1978) *Proc. Natl. Acad. Sci. USA* **75**, 4194; Mauk and Gamble (1979) *Anal. Biochem.* **94**, 302-307; Foressen *et al* (1992) *Cancer Res.* **52**, 3255-3261; and Perez-Soler and Khokhar (1992) *Cancer Res.* **52**, 6331-6347, all incorporated herein by reference.

It is preferred if the liposomes are able to target particular organs.

A second aspect of the invention provides a method of destroying target cells in a host, the method comprising the steps of administering to the host (a) a compound comprising a target cell-specific portion and a portion capable of converting a substantially non-toxic substance into another substance which is cytotoxic; (b) a molecule capable of substantially inhibiting the conversion of said substantially non-toxic substance, or a precursor of said molecule; and (c) the substantially non-toxic substance.

Preferably, the compound comprising a target cell-specific portion and a portion capable of converting a substantially non-toxic substance into another substance which is cytotoxic is administered and, once there is an optimum balance between the target cell to normal cell ratio of the compound and the absolute level of compound associated with the target, the molecule capable of substantially inhibiting the conversion of said substantially non-toxic substance, or a precursor of said molecule, is administered. Then the substantially non-toxic substance (such as a pro-drug) is administered. The interval between administration of the target

cell-specific portion and a portion capable of converting a substantially non-toxic substance into another substance which is cytotoxic (for example, an antibody-enzyme conjugated) and the inhibitor molecule will depend on the target cell localisation characteristics of the compound, but typically it will be between 6 and 48 hours.

Suitably, pro-drug administration commences as soon as the plasma activity of enzyme and, by inference, the activity in normal tissues, is insufficient to catalyse enough pro-drug to cause toxicity. In the case of carboxypeptidase G2, the enzyme activity is preferably below 0.1 enzyme units/ml, more preferably below 0.02 enzyme units/ml and most preferably zero. One enzyme unit of carboxypeptidase G2 is defined as the amount of enzyme which hydrolyses 1 μ mol of methotrexate/min at pH 7.0 and 25°C.

Preferably, the target cell is a tumour cell.

A third aspect of the invention provides a method of treating a mammal harbouring a tumour, the method comprising the steps of administering to the mammal (a) a compound comprising a tumour cell-specific portion and a portion capable of converting a substantially non-toxic substance into another substance which is cytotoxic; (b) a molecule capable of substantially inhibiting the conversion of said substantially non-toxic substance, or a precursor of said molecule; and (c) the substantially non-toxic substance.

Thus, in the second and third aspects of the invention the cytotoxic compound is released in relatively high concentration at the target or tumour site but not at non-tumour sites.

A fourth aspect of the invention provides a method of destroying a target cell in a host, the method comprising administering to the host (a) a compound comprising a target cell-specific portion and a portion capable of converting a substance which, in its native state, is able to inhibit the effect of a cytotoxic agent into another substance which has less effect against said cytotoxic agent; (b) a molecule capable of substantially inhibiting the conversion of said substance, or a precursor of said molecule; (c) a cytotoxic agent; and (d) said substance.

Preferably, the compound comprising a target cell-specific portion and a portion capable of converting a substance which, in its native state, is able to inhibit the effect of the cytotoxic agent into a substance which has less effect against said cytotoxic agent is administered and, once there is an optimum balance between the target cell to normal cell ratio of the compound and the absolute level of compound associated with the target, the cytotoxic agent together with the substance capable of blocking the effect of the cytotoxic agent are administered. However, an alternative method of administration would be possible. The amount of the compound of the invention circulating in the blood may be determined by measuring the activity of the enzymatic portion. Conveniently, the molecule capable of substantially inhibiting the conversion of said substance, or a precursor of said molecule, is administered to the patient before administration of the cytotoxic agent or the substance capable of blocking the effect of the cytotoxic agent.

Preferably, the present invention provides a method of treating a mammal harbouring a tumour. Suitably, the mammal is first prepared for tumour therapy by administering a compound comprising a target cell-specific portion and a portion capable of converting a substance which, in its native state, is able to inhibit the effect of the cytotoxic agent into a

substance which has less effect against said cytotoxic agent and allowing the ratio of compound bound to target cells to compound not bound to target cells to reach a desired value. The method then further comprises administering to the mammal a cytotoxic agent and a substance which in its native state is capable of inhibiting the effect of said cytotoxic agent from which a substance which has less effect on the cytotoxic agent can be generated by the inactivating portion of the said compound. Conveniently the molecule capable of substantially inhibiting the conversion of said substance, or a precursor of said molecule, are administered to the patient before administration of the cytotoxic agent or the substance capable of blocking the effect of the cytotoxic agent.

Thus, a fifth aspect of the invention provides a method of treating a mammal harbouring a tumour, the mammal having been prepared for treatment by administering a compound comprising a target cell-specific portion and a portion capable of converting a substance which, in its native state, is able to inhibit the effect of a cytotoxic agent into another substance which has less effect against said cytotoxic agent and allowing the ratio of compound bound to target cells to compound not bound to target cells to reach a desired value, the method comprising administering to the mammal (a) a cytotoxic agent; (b) a molecule capable of substantially inhibiting the conversion of said substance, or a precursor of said molecule; and (c) a substance which in its native state is capable of inhibiting the effect of said cytotoxic agent from which a substance which has less effect on the cytotoxic agent can be generated by the portion capable of converting a substance.

For the fourth and fifth aspects of the invention it is preferred that the molecule capable of substantially inhibiting the conversion of said substance, or a precursor of said molecule, are administered to the host

or patient 6 to 48 hours after the administration of the said compound.

Thus, the fourth and fifth aspects of the invention provide a means to allow continuous action of a cytotoxic agent at target sites (such as tumour sites) whilst protecting normal tissue from the effects of the cytotoxic agent. The substance which in its native state is capable of inhibiting the effect of the cytotoxic agent is given at a dose level sufficient to protect the normal tissues. However, the substance reaching tumour sites is inactivated before it can enter the cells and protect them from the cytotoxic agent. In this way, normal tissues are protected from the effects of the cytotoxic agent whereas the protective molecule is rapidly degraded at tumour sites.

In the second, third, fourth and fifth aspects of the invention the components can be administered in any suitable way, usually parenterally, for example intravenously, intraperitoneally or intravesically, in standard, sterile, non-pyrogenic formulations of diluents and carriers, for example isolonic saline (when administered intravenously).

In the second and third aspects of the invention the preferred molecules capable of substantially inhibiting the conversion of said substantially non-toxic substance into another substance which is cytotoxic are the same as those preferred in the first aspect of the invention and, especially, those preferred in the first particularly preferred embodiment.

In the fourth and fifth aspects of the invention the preferred molecules capable of substantially inhibiting the conversion of said substance are the same as those preferred in the first aspect of the invention and, especially, those preferred in the second particularly preferred embodiment.

When treating a host, such as a patient with a tumour, it is preferred if the ratio administered, in molar terms, of the compound comprising a target cell-specific portion and a portion capable of converting a substance into another substance and the molecule capable of substantially inhibiting the conversion of said substance is between 100:1 and 1:100, more preferably between 10:1 and 1:10, more preferably still between 5:1 and 1:5 and most preferably 1:1. In other words, it is most preferred if the same molar amount is administered. The most suitable ratio can, however, be determined by clinician having regard to the nature of said compound and said molecule.

A sixth aspect of the invention provides a pharmaceutical composition comprising a molecule capable of substantially inhibiting the conversion of a substance as defined in the first aspect of the invention and a pharmaceutically acceptable carrier.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient (compound of the invention) with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose

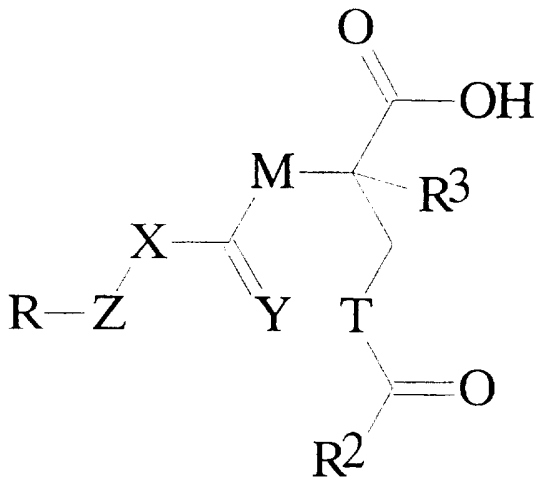
containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose or an appropriate fraction thereof, of an active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question.

It is preferred if the pharmaceutical composition comprises an inhibitor of any of the previously mentioned enzymes for use in the methods of the invention. It is particularly preferred if the enzyme is any one of carboxypeptidase G2, carboxypeptidase A, glucuronidase or β-lactamase.

It is particularly preferred if the pharmaceutical composition comprises any one of



wherein R is selected from alkyl, haloalkyl, cycloalkyl, aryl, substituted aryl (1 to 5 substituents selected from halogen, alkyl, alkoxy, NH_2, OH, NR_2, COOH, CN, $CONH_2$), heteroaryl (5 or 6 membered ring containing 1 to 3 heteroatoms selected from N or S), OH, alkoxy, H, halogen, NH_2, O-sugar, O-amino acid, N-sugar, N-amino acid, aminopyridine N-oxide (aminopy N^+O^-), alkenyl, phenyl, nitro, nitroso, carbohydrate, aminoacid, lipid, pteridine derivative; R^2 is selected from OH, aminopyridine, aminopyridine N-oxide and amide; R^3 is H or CH_3; M is selected from NH, CH_2, O and S; T is selected from NH, CH_2, O, S and PO; X is selected from S, Se, O, NH or is absent; Y is selected from O and S; and Z is a six-membered carbon ring, a five-membered carbon ring, a seven-membered carbon ring or a heterocyclic five-, six- or seven-membered ring or a fused ring system consisting of 1 to 3 rings (either aryl or heteroaryl) and R is substituted on the ring or ring system.

Preferred compounds are those discussed above in relation to the first aspect of the invention.

A particularly preferred molecule is wherein R is methoxy, Z is a benzene ring where R is *para* to X, M is NH, T is CH_2, X is S, Y is O, R_2 is OH

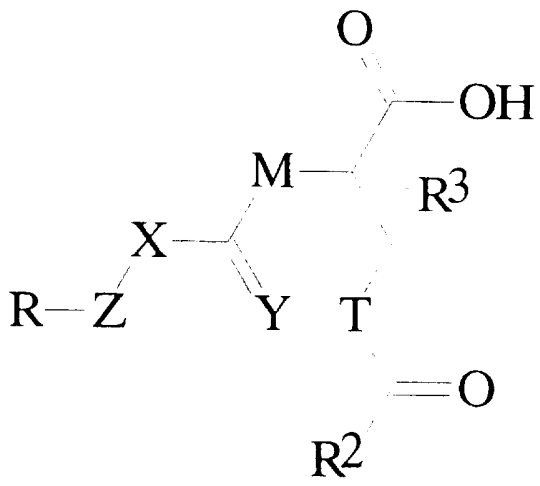
and R_3 is H.

A seventh aspect of the invention provides a compound as defined in the sixth aspect of the invention for use in medicine.

An eighth aspect of the invention provides use of a compound of the sixth aspect of the invention in the manufacture of a medicament for treating a patient with cancer.

Preferably, the patient has been, is being, or will be administered a compound as defined in the first aspect of the invention.

A ninth aspect of the invention provides a compound:



wherein R is selected from alkyl, haloalkyl, cycloalkyl, aryl, substituted aryl (1 to 5 substituents selected from halogen, alkyl, alkoxy, NH_2, OH, NR_2, COOH, CN, $CONH_2$), heteroaryl (5 or 6 membered ring containing 1 to 3 heteroatoms selected from N or S), OH, alkoxy, H, halogen, NH_2, O-sugar, O-amino acid, N-sugar, N-amino acid, aminopyridine N-oxide

(aminopy N^+O^-), alkenyl, phenyl, nitro, nitroso, carbohydrate, aminoacid, lipid, pteridine derivative; R^2 is selected from OH, aminopyridine, aminopyridine N-oxide and amide; R^3 is H or CH_3; M is selected from NH, CH_2, O and S; T is selected from NH, CH_2, O, S and PO; X is selected from S, Se, O, NH or is absent; Y is selected from O and S; and Z is a six-membered carbon ring, a five-membered carbon ring, a seven-membered carbon ring or a heterocyclic five-, six- or seven-membered ring or a fused ring system consisting of 1 to 3 rings (either aryl or heteroaryl) and R is substituted on the ring or ring system.

Preferred compounds are those discussed above in relation to the first aspect of the invention.

Preferably, R is methoxy, Z is a benzene ring where R is *para* to X, M is NH, T is CH_2, X is S, Y is O, R_2 is OH and R_3 is H.

Synthetic methods for producing these molecules is given in Example 1. At least some of the molecules are inhibitors of carboxypeptidase G2 thus a tenth aspect of the invention provides a method of inhibiting carboxypeptidase G2 comprising providing a compound according to the ninth aspect of the invention.

The invention will now be described in more detail with reference to the following Examples and Figures wherein

Figure 1 shows the *in vitro* cytotoxicity of In-1 on LS1747 cells.

Figure 2 shows the effect of In-1 on CPG2 enzyme activity.

Figure 3 shows a Lineweaver-Burke plot which indicates that In-1 is a

non-competitive inhibitor. V_{max} has been reduced but K_m is similar to the substrate, methotrexate (MTX). This indicates that increasing concentration of MTX would not displace the inhibitor. More importantly, during therapy high dose of pro-drug should not displace the inhibitor from inhibited enzyme.

Figure 4 shows a synthetic scheme for an inhibitor for CPG2 In-1.

Figure 5 shows in general scheme for synthesis of inhibitors for CPG2.

Figure 6 shows the structure of a substrate for carboxypeptidase A.

Figure 7 shows the structures of possible inhibitors of carboxypeptidase A: these are inhibitors because of the sulphur substituents in compounds 140 and 142, and because of the carbocyclic structure in place of -NH- in compound 141.

Figure 8 describes macromolecule-supported inhibitors on biologically cleavable systems. HSA is human serum albumin.

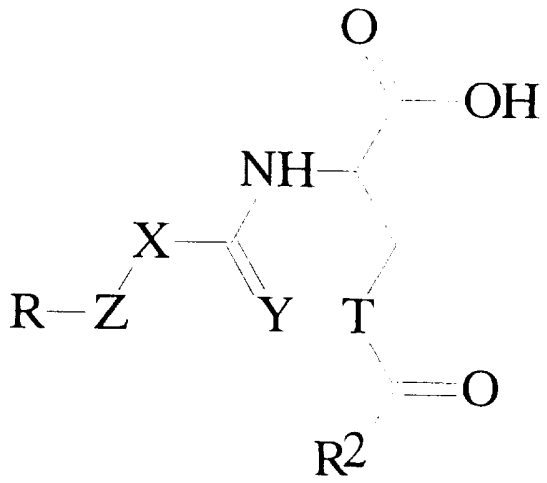
Example 1: Inhibitors of carboxypeptidase G2 (CPG2)

The synthesis of inhibitors for CPG2 was based on the knowledge of compounds for activation by CPG2 and their K_M and k_{cat} values as substrates for CPG2. It was known that the enzyme required $zinc^{2+}$ for its activity thus, preferably, a sulphur atom is present in the inhibitor, the said sulphur atom having a high affinity for zinc. The inhibitors in general are irreversible inhibitors or ones with such a low k_{cat} that it would allow non-specifically targeted enzyme to be inhibited and cleared before toxic levels of active drug were generated. Conveniently, the inhibitors

are active site inhibitors of CPG2 which bind to the catalytic site. It is known that a glutamic acid moiety is present on the best substrates of CPG2, however, gamma substituted derivatives were also found to be substrates (such as pyridyl derivatives). The phenyl ring is also desirable for this particular enzyme. There is considerable flexibility in the *para* position of the phenyl ring (compare methotrexate structure) but only some *meta* and *ortho* substitutions on the benzene ring may be acceptable. The linkage between the phenyl ring and the glutamic acid is very important as this position is at the active site. Thus, preferred inhibitors have particular characteristics, quite distinct from pro-drugs. The inhibitor should have a low K_i, and should remain bound in the active site resulting in a k_{cat} that is as low as possible, ideally zero as for an irreversible inhibitor. A thiocarbamate linkage satisfies these requirements, since this linkage resulted in lower k_{cat} values compared to benzoic acid mustard pro-drugs (Springer *et al* (1991) *Eur. J. Cancer* 27, 1361-1366). A series of potential compounds was designed to investigate our hypothesis, these are given in scheme (1) and Figure 5. The chemical synthesis of In-1(No 1 in Scheme 1) is given in scheme 2 and Figure 4. This derivative was chosen on the basis that the sulphur atom was present in the molecule and this would complex with the zinc ion in the active site of the enzyme. The *p*-methoxy-benzene thiol moiety was chosen as this did not require protection, fewer by-products would result, the product (23), scheme (2), would be lipophilic and easily purified by chromatography. The compound (1) was also a good model to quickly check the efficiency of the chemistry for the synthesis of thiocarbamate derivatives. Two derivatives were successfully synthesised: the *p*-hydroxy-thiophenol (2) and the *p*-methoxy-thiophenol (1). These derivatives were used to determine K_i and k_{cat} values with CPG2, IC50 towards LS174T cells and to test the inhibitor/ADEPT hypothesis *in vivo*. Data is reported here for (1). The inhibitors presented in scheme 1, include the optical isomers

(D/L) of the amino acid (glutamic acid) and structural isomers of various substitutions on the aromatic ring. In addition, glutamic acid analogues and α-methyl glutamic acid analogues are also included. Some γ-derivatised glutamic acid analogues are also given as examples.

Scheme 1



No	R	D/L	R^2	T	X	Y	Z
1	**OMe**	**L**	**OH**	**CH_2**	**S**	**O**	**C**
2	**OH**	**L**	**OH**	**CH_2**	**S**	**O**	**C**
3	H	D/L	OH	CH_2	S	O	C
4	I	D/L	OH	CH_2	S	O	C
5	**NH_2**	**D/L**	**OH**	**CH_2**	**S**	**O**	**C**
6	**O-Sugar**		**OH**	**CH_2**	**S**	**O**	**C**
7	**O-Aminoacid**		**OH**	**CH_2**	**S**	**O**	**C**
8	**N-Sugar**		**OH**	**CH_2**	**S**	**O**	**C**
9	**N-Aminoacid**		**OH**	**CH_2**	**S**	**O**	**C**
10	OMe	D/L	OH	CH_2	O	S	C
12	OH	D/L	OH	CH_2	O	S	C
13	**H**	**D/L**	**OH**	**CH_2**	**O**	**S**	**C**
14	**I**	**D/L**	**OH**	**CH_2**	**O**	**S**	**C**
15	NH_2	D/L	OH	CH_2	O	S	C

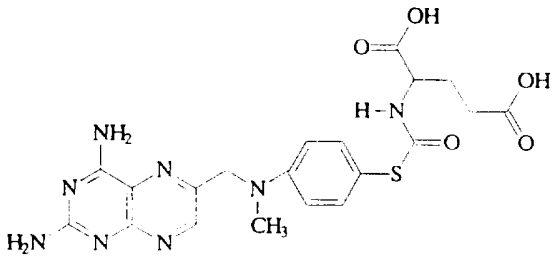
	16	O-Sugar		OH	CH_2	O	S	C
	17	O-Aminoacid		OH	CH_2	O	S	C
	18	**N-Sugar**		**OH**	**CH_2**	**O**	**S**	**C**
	19	**N-Aminoacid**		**OH**	**CH_2**	**O**	**S**	**C**
5	20	OMe	D/L	OH	CH_2	-	S	C
	21	OH	D/L	OH	CH_2	-	S	C
	22	**H**	**D/L**	**OH**	**CH_2**	**-**	**S**	**C**
	23	**I**	**D/L**	**OH**	**CH_2**	**-**	**S**	**C**
	24	NH_2	D/L	OH	CH_2	-	S	C
10	25	O-Sugar		OH	CH_2	-	S	C
	26	O-Aminoacid		OH	CH_2	-	S	C
	27	N-Sugar		OH	CH_2	-	S	C
	28	N-Aminoacid		OH	CH_2	-	S	C
	29	**OMe**	**D/L**	**OH**	**O**	**S**	**O**	**C**
15	**30**	**OH**	**D/L**	**OH**	**O**	**S**	**O**	**C**
	31	H	D/L	OH	O	S	O	C
	32	I	D/L	OH	O	S	O	C
	33	NH_2	D/L	OH	O	S	O	C
	34	O-Sugar		OH	O	S	O	C
20	35	O-Aminoacid		OH	O	S	O	C
	36	N-Sugar		OH	O	S	O	C
	37	N-Aminoacid		OH	O	S	O	C
	38	**OMe**	**D/L**	**OH**	**S**	**S**	**S**	**C**
	39	H		aminopyridine (AP)	CH_2	S	O	C
25	**40**	**OMe**	**D/L**	**AP**	**CH_2**	**S**	**O**	**C**
	41	**OMe**	**D/L**	**AP**	**O**	**S**	**O**	**C**
	42	**OMe**		**aminopyN^+O^-(APO)**	**CH_2**	**S**	**O**	**C**
	43	**APO**	**D/L**	**OH**	**CH_2**	**O**	**S**	**Het**
	44	**APO**	**D/L**	**APO**	**CH_2**	**S**	**O**	**Het**
30	**45**	**APO**	**D/L**	**APO**	**O**	**S**	**O**	**Het**
	46	**O-Sugar**		**OH**	**CH_2**	**S**	**O**	**Het**
	47	O-Aminoacid		OH	CH_2	S	O	Het

	48	N-Sugar		OH	CH_2	S	O	Het
	49	N-Aminoacid		OH	CH_2	S	O	Het
	50	**O-Sugar**		**AP**	**CH_2**	**S**	**O**	**Het**
	51	O-Aminoacid		AP	CH_2	S	O	Het
5	52	N-Sugar		AP	CH_2	S	O	Het
	53	**N-Aminoacid**		**AP**	**CH_2**	**S**	**O**	**Het**
	54	**OMe**	**D/L**	**OH**	**CH_2**	**S**	**O**	**C5**
	55	**OH**	**D/L**	**OH**	**CH_2**	**S**	**O**	**C5**
	56	H	D/L	OH	CH_2	S	O	C5
10	57	I	D/L	OH	CH_2	S	O	C5
	58	**NH_2**	**D/L**	**OH**	**CH_2**	**S**	**O**	**C5**
	59	**O-Sugar**		**OH**	**CH_2**	**S**	**O**	**C5**
	60	**O-Aminoacid**		**OH**	**CH_2**	**S**	**O**	**C5**
	61	**N-Sugar**		**OH**	**CH_2**	**S**	**O**	**C5**
15	**62**	**N-Aminoacid**		**OH**	**CH_2**	**S**	**O**	**C5**
	63	**OMe**	**L**	**OH**	**CH_2**	**S**	**O**	**C7**
	64	**OH**	**L**	**OH**	**CH_2**	**S**	**O**	**C7**
	65	H	D/L	OH	CH_2	S	O	C7
	66	I	D/L	OH	CH_2	S	O	C7
20	**67**	**NH_2**	**D/L**	**OH**	**CH_2**	**S**	**O**	**C7**
	68	**O-Sugar**		**OH**	**CH_2**	**S**	**O**	**C7**
	69	**O-Aminoacid**		**OH**	**CH_2**	**S**	**O**	**C7**
	70	**N-Sugar**		**OH**	**CH_2**	**S**	**O**	**C7**
	71	**N-Aminoacid**		**OH**	**CH_2**	**S**	**O**	**C7**
25	**72**	**OMe**	**L**	**amide**	**CH_2**	**S**	**O**	**C**
	73	**OH**	**L**	**amide**	**CH_2**	**S**	**O**	**C**
	74	H	D/L	amide	CH_2	S	O	C
	75	I	D/L	amide	CH_2	S	O	C
	76	**NH_2**	**D/L**	**amide**	**CH_2**	**S**	**O**	**C**
30	**77**	**O-Sugar**		**amide**	**CH_2**	**S**	**O**	**C**
	78	**O-Aminoacid**		**amide**	**CH_2**	**S**	**O**	**C**
	79	**N-Sugar**		**amide**	**CH_2**	**S**	**O**	**C**

	80	**N-Aminoacid**		**amide**	**CH_2**	**S**	**O**	**C**
	81	OMe	D/L	OH	CH_2	-	S	HET
	82	OH	D/L	OH	CH_2	-	S	HET
	83	**H**	**D/L**	**OH**	**CH_2**	-	**S**	**HET**
5	**84**	**I**	**D/L**	**OH**	**CH_2**	-	**S**	**HET**
	85	NH_2	D/L	OH	CH_2	-	S	HET
	86	O-Sugar		OH	CH_2	-	S	HET
	87	O-Aminoacid		OH	CH_2	-	S	HET
	88	N-Sugar		OH	CH_2	-	S	HET
10	89	N-Aminoacid		OH	CH_2	-	S	HET
	90	**OMe**	**D/L**	**OH**	**O**	**S**	**O**	**HET**
	91	**OH**	**D/L**	**OH**	**O**	**S**	**O**	**HET**
	92	H	D/L	OH	O	S	O	HET
	93	I	D/L	OH	O	S	O	HET
15	94	NH_2	D/L	OH	O	S	O	HET
	95	O-Sugar		OH	O	S	O	HET
	96	O-Aminoacid		OH	O	S	O	HET
	97	N-Sugar		OH	O	S	O	HET
	98	N-Aminoacid		OH	O	S	O	HET
20	**99**	**alkoxy**		**OH**	**CH_2(R/S)**	**S**	**O**	**CHET**
	100	**alkenyl**		**OH**	**CH_2(R/S)**	**S**	**O**	**CHET**
	101	Phenyl		OH	CH_2(R/S)	S	O	CHET
	102	Halogen		OH	CH_2(R/S)	S	O	CHET
	103	**Nitro/Nitroso**		**OH**	**CH_2(R/S)**	**S**	**O**	**CHET**
25	**104**	**Carbohydrate**		**OH**	**CH_2(R/S)**	**S**	**O**	**CHET**
	105	**Aminoacid**		**OH**	**CH_2**	**S**	**O**	**CHET**
	106	**Lipid**		**OH**	**CH_2**	**S**	**O**	**CHET**
	107	**Pteridine derivative**		**OH**	**CH_2**	**S**	**O**	**CHET**
	108	**OMe**	**L**	**OH**	**CH_2**	**Se**	**O**	**CHET**
30	**109**	**OH**	**L**	**OH**	**CH_2**	**Se**	**O**	**CHET**
	110	H	D/L	OH	CH_2	Se	O	CHET
	111	I	D/L	OH	CH_2	Se	O	CHET

112	NH_2	D/L	OH	CH_2	Se	O	CHET
113	O-Sugar		OH	CH_2	Se	O	CHET
114	O-Aminoacid		OH	CH_2	Se	O	CHET
115	N-Sugar		OH	CH_2	Se	O	CHET
116	N-Aminoacid		OH	CH_2	Se	O	CHET
117	OMe	D/L(α-methyl)	OH	CH_2	S	O	CHET
118	OH	D/L(α-methyl)	OH	CH_2	S	O	CHET
119	H	D/L(α-methyl)	OH	CH_2	S	O	CHET
120	I	D/L(α-methyl)	OH	CH_2	S	O	CHET
121	NH_2	D/L(α-methyl)	OH	CH_2	S	O	CHET
122	O-Sugar(α-methyl)		OH	CH_2	S	O	CHET
123	O-Aminoacid		OH	CH_2	S	O	CHET
124	N-Sugar(α-methyl)		OH	CH_2	S	O	CHET
125	N-Aminoacid		OH	CH_2	S	O	CHET

126 D/L ISOMERS



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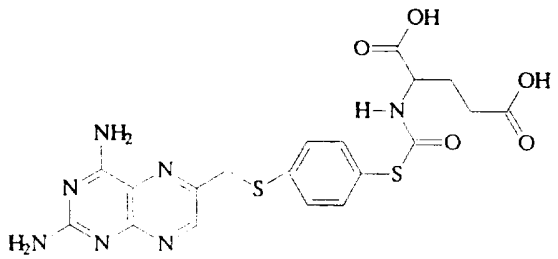


The aromatic ring size may also be changed, C5 refers to a five membered carbon ring and C7 refers to a seven membered carbon ring. C refers to the carbon skeleton of benzene ring (C6), HET refers to heterocyclic ring structure relating to 5,6 and 7 membered ring systems.

The chirality of glutamic acid or its analogue is either D or L, (strictly R or S nomenclature).

Particularly preferred inhibitors are those shown in bold.

Derivatives 107, 126 and 127 are methotrexate derivatives which would be potent CPG2 inhibitors but may be toxic as DHFR inhibitors (Rahman & Chaabra (1988).

Scheme 2

Chemistry

Di-t-butyl glutamic acid HCl (128) (2.05g, 6.95mmol) was activated with *p*-nitro phenyl chloroformate (129) (1.4g, 6.95mmol) in dichloromethane (20ml) in the presence of triethylamine (2ml). The reaction mixture was refluxed for 25min and then stirred for 1h at room temperature. The reaction mixture was flushed with argon and then a solution of p-methoxy-benzene thiol (131) (1.08g, 7.70mmol) in dichloromethane (20ml) was added, and heated to reflux for 10min. The cooled solution was then stirred for 5hs at room temperature, monitoring by TLC for disappearance of starting material. The precipitate was filtered and the filtrate concentrated *in vacuo*, and chromatographed on silica gel, eluent dichloromethane. The colourless oil (132) (2.8g, 95% yield) was treated with hexane and HCl (g) and stirred overnight. The white product was filtered, washed with hexane and dried *in vacuo* to result in 1.16g, 53% overall yield, mpt 115°C, of analytically pure In-1 (1), Scheme (2). ^{1}NMR (D_6-DMSO) δ/ppm :-1.6 (m, 2H), 2.1 (m, 2H), 3.5 (s, 3H), 3.9 (m, 1H), 6.7 (d, 2H), 7.1 (d, 2H), 8.2 (d, 1H) : % CHN analysis requires 49.83, 4.82, 4.47 found 49.5, 4.85, 4.42. Biological data:- IC50 = 122μM (1h exposure, LS174T cells, Figure 1), 95% inhibition of enzyme activity = 15 μM (Figure 2), K_i = 0.3 μM, Line Weaver-Burke Plot is shown in

Figure 3.

A general synthesis of CPG2 inhibitors is shown in Figure 5. The structures in Scheme 1 can all be made by the route shown. The starting material (133) is synthesised by standard peptide chemistry and activated with (134). The derivatives (135) or (136) may be tethered on a polymer support, containing a labile linker, and combinatorial chemistry carried out to produce many hundreds of derivatives at each end of the molecule.

A number of other potential inhibitors of carboxypeptidase G2 have been synthesised in an analogous way to IN-1 by substituting 4-methoxybenzenethiol with the appropriate thiol derivative and according to the scheme shown in Figure 4. Analytical data is given below.

(a) 4-bromophenylsulfamyl-L-glutamic acid

Mpt 142°C. CHN calculated for C_{12} $H_{12}NO_5SBr$, C 39.79, H 3.32, N 3.87: found C 39.76, H 3.38, N 3.77.

(b) 4-chlorophenylsulfamyl-L-glutamic acid

Mpt 127°C. CHN calculated for $C_{12}H_{12}NO_5SCl$, C 45.36, H 3.80, N 4.41: found C 45.59, H 3.96, N 4.31.

(c) 4-methylphenylsulfamyl-L-glutamic acid

Mpt 129°C. CHN calculated for $C_{13}H_{15}NO_5SCl$, C 52.52, H 5.08, N 4.71: found C 52.52, H 5.10, N 4.71.

Biological data: K_i = 1.05 μM.

(d) 3-methylphenylsulfamyl-L-glutamic acid monohydrate

Mpt 70°C. CHN calculated for $C_{13}H_{15}NO_5SCl$, C 49.52, H 5.43, N 4.44: found C 49.57, H 5.43, N 4.40.

(e) 3-aminophenylsulfamyl-L-glutamic acid hydrochloride

Mpt 85°C. ^{1}H NMR dmso-d_6 : δ ppm 8.8 (d,7.8 Hz, 1H), 7.6-7.2 (m, 5H), 4.2 (m, 1H), 2.3 (t,2H), 2.0 (m, 1H), 1.8 (m,1H).

(f) 4-pyridylsulfamyl-L-glutamic acid

^{1}H NMR dmso-d_6 : δ ppm 8.4 (d,7.8Hz,1H), 8.3 (d, 7.8Hz, 2H), 7.4 (d, 7.8Hz, 2H), 4.1(m,1H), 2.4 (m,2H), 2.0 (m,1H), 1.9 (m,1H).

(g) 2-pyrimidinylsulfamyl-L-glutamic acid

^{1}H NMR dmso-d_6 : δ ppm 8.8 (d,4Hz, 2H), 7.3 (m,1H), 4.3 (m,1H), 2.3-1.6 (m,4H).

(h) 4-aminophenylsulfamyl-L-glutamic acid hydrochloride

^{1}H NMR dmso-d_6 : δ ppm 8.3(s), 7.3(m), 7.1(m), 6.5 (d, 7,8Hz), 4.2 (m), 2.3(m), 2.1-1.7(m).

The following thiourethane potential inhibitor for CPG2 has been synthesised by the route described below.

(i) N-(4-trifluoromethylphenylamino-thiocarbonyl)-L-glutamic acid γ-methyl ester triethylammonium salt

To a solution of L-glutamic acid-γ-methyl ester hydrochloride (1.24 g, 7.68 mmol) in 20ml of dry dichloromethane was added 4-trifluoromethyl phenyl isothiocyanate (1.17 ml,7.68 mmol). The reaction mixture was stirred at room temperature for 10 minutes and then treated with triethylamine (2.14 ml, 14.4 mmol). The reaction mixture was stirred for 20 hrs at room temperature and the clear orange solution was concentrated *in vacuo*. The residue was triturated with ethyl acetate and the white precipitate was removed by filtration. The filtrate was concentrated *in*

vacuo and the residue was treated with di-isopropylether to give a creamy solid (1.8 g).

^{1}H NMR dmso-d_6 : δ ppm 11.0 (s,1H), 8.5 (d,7.8Hz,1H), 8.4 (s,1H), 7.9 (d,7.8Hz,1H), 7.5 (t,7.8Hz,1H), 7.4 (d,7.8Hz,1H), 4.5 (d,4Hz,1H), 3.6 (s,3H), 3.0 (q,7.8Hz,6H), 2.4-1.9 (m,4H), 1.2 (t, 7.8Hz, 9H).

Other analogous amino-thio-carbonyl-L-glutamic acid γ-methyl esters can be made using an analogous method by using an appropriate isothiocyanate.

Example 2: Biological Data for IN-1

IC50 = 122 μM, determined for a 1 hour exposure against LS174T human colon carcinoma cells. The growth curve is shown in Figure 1. The effect of IN-1 on CPG2 enzyme activity is shown in Figure 2. At an IN-1 concentration of 15 μM approximately 95% of the CPG2 activity has been inhibited as shown by its inability to convert the drug methotrexate to its deglutamylated form.

Figure 3 is a Lineweaver-Burk plot which shows the effect of IN-1 on CPG2 activity. The results suggest that IN-1 is exhibiting mixed non-competitive inhibition. The K_i of IN-1 was found to be 0.3 μM.

Figure 9 shows the *in vivo* effect of IN-1 on the enzyme activity of CPG2 conjugated to the $F(ab)_2$ fragment of the anti-carcinoembryonic antigen monoclonal antibody A5B7. In this experiment athymic nude mice were administered ca. 27 units of enzyme activity per mouse and 24 hours later some mice were administered either 2.0 mg/mouse or 6.0 mg/mouse of IN-1. One hour later the enzyme activities of a control group (no IN-1 administered) were then compared to the test groups. As shown in Figure

9 the CPG2 activity was reduced considerably in the presence of IN-1.

Example 3: Treatment of patient (I)

Stage 1. Infusion of antibody-enzyme (CPG2) conjugate intravenously, typically over a 2 hour period (5-25,000 enzyme units per m^2, depending on the pro-drug substrate).

Stage 2. 16-30 hours after Stage 1. Inhibitor given in solution by bolus or infusion, intravenously over a period of 1-4 hours (probably 1-20 mg/patient, depending on amount of enzyme given in conjugate).

Stage 3. Administration of pro-drug by multiple bolus injections I.V. or by continuous intravenous infusion, starting 1-24 hours after stage 2, (function of pro-drug with CMDA 1-3 grams daily for 2-5 days).

Example 4: Treatment of patient (II)

Stage 1 and Stage 2 as Example 2.

Stage 3. Drug and drug antagonist (for example, trimetrexate and folinic acid) starting 1-24 hours after stage 2. Trimetrexate given by I.V. infusion and continuing for 3-6 days. Folinic acid by I.V. route or by intramuscular route or by mouth. Trimetrexate dose 40-100 mg/m^2 per day. Folinic acid 10-40 mg/m^2.

CLAIMS

1. A therapeutic system comprising:

(a) a compound comprising a target cell-specific portion and a portion capable of converting a substance into another substance; and

(b) a molecule capable of substantially inhibiting the conversion of said substance, or a precursor of said molecule.

2. A system according to Claim 1 wherein said other substance is cytotoxic and said substance is substantially non-cytotoxic, the system further comprising said substance.

3. A system according to Claim 1 wherein said substance, in its native state, is able to inhibit the effect of a cytotoxic agent and said other substance has less effect against said cytotoxic agent, the system further comprising (a) a cytotoxic agent and (b) said substance.

4. A system according to any one of Claims 1 to 3 wherein said portion capable of converting a substance into another substance is an enzyme, or a macromolecule with catalytic activity.

5. A system according to any one of Claims 1 to 4 wherein the target cell-specific portion comprises an antibody or fragment or derivative thereof.

6. A system according to any one of Claims 1 to 4 wherein the molecule capable of substantially inhibiting the conversion of the substance is not an antibody.

7. A system according to Claim 6 wherein the molecule capable of substantially inhibiting the conversion of the substance has a relative molecular mass $< 10\,000$, or a precursor of said molecule.

8. A system according to Claim 7 wherein the molecule capable of substantially inhibiting the conversion of the substance has a relative molecular mass < 5000, or a precursor of said molecule.

9. A system according to Claim 8 wherein the molecule capable of substantially inhibiting the conversion of the substance has a relative molecular mass < 1000, or a precursor of said molecule.

10. A system according to any one of the preceding claims wherein the molecule capable of substantially inhibiting the conversion of the substance is non-proteinaceous.

11. A system according to Claim 4 wherein the molecule capable of substantially inhibiting the conversion of the substance binds to the active site of the enzyme, or macromolecule with catalytic activity.

12. A system according to Claim 4 wherein the molecule capable of substantially inhibiting the conversion of the substance binds to the active site of the enzyme, or macromolecule with catalytic activity, and is not exposed on the surface of said enzyme or macromolecule.

13. A system according to Claim 4 wherein the molecule capable of substantially inhibiting the conversion of the substance is a substantially irreversible inhibitor of said enzyme or said macromolecule.

14. A system according to any one of Claims 11 to 13 wherein the k_{cat} of said enzyme with respect to the molecule is less than $1s^{-1}$.

15. A system according to Claim 14 wherein the k_{cat} of said enzyme with respect to the molecule is substantially $0s^{-1}$.

16. A system according to any one of Claims 11 to 13 wherein the K_i of said enzyme with respect to the molecule is substantially zero.

17. A system according to any one of the preceding claims wherein the molecule is selective for substantially inhibiting the said conversion.

18. A system according to any one of the preceding claims wherein the molecule capable of substantially inhibiting the conversion of said substance is relatively stable to degradation in the body of a patient.

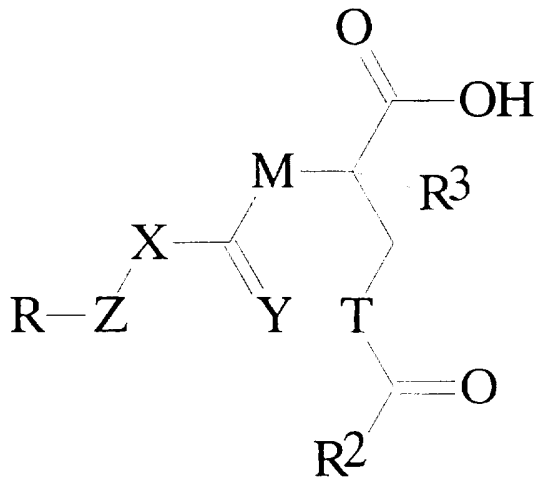
19. A system according to any one of the preceding claims wherein molecule is soluble in aqueous solutions that are suitable for pharmaceutical administration.

20. A system according to Claim 19 wherein the aqueous solution is suitable for intravenous or intramuscular administration.

21. A system according to Claim 2 wherein the portion capable of converting a substance into another substance is at least the catalytic portion of carboxypeptidase G2.

22. A system according to Claim 21 wherein the substance is a nitrogen mustard glutamate pro-drug.

23. A system according to Claim 21 or 22 wherein the molecule capable of substantially inhibiting the conversion of said substance into another substance is any one of



wherein R is selected from alkyl, haloalkyl, cycloalkyl, aryl, substituted aryl (1 to 5 substituents selected from halogen, alkyl, alkoxy, NH_2, OH, NR_2, COOH, CN, $CONH_2$), heteroaryl (5 or 6 membered ring containing 1 to 3 heteroatoms selected from N or S), OH, alkoxy, H, halogen, NH_2, O-sugar, O-amino acid, N-sugar, N-amino acid, aminopyridine N-oxide (aminopy N^+O^-), alkenyl, phenyl, nitro, nitroso, carbohydrate, aminoacid, lipid, pteridine derivative; R^2 is selected from OH, aminopyridine, aminopyridine N-oxide and amide; R^3 is H or CH_3; M is selected from NH, CH_2, O and S; T is selected from NH, CH_2, O, S and PO; X is selected from S, Se, O, NH or is absent; Y is selected from O and S; and Z is a six-membered carbon ring, a five-membered carbon ring, a seven-membered carbon ring or a heterocyclic five-, six- or seven-membered ring or a fused ring system consisting of 1 to 3 rings (either aryl or heteroaryl) and R is substituted on the ring or ring system.

24. A system according to Claim 23 wherein R is substituted furthest from X on ring Z.

25. A system according to Claim 23 wherein Z is a benzene ring.

26. A system according to Claim 25 wherein R is substituted *para* to X.

27. A system according to Claim 23 wherein M is CH_2 or NH.

28. A system according to Claim 23 wherein T is CH_2 or NH.

29. A system according to Claim 23 wherein R is alkoxy, preferably methoxy.

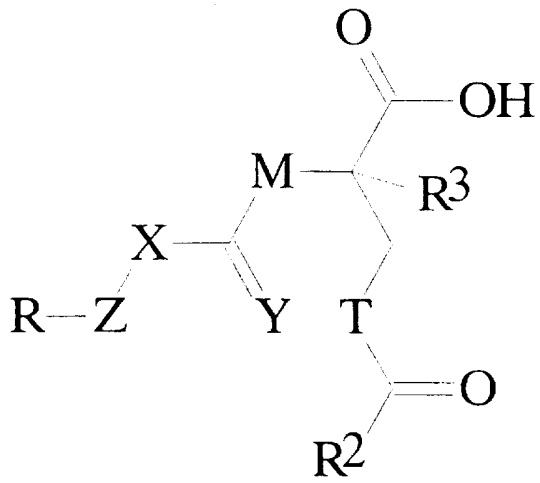
30. A system according to Claim 23 wherein R is methoxy, Z is a benzene ring where R is *para* to X, M is NH, T is CH_2, X is S, Y is O, R^2 is OH and R^3 is H.

31. A system according to Claim 3 wherein the portion capable of converting a substance which, in its native state, is able to inhibit the effect of a cytotoxic agent to said other substance which has less effect against said cytotoxic agent is at least the catalytic portion of carboxypeptidase G2.

32. A system according to Claim 31 wherein said substance is folinic acid.

33. A system according to Claim 31 or 32 wherein said cytotoxic agent is trimetrexate.

34. A system according to any one of Claims 31 to 33 wherein the molecule capable of substantially inhibiting the conversion of said substance into another substance is any one of



wherein R is selected from alkyl, haloalkyl, cycloalkyl, aryl, substituted aryl (1 to 5 substituents selected from halogen, alkyl, alkoxy, NH_2, OH, NR_2, COOH, CN, $CONH_2$), heteroaryl (5 or 6 membered ring containing 1 to 3 heteroatoms selected from N or S), OH, alkoxy, H, halogen, NH_2, O-sugar, O-amino acid, N-sugar, N-amino acid, aminopyridine N-oxide (aminopy N^+O^-), alkenyl, phenyl, nitro, nitroso, carbohydrate, aminoacid, lipid, pteridine derivative; R^2 is selected from OH, aminopyridine, aminopyridine N-oxide and amide; R^3 is H or CH_3; M is selected from NH, CH_2, O and S; T is selected from NH, CH_2, O, S and PO; X is selected from S, Se, O, NH or is absent; Y is selected from O and S; and Z is a six-membered carbon ring, a five-membered carbon ring, a seven-membered carbon ring or a heterocyclic five-, six- or seven-membered ring or a fused ring system consisting of 1 to 3 rings (either aryl or heteroaryl) and R is substituted on the ring or ring system.

35. A system according to Claim 34 wherein R is substituted furthest from X on ring Z.

36. A system according to Claim 34 wherein Z is a benzene ring.

37. A system according to Claim 36 wherein R is substituted *para* to X.

38. A system according to Claim 34 wherein M is CH_2 or NH.

39. A system according to Claim 34 wherein T is CH_2 or NH.

40. A system according to Claim 34 wherein R is alkoxy, preferably methoxy.

41. A system according to Claim 34 wherein R is methoxy, Z is a benzene ring where R is *para* to X, M is NH, T is CH_2, X is S, Y is O, R^2 is OH, and R^3 is H.

42. A system according to any one of the preceding claims wherein the precursor of said molecule comprises said molecule in a form capable of releasing said molecule in a host such as a patient.

43. A system according to Claim 42 wherein the precursor comprises said molecule bound to an entity through a linkage, said linkage being biodegradable.

44. A system according to Claim 42 wherein the precursor comprises said molecule bound to an entity, whether or not through a linkage, said entity being biodegradable.

45. A system according to Claim 42 wherein said precursor comprises a liposome.

46. A method of destroying target cells in a host, the method comprising the steps of administering to the host

(a) a compound comprising a target cell-specific portion and a portion capable of converting a substantially non-toxic substance into another substance which is cytotoxic;

(b) a molecule capable of substantially inhibiting the conversion of said substantially non-toxic substance, or a precursor of said molecule; and

(c) the substantially non-toxic substance.

47. A method of treating a mammal harbouring a tumour, the method comprising the steps of administering to the mammal

(a) a compound comprising a tumour cell-specific portion and a portion capable of converting a substantially non-toxic substance into another substance which is cytotoxic;

(b) a molecule capable of substantially inhibiting the conversion of said substantially non-toxic substance, or a precursor of said molecule; and

(c) the substantially non-toxic substance.

48. A method of destroying a target cell in a host, the method comprising administering to the host

(a) a compound comprising a target cell-specific portion and a portion capable of converting a substance which, in its

native state, is able to inhibit the effect of a cytotoxic agent into another substance which has less effect against said cytotoxic agent;

(b) a molecule capable of substantially inhibiting the conversion of said substance, or a precursor of said molecule;

(c) a cytotoxic agent; and

(d) said substance.

49. A method of treating a mammal harbouring a tumour, the mammal having been prepared for treatment by administering a compound comprising a target cell-specific portion and a portion capable of converting a substance which, in its native state, is able to inhibit the effect of a cytotoxic agent into another substance which has less effect against said cytotoxic agent and allowing the ratio of compound bound to target cells to compound not bound to target cells to reach a desired value, the method comprising administering to the mammal

(a) a cytotoxic agent;

(b) a molecule capable of substantially inhibiting the conversion of said substance, or a precursor of said molecule; and

(c) a substance which in its native state is capable of inhibiting the effect of said cytotoxic agent from which a substance which has less effect on the cytotoxic agent can be generated by the portion capable of converting a substance.

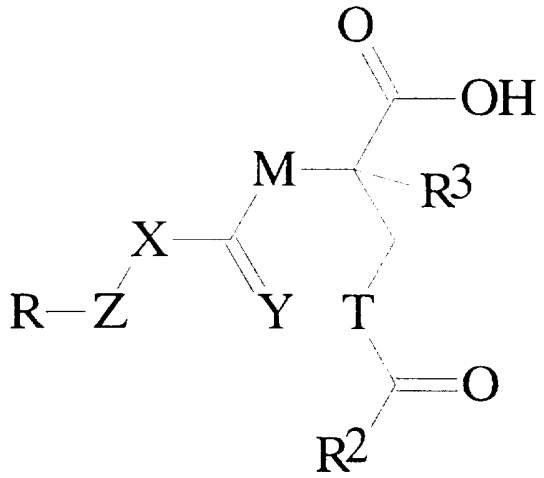
50. A method according to Claim 46 or Claim 47 wherein the amount, on a molar basis, of the compound of step (a) and molecule of step (b) administered is substantially the same.

51. A method according to Claim 48 or Claim 49 wherein the amount, on a molar basis, of said compound and molecule of step (b) administered is substantially the same.

52. A pharmaceutical composition comprising a molecule capable of substantially inhibiting the conversion of a substance as defined in any one of Claims 1 to 45 and a pharmaceutically acceptable carrier.

53. A pharmaceutical composition according to Claim 52 comprising an inhibitor of any one of carboxypeptidase G2, carboxypeptidase A, glucuronidase or β-lactamase and a pharmaceutically acceptable carrier.

54. A pharmaceutical composition according to Claim 52 or 53 comprising any one of



wherein R is selected from alkyl, haloalkyl, cycloalkyl, aryl, substituted aryl (1 to 5 substituents selected from halogen, alkyl, alkoxy, NH_2, OH, NR_2, COOH, CN, $CONH_2$), heteroaryl (5 or 6 membered ring containing 1 to 3 heteroatoms selected from N or

S), OH, alkoxy, H, halogen, NH_2, O-sugar, O-amino acid, N-sugar, N-amino acid, aminopyridine N-oxide (aminopy N^+O^-), alkenyl, phenyl, nitro, nitroso, carbohydrate, aminoacid, lipid, pteridine derivative; R^2 is selected from OH, aminopyridine, aminopyridine N-oxide and amide; R^3 is H or CH_3; M is selected from NH, CH_2, O and S; T is selected from NH, CH_2, O, S and PO; X is selected from S, Se, O, NH or is absent; Y is selected from O and S; and Z is a six-membered carbon ring, a five-membered carbon ring, a seven-membered carbon ring or a heterocyclic five-, six- or seven-membered ring or a fused ring system consisting of 1 to 3 rings (either aryl or heteroaryl) and R is substituted on the ring or ring system.

55. A composition according to Claim 54 wherein R is substituted furthest from X on ring Z.

56. A composition according to Claim 54 wherein Z is a benzene ring.

57. A composition according to Claim 56 wherein R is substituted *para* to X.

58. A composition according to Claim 54 wherein M is CH_2 or NH.

59. A composition according to Claim 54 wherein T is CH_2 or NH.

60. A composition according to Claim 54 wherein R is alkoxy, preferably methoxy.

61. A pharmaceutical composition according to Claim 54 wherein R is methoxy, Z is a benzene ring where R is *para* to X, M is NH, T

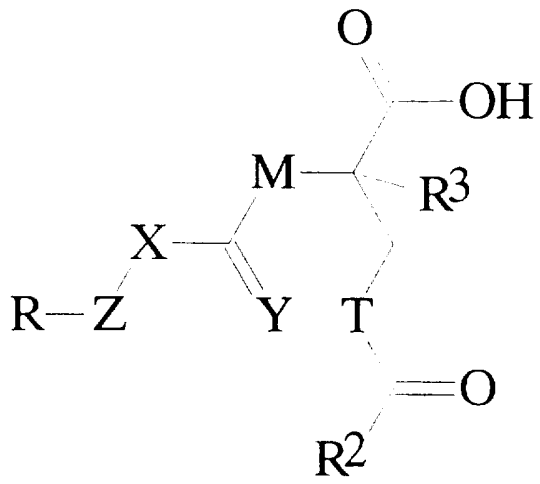
is CH_2, X is S, Y is O, R^2 is OH and R^3 is H.

62. A compound as defined in any one of Claims 52 to 61 for use in medicine.

63. Use of a compound as defined in any one of Claims 52 to 61 in the manufacture of a medicament for treating a patient with cancer.

64. Use according to Claim 63 wherein the patient has been, is being or will be administered a compound as defined in any one of Claims 1 to 3.

65. A compound:



wherein R is selected from alkyl, haloalkyl, cycloalkyl, aryl, substituted aryl (1 to 5 substituents selected from halogen, alkyl, alkoxy, NH_2, OH, NR_2, COOH, CN, $CONH_2$), heteroaryl (5 or 6 membered ring containing 1 to 3 heteroatoms selected from N or S), OH, alkoxy, H, halogen, NH_2, O-sugar, O-amino acid, N-sugar, N-amino acid, aminopyridine N-oxide (aminopy N^+O^-),

alkenyl, phenyl, nitro, nitroso, carbohydrate, aminoacid, lipid, pteridine derivative; R^2 is selected from OH, aminopyridine, aminopyridine N-oxide and amide; R^3 is H or CH_3; M is selected from NH, CH_2, O and S; T is selected from NH, CH_2, O, S and PO; X is selected from S, Se, O, NH or is absent; Y is selected from O and S; and Z is a six-membered carbon ring, a five-membered carbon ring, a seven-membered carbon ring or a heterocyclic five-, six- or seven-membered ring or a fused ring system consisting of 1 to 3 rings (either aryl or heteroaryl) and R is substituted on the ring or ring system.

66. A compound according to Claim 65 wherein R is substituted furthest from X on ring Z.

67. A compound according to Claim 65 wherein Z is a benzene ring.

68. A compound according to Claim 67 wherein R is substituted *para* to X.

69. A compound according to Claim 65 wherein M is CH_2 or NH.

70. A compound according to Claim 65 wherein T is CH_2 or NH.

71. A compound according to Claim 65 wherein R is alkoxy, preferably methoxy.

72. A compound according to Claim 65 wherein R is methoxy, Z is a benzene ring where R is *para* to X, M is NH, T is CH_2, X is S, Y is O, R^2 is OH and R^3 is H.

73. A method of inhibiting carboxypeptidase G2 comprising providing a compound according to any one of Claims 65 or 72.

74. Any novel feature or combination of features disclosed herein.

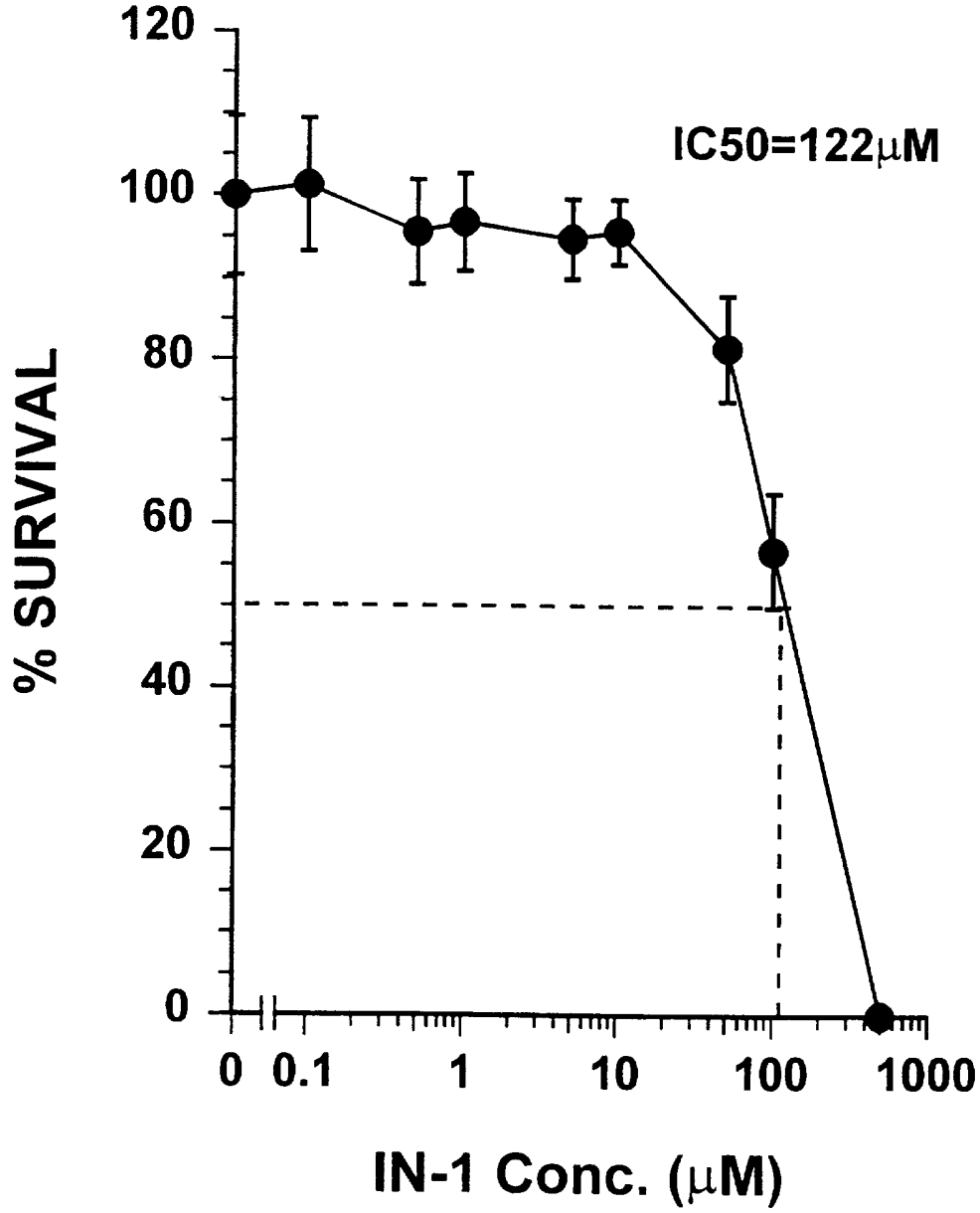


Figure 1. In vitro cytotoxicity of IN-1 on LS174T cells

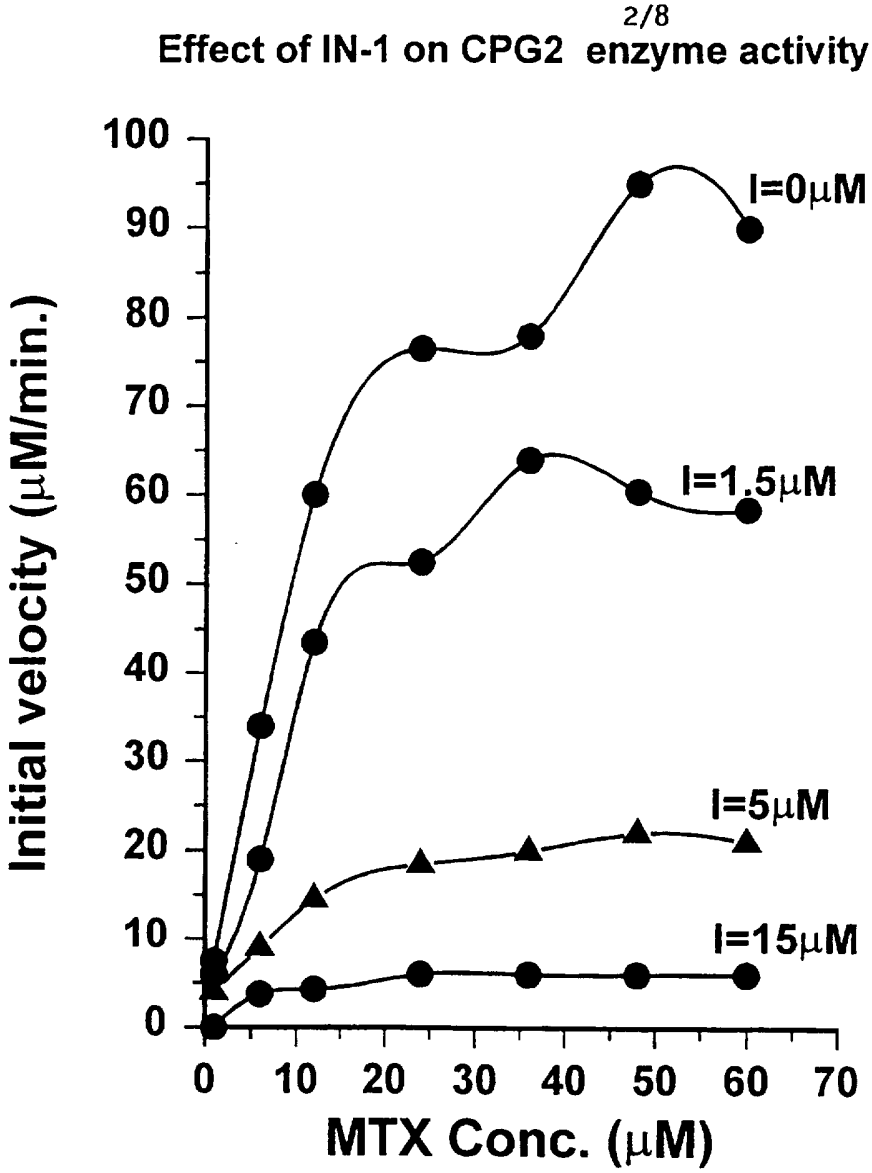


Figure 2. Effect of IN-1 on CPG2 enzyme activity

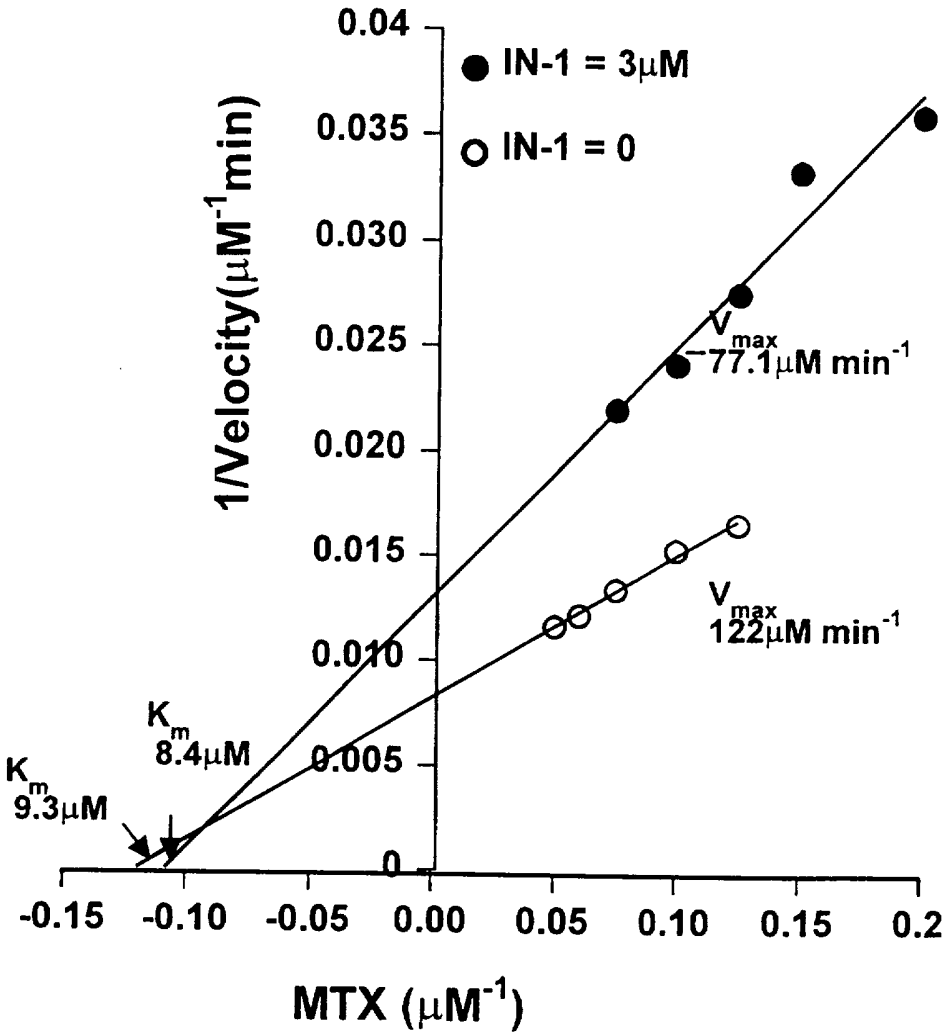


Figure 3. Lineweaver-Burk Plot

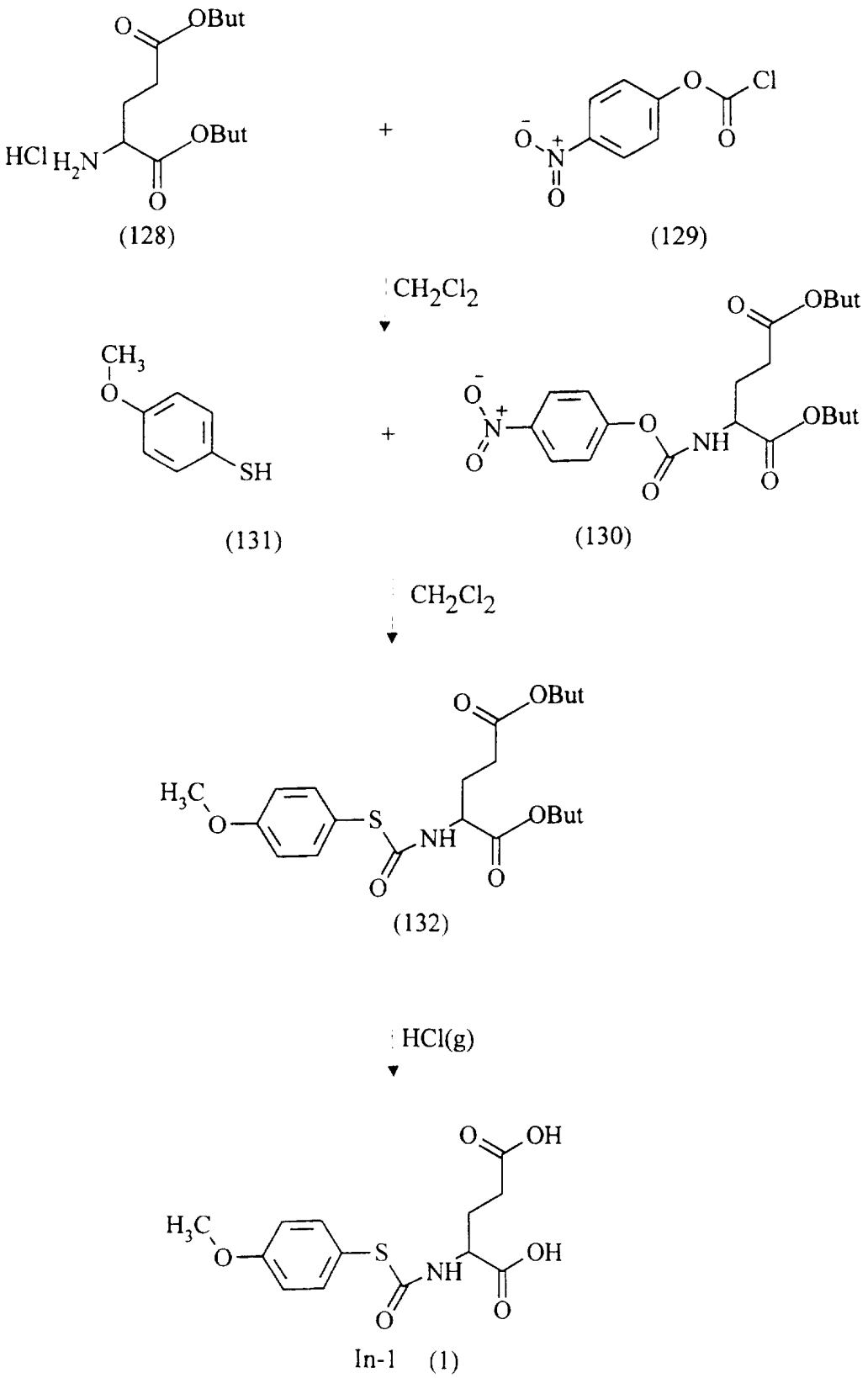


Figure 4 : Synthesis of inhibitor for CPG2- In-1 (1)

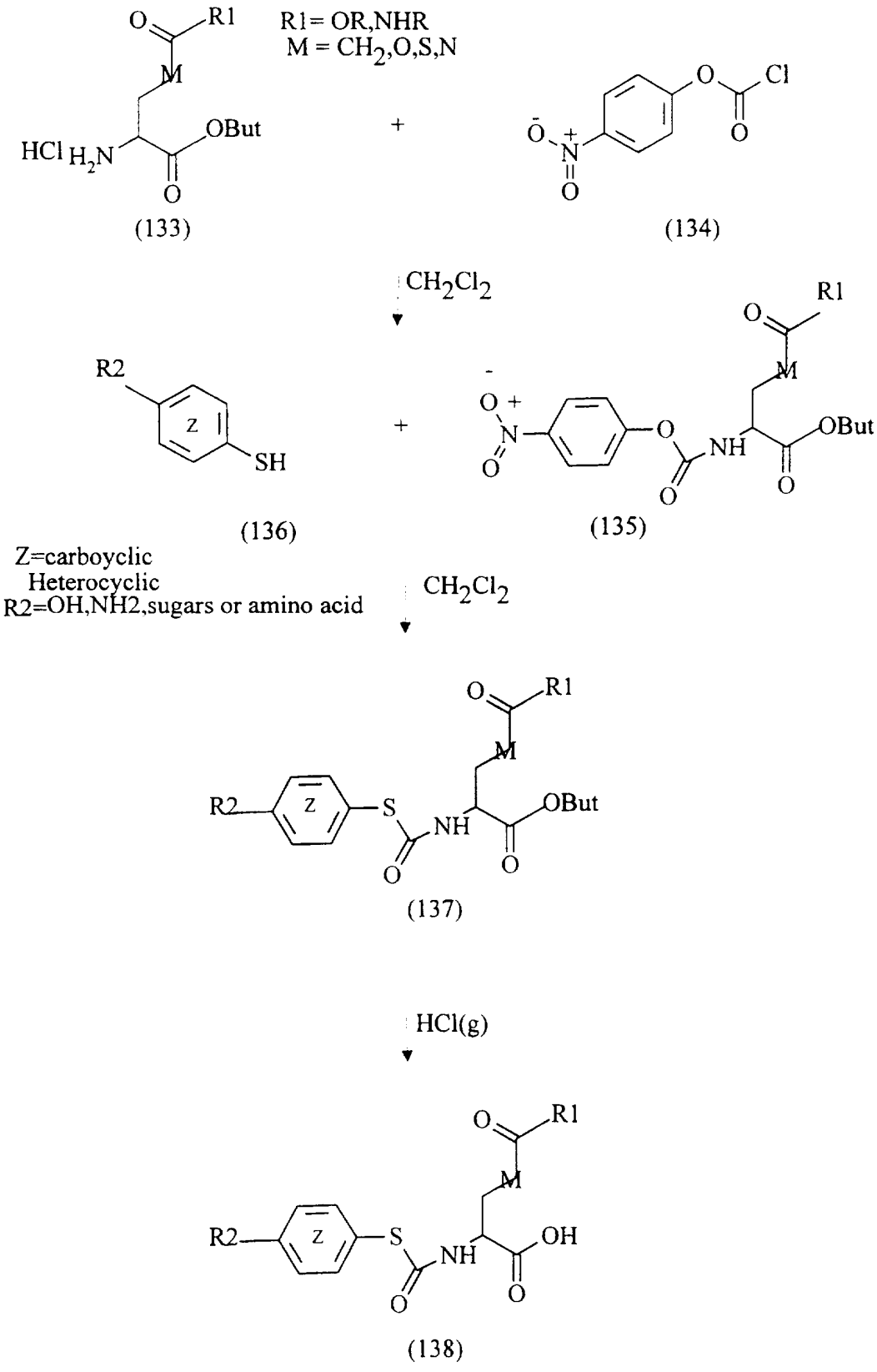
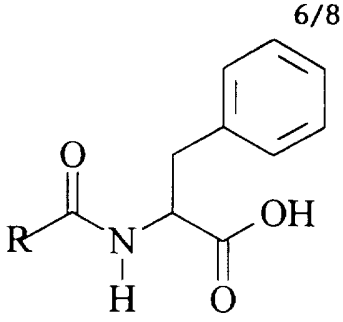


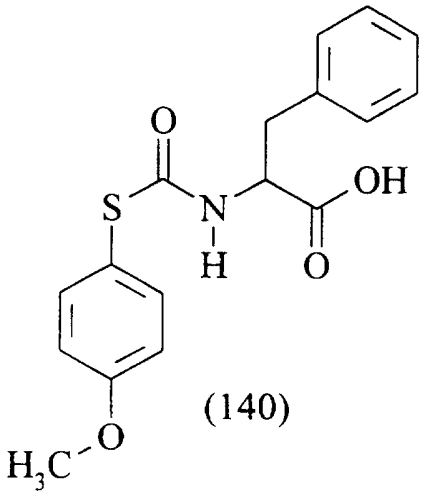
Figure 5: General synthesis of inhibitors for CPG2



R=Drug

(139)

Figure 6:- Substrate for Carboxypeptidase A



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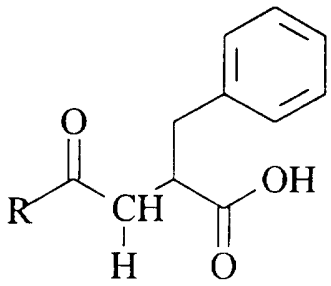

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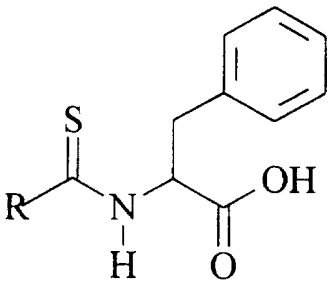
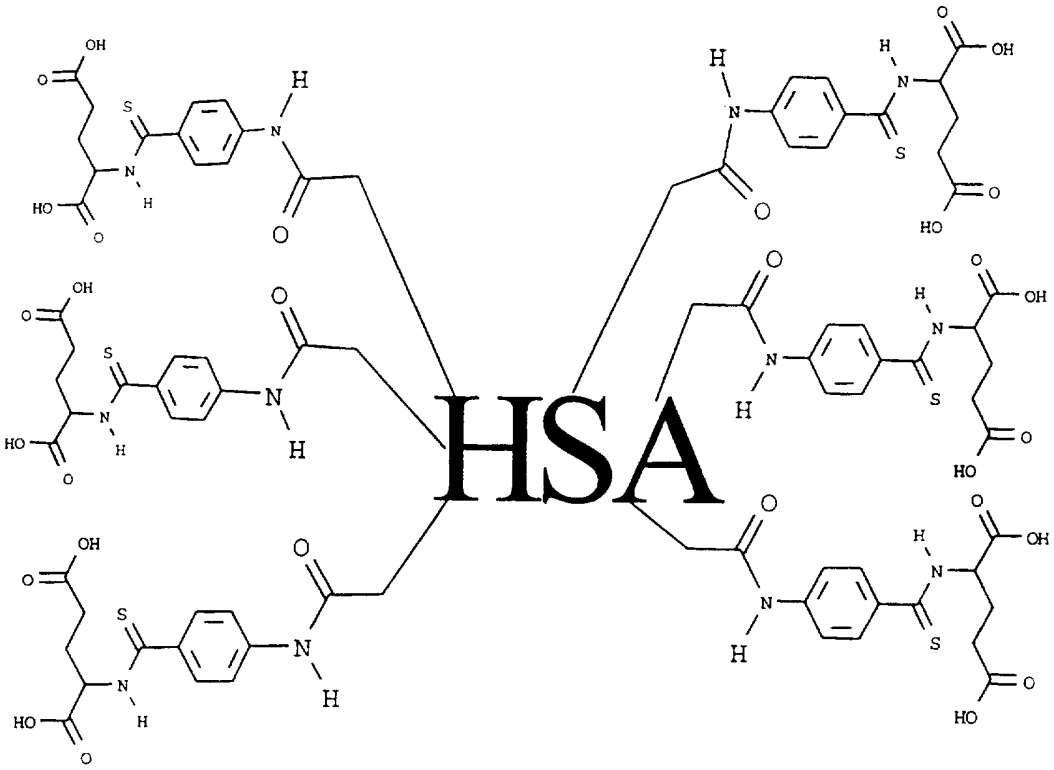


(142)

Figure 7:- Possible inhibitors for Carboxypeptidase A

Macromolecule supported inhibitors on biologically cleavable system



M.W. 70692

FIGURE 8

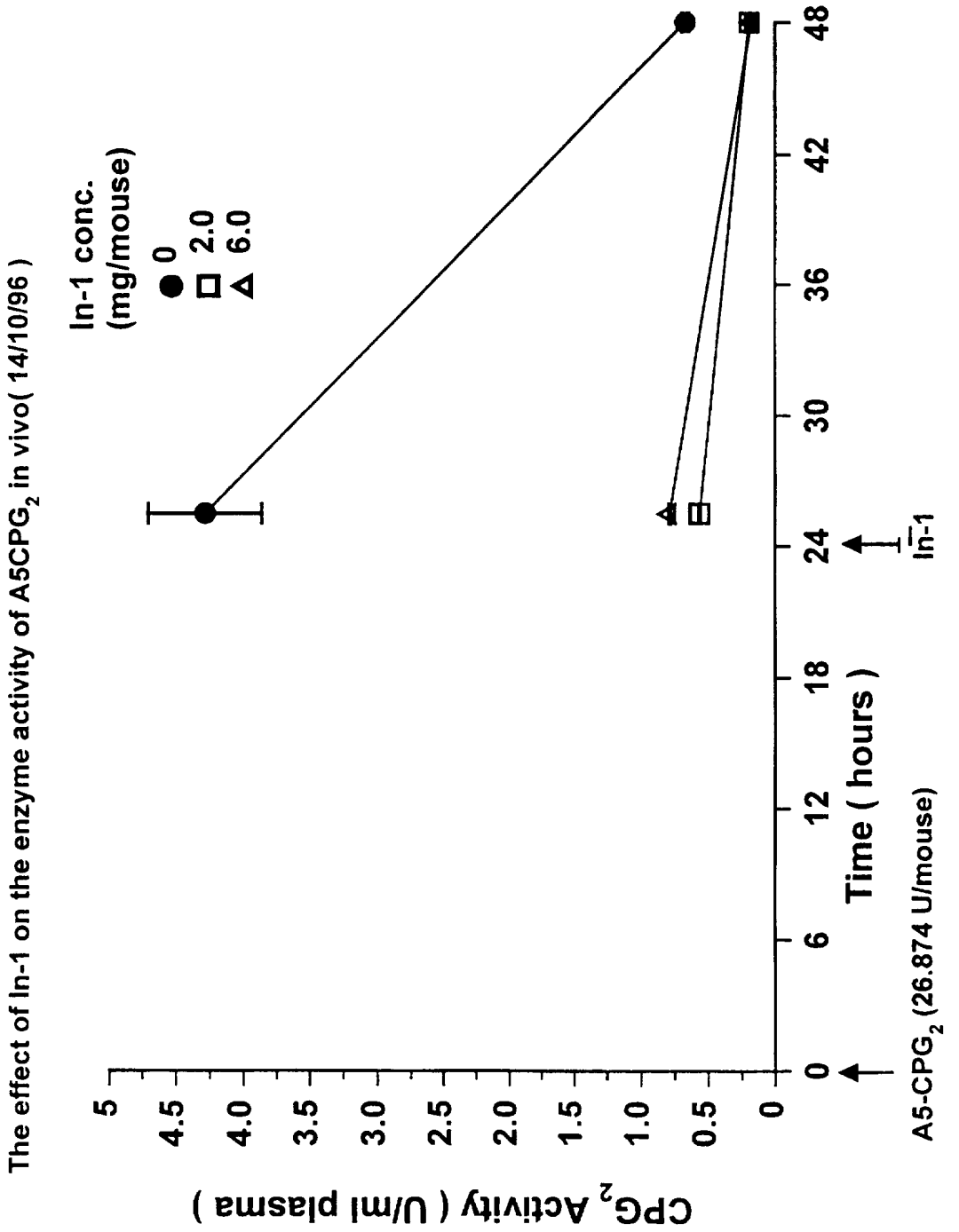


Figure 9: The effect of IN-1 on the enzyme activity of CPG2 in vivo

INTERNATIONAL SEARCH REPORT

In tional Application No
PCT/GB 96/03000

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K47/48

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

B. FIELDS SEARCHED

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

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,Y	WO 93 13805 A (BAGSHAWE KENNETH DAWSON) 22 July 1993 cited in the application see figure 2 ---	1-28, 31-39, 42-59, 62-70, 73,74
A	WO 88 07378 A (CANCER RES CAMPAIGN TECH) 6 October 1988 cited in the application --- -/--	1-74

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

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
11 March 1997	21.03.97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer Berte, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

In tional Application No
PCT/GB 96/03000

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF CONTROLLED RELEASE, vol. 28, no. 1/03, 1 January 1994, pages 187-193, XP000435249 BAGSHAWE K D: "ANTIBODY-DIRECTED ENZYME PRODRUG THERAPY (ADEPT)"	1
Y		1-28, 31-39, 42-59, 62-70, 73,74

A	BIOCONJUGATE CHEMISTRY, vol. 3, no. 2, 1 March 1992, pages 176-181, XP000262174 SVENSSON H P ET AL: "MONOCLONAL ANTIBODY-B-LACTAMASE CONJUGATES FOR THE ACTIVATION OF A CEPHALOSPORIN MUSTARD PRODRUG" see page 176, column 2, paragraph 1; figure 1	1-74

P,X	J. MED. CHEM. (1995), 38(26), 5051-65 CODEN: JMCMAR;ISSN: 0022-2623, 1995, XP002027293 SPRINGER, CAROLINE J. ET AL: "Optimization of Alkylating Agent Prodrugs Derived from Phenol and Aniline Mustards: A New Clinical Candidate Prodrug (ZD2767) for Antibody-Directed Enzyme Prodrug Therapy" see figures	1-28, 31-39, 42-59, 62-70, 73,74

P,X	J. MED. CHEM. (1996), 39(5), 1100-5 CODEN: JMCMAR;ISSN: 0022-2623, 1996, XP002027294 DOWELL, ROBERT I. ET AL: "New Mustard Prodrugs for Antibody-Directed Enzyme Prodrug Therapy: Alternatives to the Amide Link" see figures	1-28, 31-39, 42-59, 62-70, 73,74

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 96/03000

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

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

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

Remark: Although claim(s) 46-51
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

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

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

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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

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

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

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

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

Remark on Protest

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

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

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 96/03000

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		EP 0620741 A	26-10-94
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		JP 7506338 T	13-07-95
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