

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
17 March 2005 (17.03.2005)

PCT

(10) International Publication Number
WO 2005/023232 A2

(51) International Patent Classification⁷: **A61K 31/00**,
31/454, 31/5383, 31/164, A61P 35/00, G01N 33/94,
A61K 39/395

CHRISTENSSON, Birger [SE/SE]; Örnbergsvägen
16, S-146 40 Tullinge (SE). **SMITH, Edvard** [SE/SE];
Naumannsvägen 23, S-129 38 Hägersten (SE). **KIMBY,**
Eva [SE/SE]; Belevägen 10, S-182 64 Djursholm (SE).

(21) International Application Number:
PCT/SE2004/001277

(74) Agent: **AWAPATENT AB**; Box 45086, S-104 30 Stock-
holm (SE).

(22) International Filing Date:
6 September 2004 (06.09.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/499,901 4 September 2003 (04.09.2003) US

(81) Designated States (*unless otherwise indicated, for every
kind of national protection available*): AE, AG, AL, AM,
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM,
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM,
ZW.

(71) Applicant (*for all designated States except US*): **AFFI-
BODY AB** [SE/SE]; Box 20137, S-161 02 Bromma (SE).

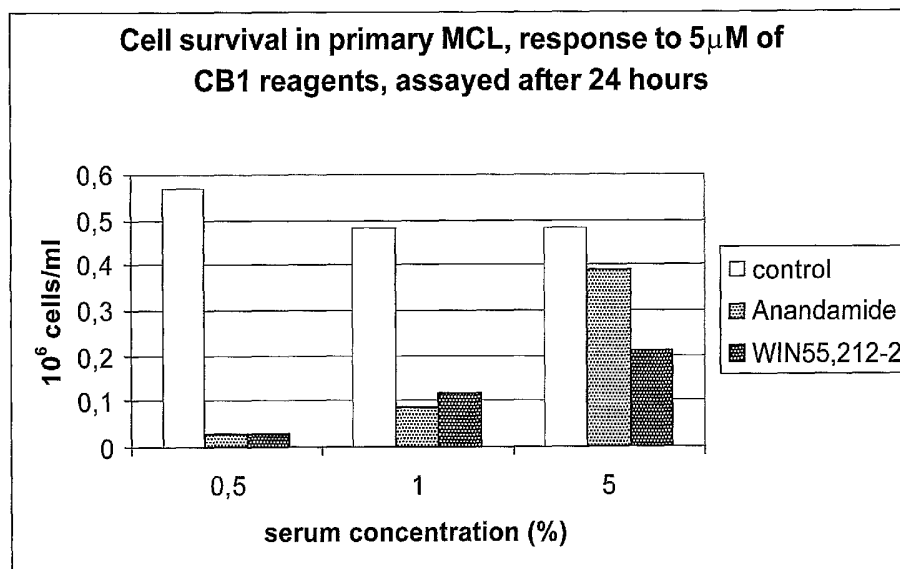
(84) Designated States (*unless otherwise indicated, for every
kind of regional protection available*): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **SANDER, Birgitta**
[SE/SE]; Örnbergsvägen 16A, S-146 40 Tullinge (SE).

[Continued on next page]

(54) Title: NEW USE AND METHODS



(57) Abstract: The invention provides use of a compound capable of modulating CB1 receptor signaling, or of a polypeptide with a biospecific affinity for the CB1 receptor, for the preparation of a medicament for treatment of a lymphoproliferative disorder involving cells with abnormal CB1 receptor function. Furthermore, the invention provides methods for identification of a compound capable of modulating CBI receptor signaling, a method for the diagnosis of a lymphoproliferative disorder involving cells with abnormal CBI receptor function in a subject, a method for treatment of a lymphoproliferative disorder involving cells with abnormal CB1 receptor function, and a method for elimination of malignant lymphocytes from a cell population.



FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI,
SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished
upon receipt of that report*

Declaration under Rule 4.17:

— *of inventorship (Rule 4.17(iv)) for US only*

*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

NEW USE AND METHODS

Field of the invention

The present invention relates to the elucidation of a novel association between modulation of the CB1 receptor and an effect on cells involved in lymphoproliferative disorders. More specifically, the present invention relates to methods and uses connected to this association, which enable the treatment and diagnosis of said disorders, the identification of new effective substances for their treatment, and other aspects apparent from the following disclosure.

Background15 *Early studies*

Prior to the discovery of cannabinoid receptors, numerous studies examined the effect of tetrahydrocannabinol (THC) or marihuana on immune function. Mostly, a suppressive effect on the immune system has been reported (reviewed in Klein TW et al (2003), J Leukoc Biol 74(4):486-496), seen as suppressive effects on T cells, B cells, NK cells and macrophages. Since the cloning of the cannabinoid receptors (CB), it has been clear that lymphocytes express both CB1 and CB2. The cannabinoid receptor type 1 (CB1) was cloned in 1991 (Gerard CM et al (1991), Biochem J 279(Pt 1):129-34) and the peripheral cannabinoid receptor (CB2) in 1993 (Munro S et al (1993), Nature 365(6441):61-5). At that time, and during the years thereafter, many functional studies of the immune system were performed, and showed inhibition of both B and T lymphocyte activation and immunoglobulin production. At this time, however, ligands incapable of discriminating between CB1 and CB2, such as $\Delta(9)$ -tetrahydrocannabinol (THC), CP 55,940 and HU-210, were

used, and no specific inhibitors to CB1 or CB2 were available. It is therefore not clear whether CB1 or CB2 was stimulated in these experiments, or if the effects were mediated through non-CB1/CB2 receptors or binding to other molecules (reviewed in Klein TW et al (2003), *supra*). As an example of this type of study, it was reported that anandamide and THC were found to induce apoptosis in human B and T lymphocytes already in 1994. Since it was shown by RT-PCR that these cells expressed CB1 and CB2 receptors, it was suggested that the effect was mediated by cannabinoid receptors (Schwarz H et al (1994), J Neuroimmunol 55(1):107-15). However, later studies have shown that the apoptosis-inducing effect of anandamide is mediated by vanilloid receptors (VR) and that CB receptors, conversely, protect against anandamide induced cell death in human lymphoma cells (Maccarrone M et al (2000), J Biol Chem 275(41):31938-45).

Specific ligands to CB1 and CB2 facilitated further studies

A specific and potent CB1 antagonist, SR141716A, was described in 1994. Subsequently, a CB2 specific antagonist, SR144528, was described in 1998. Later on in 1997, it was found that human CB1 receptor, transfected into hamster ovary CHO cells, had a high constitutive activity at both the MAPK and adenylyl cyclase pathways. These activities were blocked by SR141716A, which therefore was described to function as an inverse agonist. Similar activities were described in neural cells, where SR141716A could induce Ca^{2+} currents by reversing tonic CB1 receptor activity. The CB2 receptor is also autoactivated, and its activity inhibited by pertussis toxin or SR144528, which functions as an inverse agonist. The activities and interactions of the CB receptors with SR141716A and SR144528 have been reviewed in Shire D et al (1999), Life Sci 65(6-7):627-35. These two highly specific substances have been of tremendous value for studying various as-

pects of cannabinoid interactions with human, murine and rat cells.

Expression of CB1 and CB2 in lymphoid tissue

5 Expression of CB1 was initially described only in brain and testis, but was later investigated in lymphocytes. Expression of cannabinoid receptors in human leukocytes were investigated by PCR already in 1993 (Bouaboula M et al (1993), Eur J Biochem 214(1):173-80).
10 According to this report, the highest expression was seen in human B cells. In 1995, the same research group repeated their experiments with RT-PCR specific for CB1 and CB2, and could then detect CB1 mRNA expression in human immune tissue, but to much lower levels than in the brain
15 (Galiegue S et al (1995), Eur J Biochem 232(1):54-61). By RT-PCR, the expression of CB2 in immune cells was 10-100 fold higher than that of CB1, and in fact the expression levels of CB2 in human spleen and tonsils was as high as CB1 expression in the brain, strongly suggesting that the
20 CB2 receptor has important functions in the immune system. Later studies have confirmed that the amount of mRNA for CB2 exceeds CB1 mRNA in human peripheral blood mononuclear cells by approximately 3-fold (Nong L et al (2002), J Neuroimmunol 127(1-2):169-76).

25

Expression of CB1 and CB2 in malignant lymphoma

 In murine and human T cell lymphoma/leukaemia cell lines, expression of CB2 but not CB1 has been reported (McKallip RJ et al (2002), Blood 100(2):627-34).
30 In human mantle cell lymphoma (MCL), a non-Hodgkin's lymphoma of B cell type, increased expression of CB1 mRNA in comparison with reactive B cells was demonstrated (Ek S et al (2002), Cancer Res 62(15):4398-405). No functional studies on CB1 receptor activity were reported by
35 Ek et al.

Effects of cannabinoids on cell survival

In murine cells, THC induces apoptosis in both T cells and B cells (McKallip RJ et al (2002), J Pharm Exp Ther 302(2):451-65). The apoptotic effect of THC could be inhibited by SR144528 but not by SR141716, and was thus attributed to CB2 specific effects. In freshly isolated human T cell lymphoblastic leukaemia cells, THC induced reduced proliferation and apoptosis (McKallip RJ et al (2002), Blood 100(2):627-34). In human PBMC, anandamide, THC or the CB1/CB2 agonist CP 55,940 reduced proliferation in response to mitogen stimulation of T or B lymphocytes. The cannabinoid receptor agonist WIN 55,212-2 did not induce apoptosis in these cell lines. At higher concentrations, anandamide and THC, but not CP55,940, induced apoptosis (Schwarz H et al (1994), *supra*). Production of both 2-AG and anandamide has been found in human lymphoma cells such as U937 (Maccarrone M et al (2001), J Neurochem 76(2):594-601). Exogenous anandamide induced apoptosis in U937, an effect that was attributed to action via vanilloid receptors (Maccarrone M et al (2000), *supra*) and that could not be inhibited by SR141716A or SR144528. In fact, binding studies described in this publication showed that U937 lacked CB receptors on the surface. However, in cells that expressed CB1 (rat C6 glioma) or CB2 (human leukaemia Daudi), anandamide did not induced programmed cell death by itself, only if cells were co-incubated with SR141716 (C6) or SR144528 (Daudi). It was therefore suggested that CB receptors protect against VR-receptor mediated cell death induced by anandamide. It has since been confirmed, in other celltypes, that the apoptosis inducing effects by anandamide is mediated via VR and not by the CB1/CB2 system (Contassot E et al (2004), Gynecol Oncol 93(1):182-8).

Treatment of malignant disease by cannabinoids

It has been suggested that cannabinoids can be used in treatment of malignant cancer diseases, mainly in

cells of epithelial origin (reviewed in Bifulco M and Di
Marzo V (2002), *Nature Medicine* 8(6):547-50), but no con-
nection between the CB1 system and disorders related to
lymphocytes has been demonstrated. In T lymphoblastic
5 disorders, targeting of CB2 receptors induced apoptosis
(McKallip RJ et al (2002), *Blood* 100(2):627-34). In
glioma, the cannabinoid CB2 specific ligand JWH133 re-
duced angiogenesis and this effect was also seen with THC
and other cannabinoids and mediated via VEGF pathway
10 (Blazquez C et al (2003), *Faseb J* 17(3):529-31).

Mantle cell lymphoma

Mantle cell lymphoma (MCL) is a rare type of lym-
phoma (5-10% of all malignant lymphomas). The normal cel-
15 lular counterpart is presumed to be a B cell found in
small numbers in the mantle zone of lymphoid follicles.
MCL was originally classified as a low-grade lymphoma,
but recent reports of outcome indicate that most cases
behave as an intermediate-grade lymphoma. It has neither
20 the long survival of low-grade tumors nor the response to
aggressive chemotherapy of high-grade tumors. One excep-
tion is the infrequent "mantle zone" variant, which may
have a more indolent course. These tumors present in
older adults with a median age of 65. Therapy varies with
25 extent of disease from radiation alone for limited dis-
ease to multiagent chemotherapy ± local radiation for
more aggressive disease. In one study, the overall median
survival of 600 patients was 36 months. This lymphoma re-
sponds to chemotherapy but usually recurs within 2 years.
30 It is classified as an intermediate-grade lymphoma. For a
review, see for example Decaudin D (2002), *Leuk Lymphoma*
43(4):773-81.

Today, there exists no curative treatment options
for such lymphoproliferative disorders as MCL, with the
35 exception of bone marrow transplantation, which is only
an option for young patients. New therapeutic approaches

are needed to extend survival of, or cure, patients suffering from this category of diseases.

Disclosure of the invention

5

It is an object of the invention to provide a new approach towards curative treatment of lymphoproliferative disorders, such as lymphomas.

10 It is a related object of the present invention to provide pharmaceutical products for treatment of lymphoproliferative disorders, which act to kill, or reduce viability, of affected cells only, and which do not significantly influence normal cells.

15 It is another object of the invention to identify signaling pathways, in which it is possible to intervene to obtain a biological effect which is useful for curative treatment of lymphoproliferative disorders.

20 It is a further object of the invention to provide new uses for compounds which affect such signaling pathways.

Still another object of the invention is to provide methods for identifying new compounds which affect such signaling pathways.

25 Another object of the invention is to provide diagnostic methods, by which it is possible to identify and diagnose lymphoproliferative disorders, such as lymphomas.

These and additional objects are met by the different aspects of the invention as claimed.

30 In a first aspect, the invention relates to use of a compound capable of modulating CB1 receptor signaling for the preparation of a medicament for treatment of a lymphoproliferative disorder involving cells with abnormal CB1 receptor function. Thus, the invention provides a
35 novel linkage between modulation of the CB1 receptor and an effect on cells involved in a lymphoproliferative disorder characterized by abnormal function of this recep-

tor. In embodiments of the invention, the effect on these cells of modulating the CB1 receptor is to reduce or eliminate the negative effects of the cells in the progress of the disorder. In one embodiment, this means that modulation of the CB1 receptor leads to reduced viability of those cells having abnormal CB1 receptor function as compared with cells with normal CB1 receptor function. In another embodiment, it leads to the death of such cells, for example through induction of apoptosis.

10 Importantly, the present inventors have shown, for the first time, that lymphoid cells affected by certain lymphoproliferative disorders display an increased amount of the CB1 receptor on their surface, and that the amount of CB1 receptor in fact exceeds the amount of the CB2 re-
15 ceptor. This is surprising, in view of the long known fact that normal such cells exhibit a significant excess of CB2 receptor over CB1 receptors. Significantly, the observations extend to the fact that these receptors, in particular CB1, are important for cell survival. There-
20 fore, interference with CB1 ligand binding/CB1 signaling is a possible new therapeutic approach in lymphoproliferative disorders. Such a therapeutic approach would, among other things, have the advantage that administra-
25 tion of compounds capable of modulating CB1 receptor signaling would only affect those cells that are affected by the disorder, leaving normal, healthy cells substantially unaffected.

 In one embodiment of the present invention, the compound capable of modulating CB1 receptor signaling is a
30 compound characterized as a "CB1 agonist". In another embodiment, it is a compound characterized as a "CB1 inverse agonist". In another embodiment, it is a compound characterized as a "CB1 antagonist". In yet an embodiment, the compound has been identified as having activity
35 on the CB1 receptor through the use of a screening assay according to the second aspect of this invention, as described below. The number of possible, known and unknown,

compounds with the requisite activity is great. A non-limiting listing of compounds that have been described in the literature as capable of modulating CB1 receptor signaling is presented below. In one embodiment of the invention, the compound capable of modulating CB1 receptor signaling is chosen from this listing. From the information given herein regarding the previously unknown association between CB1 receptor function and lymphoproliferative disorder, the skilled person will be able to select suitable CB1 ligands without having to exercise any undue experimentation or inventive skill. For example, the compound could be one selected from the group consisting of WIN 55,212-2, anandamide and AM-251.

As an alternative to conventional, small molecule CB1 agonists, antagonists and other modulators, it is also contemplated, within the bounds of the present invention, that the compound capable of modulating CB1 receptor signaling is a polypeptide, which has a biospecific affinity for the CB1 receptor. In one embodiment, such a polypeptide could be an antibody, or functional fragment thereof, which recognizes one or more epitopes on the CB1 receptor structure. In another embodiment, the polypeptide is based on another protein. For example, it could be a variant of a certain scaffold protein, which has been selected from a library of many different, randomly created such variants, wherein the selection has been performed using the CB1 receptor as target. Such selection procedures, for example phage display, protein complementation assay, selection on bacterial surfaces etc are known to the skilled person, as are suitable starting scaffolds for creating affinity ligands against a target molecule. Such engineered proteins for use as affinity ligands to CB1 in the invention may be constructed using as scaffold a protein domain selected from the group consisting of domains of bacterial receptors, fibronectins, protease inhibitors, retinol binding proteins, bilin binding proteins, amylase inhibitors, CTLA-

4, cytochromes and cellulose binding proteins. As examples of preferred scaffolds, domains from bacterial receptors are mentioned. Of such domains, domains derived from the group consisting of staphylococcal protein A, streptococcal protein G and *Peptostreptococcus magnus* protein L are especially preferred.

The present invention also provides use of a polypeptide with a biospecific affinity for the CB1 receptor for the preparation of a medicament for treatment of a lymphoproliferative disorder involving cells with abnormal CB1 receptor function. The polypeptide with a biospecific affinity for the CB1 receptor is suitably as described immediately above.

As is readily understood by the person of skill in the art, the non-limiting examples given herein of compounds capable of modulating CB1 receptor signaling may be used singly or in any combination or mixture thereof. Also, for administration to a subject, they may be admixed with any conventional pharmaceutical excipients in accordance with known practices.

The lymphoproliferative disorder to be treated may be any disorder or disease which fulfils the definition given herein. Thus, the invention is applicable for example to lymphomas, such as B cell lymphomas, for example mantle cell lymphoma. Other examples of such disorders are posttransplantational lymphoproliferative disorders, Castleman's disease, atypical EBV infection, HTLV1 infection, infection with Herpes virus type 8, angioimmunoblastic lymphadenopathy and lymphomatoid granulomatosis. In one embodiment of the invention, the cells with abnormal CB1 receptor function involved in the lymphoproliferative disorder are B lymphocytes.

In some embodiments of the invention, the treatment of lymphoproliferative disorder for which the medicament to be prepared using the compound capable of modulating CB1 receptor signaling is intended, is performed in com-

ination with at least one other treatment of the lymphoproliferative disorder.

In some embodiments, the treatment is carried out as maintenance therapy under a long period of time, such as
5 from months to years.

In a further aspect of the present invention, a method for screening for, or identification of, compounds that have the desired effect on the CB1 receptor is presented, which method makes use of the association described above between modulation of CB1 receptor and effect on cells affected by a lymphoproliferative disorder. Thus, in one such embodiment, the invention provides a method for identification of a compound capable of modulating CB1 receptor signaling, which method comprises:

15 - providing a compound suspected of being capable of modulating CB1 receptor signaling;

- applying said compound to a test population of cells with abnormal CB1 receptor function;

20 - applying said compound to a control population of cells with normal CB1 receptor function;

- analyzing the viability of cells in said test population and in said control population;

whereby reduced viability in the test population relative to the control population identifies said compound as capable of modulating CB1 receptor signaling. Another embodiment of this aspect of the invention provides a method for identification of a compound capable of modulating CB1 receptor signaling, which method comprises:

30 - providing a compound suspected of being capable of modulating CB1 receptor signaling;

- providing a test population and a control population of cells with abnormal CB1 receptor function;

35 - applying said compound to said test population but not to said control population; and

- analyzing the viability of cells in said test population and in said control population;

whereby reduced viability in the test population relative to the control population identifies said compound as capable of modulating CB1 receptor signaling.

In these methods, the discovered association is exploited when investigating the effects on CB1 signaling of a newly synthesized or previously known compound. If the result of the method implies that the compound tested has an effect as a CB1 ligand, the compound could be investigated further, as an interesting candidate substance. For example, it could be tested as a compound capable of modulating CB1 receptor signaling in a medicament for treating lymphoproliferative disorders according to the present invention. Other alternatives as to the uses of such a compound are readily apparent to the person skilled in the biology of cannabinoid systems, since there are many areas of research where such a CB1 ligand could be useful.

In embodiments of the two different set-ups of the screening method described above, cells with abnormal CB1 receptor function may be transformed B lymphocytes, in particular mantle cell lymphoma cells. Where a control population with normal CB1 receptor function is needed, it is then preferred to use non-transformed B lymphocytes. It is to be noted that the precise order in which some of the method steps is carried out is not material to the working of this aspect of the invention. For example, the application of the substance suspected of having CB1 modulating effect to the test and control cell populations can be performed in any order, or simultaneously.

Another aspect of the present invention provides a method for the manufacture of a medicament for treatment of a lymphoproliferative disorder involving cells with abnormal CB1 receptor function, comprising admixing a compound capable of modulating CB1 receptor signaling with at least one pharmaceutically acceptable excipient. Various different embodiments of this aspect of the invention, in terms of what compounds may be used, what

disorders may be specifically targeted by such a medication etc, are derivable from the foregoing discussion of the first aspect of the invention.

Yet another aspect of the present invention provides
5 a method for treatment of a lymphoproliferative disorder involving cells with abnormal CB1 receptor function, which method comprises administering, to a subject in need thereof, a pharmaceutically active amount of a compound capable of modulating CB1 receptor signaling. Various
10 different embodiments of this aspect of the invention, in terms of what compounds may be used, what disorders may be specifically targeted by such a method of treatment etc, may, again, be derived from the foregoing discussion of the first aspect of the invention.

15 Another aspect of the invention provides a method for the diagnosis of a lymphoproliferative disorder involving cells with abnormal CB1 receptor function in a subject, which method comprises
- taking cells from said subject;
20 - culturing said cells to obtain a first cell population;
- dividing said first cell population into a test population and a control population;
- applying a compound capable of modulating CB1 receptor signaling to said test population but not to said
25 control population; and
- analyzing the viability of cells in said test and control populations;

whereby reduced viability in the test population
30 relative to the control population implies the presence of said disorder. Various different embodiments of this aspect of the invention, in terms of what compounds may be used, what disorders may be specifically diagnosed by such a diagnostic method etc, may be derived from the
35 foregoing discussion of the first aspect of the invention.

A further aspect of the present invention is a method for elimination of malignant lymphocytes from a cell population comprising malignant lymphocytes and non-malignant cells, comprising treating said cell population with a compound capable of modulating CB1 receptor signaling. Such a "purging" method serves to eliminate or reduce in number unwanted, malignant cells from cell preparations that are to be used in a context where it is of importance that no malignant cells are present. For example, such a context could be the preparation of cells for stem cell transplantation.

For a better understanding of the terms used throughout the present disclosure, the following definitions are provided. Thereafter, a non-limiting listing of compounds capable of modulating CB1 receptor signaling is provided.

Definitions

As used herein, "a compound capable of modulating CB1 receptor signaling" is a compound, synthetic or biological or modified biological, which binds to the CB1 receptor, or in other ways modifies the structure or localization of the CB1 receptor or its association with other molecules, in such a way that the effect of the receptor on signaling pathway(s) is modified so that the cell's fate or function is changed. In the present text, "a compound capable of modulating CB1 receptor signaling" is sometimes also referred to as "a CB1 receptor ligand" or "a CB1 ligand". Furthermore, compounds that are presented in the literature as "agonists", "inverse agonists" or "antagonists" to the CB1 receptor are contemplated to fall within this definition. In embodiments of the invention, such "compound capable of modulating CB1 receptor signaling" may be selective for the CB1 receptor over other receptors, notably the CB2 receptor, but it is equally possible that the compound is capable also of modulating other receptors.

The term "a lymphoproliferative disorder" is used in this context to indicate a group of diseases, which includes lymphoid proliferations with proven and non-proven malignant potential. Non-limiting examples of such disorders include lymphomas, such as B cell lymphomas, for example mantle cell lymphoma; posttransplantational lymphoproliferative disorders; Castleman's disease; atypical EBV infections; HTLV1 infection; infection with Herpes virus type 8; angioimmunoblastic lymphadenopathy and lymphomatoid granulomatosis.

As used herein, "a lymphoproliferative disorder involving cells with abnormal CB1 receptor function" is a disease or disorder as defined above, in which individual lymphocytes or groups of lymphocytes have an increased amount of CB1 (functional or non-functional) within, on or around the cell and/or in which altered CB1 function is instrumental in, or associated with, the disease or disorder, in such a way that without the increase in CB1 and/or altered CB1 function no disease or disorder would be discernible.

As used herein, a compound is said to be "selective for the CB1 receptor over the CB2 receptor" if the compound binds to, or in other ways modifies the structure, localization or the association with other molecules of, the CB1 receptor in such a way that the effect of the receptor on signaling pathway(s) is modified so that the cell's fate or function is changed, whereas, in the presence of the CB2 receptor only, this effect is not discernible.

As used herein, a polypeptide has "a biospecific affinity for the CB1 receptor" if the compound interacts with the CB1 receptor through specific recognition between the polypeptide and the receptor, which recognition is based on the specific tertiary structure and/or charge resulting from the specific amino acid sequence of the polypeptide. In the context of the present invention, the interaction of polypeptide with receptor through bio-

specific affinity leads to a biological result which is such that the effect of the receptor on signaling pathway(s) is modified so that the cell's fate or function is changed. Alternatively, the interaction of polypeptide with receptor through biospecific affinity inhibits the binding of natural or modified CB1 specific ligands so that the receptor's effect on the cell function is not discernible. As a third alternative, the interaction of polypeptide with receptor through biospecific affinity inhibits the binding of natural or modified CB1 specific ligands so that the receptor's effect on the cell function is changed or the endogenous activity of the receptor is blocked.

As used herein, "a functional fragment" of an antibody is a part of the antibody molecule that retains some or all of the antibody molecule's capacity to associate with the antigen.

Compounds capable of modulating CB1 receptor signaling

The present invention contemplates use of a compound capable of modulating CB1 receptor signaling. Ample examples of such compounds, which are suggested herein to be of use in the context of the present invention, can be found in the literature. Furthermore, the present invention provides screening methods by which it is possible to identify new such compounds. In the following, compounds and general guidelines on the preparation and identification of compounds are presented.

In one study, it was suggested that a CB1 binding ligand has to be highly flexible, and that acyl chains in the ligand should be able to assume a tightly folded conformation (U-shaped). Furthermore, there should be an aliphatic chain of 20-22 carbons with at least three non-conjugated *cis* double bonds with a saturated tail of at least the five last carbons. The head can be either polar or non-polar but should not be bulky (Reggio PH and Traore H (2000), Chem Phys Lipids 108(1-2):15-35).

As a non-exhaustive listing, the following compounds have been shown to exert an effect on the CB1 receptor:

- AM 281 (1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-4-morpholinyl-1*H*-pyrazole-3-carboxamide)
5 (Cosenza *et al* (2000), *Synapse* 38:477);
- AM 251 (*N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide) (Gatley *et al* (1996), *Eur J Pharmacol* 307:331);
- AM 630 (6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-
10 1*H*-indol-3-yl](4-methoxyphenyl)methanone) (Hosohata *et al* (1997), *Life Sci.* 61:PL115);
- Virodhamine (*O*-(2-Aminoethyl)-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenoate) (Porter *et al* (2002), *J Pharmacol Exp Ther* 301:1020);
- 15 O-2050 ((6*aR*,10*aR*)-3-(1-methanesulfonylamino-4-hexyn-6-yl)-6*a*,7,10,10*a*-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b,d*]pyran) (Martin *et al* (2002), "Symposium on the Cannabinoids", International Cannabinoid Research Society);
- 20 CP 55,940 ((-)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol) (Wiley *et al* (1995), *Neuropharmacology* 34:669);
- HU 210 ((6*aR*)-*trans*-3-(1,1-dimethylheptyl)-6*a*,7,10,10*a*-tetrahydro-1-hydroxy-6,6-dimethyl-6*H*-
25 dibenzo[*b,d*]pyran-9-methanol (Felder *et al* (1995), *Mol Pharmacol* 48:443);
- Anandamide (arachidonylethanolamide; *N*-(2-hydroxyethyl)-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenamide) (Ross *et al* (2001), *Br J Pharmacol* 132:631);
- 30 WIN 55,212-2 mesylate ((*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate) (D'Ambra *et al* (1992), *J Med Chem* 35:124);
- (*R*)-(+)-Methanandamide ((*R*)-*N*-(2-hydroxy-1-
35 methylethyl)-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenamide (Abadji *et al* (1994), *J Med Chem* 37:1889);

- 2-Arachidonyl glycerol ((5Z,8Z,11Z,14Z)-5,8,11,14-eicosatetraenoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester) (Mechoulam *et al* (1995), *Biochem Pharmacol* 50:83);
- ACPA (arachidonylcyclopropylamide; *N*-(cyclopropyl)-5Z,8Z,11Z,14Z-eicosatetraenamide) (Hillard *et al* (1999), *J Pharmacol Exp Ther* 289:1427);
- ACEA (arachidonyl-2'-chloroethylamide; *N*-(2-chloroethyl)-5Z,8Z,11Z,14Z-eicosatetraenamide) (Hillard *et al* (1999), *J Pharmacol Exp Ther* 289:1427);
- 10 Arvanil (*N*-[(4-hydroxy-3-methoxyphenyl)methyl]-5Z,8Z,11Z,14Z-eicosatetraenamide) (Melck *et al* (1999), *Biochem Biophys Res Comm* 262:275);
- Noladin ether (HU 310; 2-arachidonyl glycerol ether) (Suhara *et al* (2000), *Chem Pharm Bull* 48:903);
- 15 DEA (*N*-(2-hydroxyethyl)-7Z,10Z,13Z,16Z-docosatetraenamide; docosatetraenylethanolamide) (Hanus *et al* (1993), *J Med Chem* 36:3032)
- NADA (*N*-[2-(3,4-dihydroxyphenyl)ethyl]-5Z,8Z,11Z,14Z-eicosatetraenamide; *N*-arachidonyldopamine) (Bisogno *et al* (2000), *Biochem J* 351:817);
- 20 Oleamide (*cis*-9-octadecenoamide);
- MAFP ((5Z,8Z,11Z,14Z)-5,8,11,14-eicosatetraenyl-methyl ester phosphonofluoridic acid) (Martin *et al* (2000), *J Pharmacol Exp Ther* 294:1209);
- 25 (-)-Cannabidiol (2-[(1*R*,6*R*)-3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5-pentyl-1,3-benzenediol (Martin *et al* (1987), *NIDA Res Monogr* 79:48);
- OMDM-2 ((9Z)-*N*-[1-((*R*)-4-hydroxibenzyl)-2-hydroxyethyl]-9-octadecenamide) (Ortar *et al* (2003), *Biochem Pharmacol* 7624:1);
- 30 HU-211 (Yoles E *et al* (1996), *J Neurotrauma* 13(1):49-57);
- cis*-9,10-Octadecenoamide (CODA) (Langstein J *et al* (1996), *Res Immunol* 147(6):389-96);
- 35 Arvanil (Sancho R *et al* (2003), *Br J Pharmacol* 140(6):1035-1044);

Ajumelic acid (Bidinger B *et al* (2003), Clin Immunol 108(2):95-102);

Cannabidiol (Malfait AM *et al* (2000), Proc Natl Acad Sci U S A 97(17):9561-6);

5 Quinonoid cannabinoid derivatives (Kogan NM *et al* (2004), J Med Chem 47(15):3800-6);

DML20 (3-(2-ethylmorpholino)-5,5'-di(p-bromophenyl)-imidazolidinedione), DML21 (3-(1-hydroxypropyl)-5,5'-di(p-bromophenyl)-imidazolidinedione) and DML23 ((3-
10 heptyl-5,5'-di(p-bromophenyl)-imidazolidinedione)) (Govaerts SJ *et al* (2004), Eur J Pharmacol 495(1):43-53);

Derivatives of arachidyl alcohol with CB1 receptor activity according to Parkkari T *et al* (2004), Bioorganic & Medicinal Chemistry Letters 14(12):3231-3234 (sub-
15 stances 4a and 4b);

AM411, AM782, AM1902, AM2233, 2-arachidonoyl ester, 2-arachidonoyl ether (Luk T *et al* (2004), Br J Pharmacol 142(3):495-500);

3-[2-Cyano-3-(trifluoromethyl)phenoxy]phenyl-4,4,4-trifluoro-1-butanesulfonate (BAY 59-3074) (De Vry J *et al* (2004), J Pharmacol Exp Ther 310(2):620-32);

[O-Methyl-¹¹C]1-(2-chlorophenyl)-5-(4-methoxyphenyl)-4-methyl-1H-pyrazole-3-carboxylic acid piperidin-1-ylamide ([¹¹C]-1) (Kumar JS *et al* (2004), Bioorg Med Chem
25 Lett 14(10):2393-6);

O-2654 (6'-azidohept-2'-yne-cannabidiol) (Thomas A *et al* (2004), Eur J Pharmacol 487(1-3):213-21);

(Dimethylheptyl)anandamide [(16,16-dimethyldocosa-cis-5,8,11,14-tetraenoyl)ethanolamine] (17a) (Seltzman HH
30 *et al* (1997), J Med Chem 40(22):3626-34);

Diarylimidazolecarboxamides and diaryltriazolecarboxamides related to SR141716, Diarylpyrazoles related to SR141716, triazoles related to SR141716, imidazoles related to SR141716 presented in (Dyck B *et al* (2004), Bio-
35 org Med Chem Lett 14(5):1151-4);

3,4-Diarylpyrazolines; eutomer 80 (SLV319) (Lange JH *et al* (2004), J Med Chem 47(3):627-43);

N1 and C5 substituted cycloalkyl and C5 4-methylphenyl analogues of the *N*-(piperidin-1-yl)-4-methyl-1*H*-pyrazole-3-carboxamide class of cannabinoid ligands (Krishnamurthy M *et al* (2004), *Bioorg Med Chem* 12(2):393-404);

BAY 38-7271 (Mauler F *et al* (2003), *CNS Drug Rev* 9(4):343-58);

Δ 8-THC analogues with phenyl side chains (Krishnamurthy M *et al* (2003), *Bioorg Med Chem Lett* 13(20):3487-90);

Arachidonylsulfonyl fluoride (Segall Y *et al* (2003), *Bioorg Med Chem Lett* 13(19):3301-3);

O-isopropyl dodecylfluorophosphonate (compound 2), dodecanesulfonyl fluoride (compound 14) and dodecylbenzodioxaphosphorin oxide as described by (Quistad GB *et al* (2002), *Toxicol Lett* 135(1-2):89-93);

The present invention will now be further illustrated by the description of experiments conducted in accordance therewith, with reference to the attached figures. This description is not to be construed as limiting.

Brief description of the figures

Figure 1. High expression of cannabinoid receptor mRNA in mantle cell lymphomas as analyzed by gene expression analysis using the Affymetrix U95Av.2 chip or U133A chip. All mantle cell lymphomas (n=20) have high or very high expression levels of CB1 but also CB2 expression is higher in mantle cell lymphomas compared to controls (n=9). Y-axis represents absolute signal values.

Figure 2. Analysis of expression of CB1 (A) and CB2 (B) by gene expression analysis using the Affymetrix u95Av.2 chip or U133A chip in 27 MCL tumor biopsies and 9 reactive lymphoid tissues demonstrates a significant

over-expression of CB1 in MCL compared to reactive lymphoid tissue ($p < 0.0001$ Wilcoxon/Kruskall-Wallis test).

Figure 3. Immunoblot for CB1 (A) shows high protein expression in the Granta 519 mantle cell lymphoma cell line compared to no expression in the SK-MM-2 plasma cell leukaemia cell line. Whole cell lysates of Granta 519 and SK-MM-2 was prepared, resolved by SDS-PAGE, and immunoblotted with anti-CB1 antibody as indicated. The CB1 transfected AtT20 cell line stably expressing CB1 receptors (C) and the mantle cell lymphoma cell line Granta 519 (B) were stained for CB1 as described in "Materials and methods". A strong specific staining (red) is detected in both cell lines as compared to cells stained with an irrelevant control antibody (D). Nuclei are stained blue by DAPI. Imprints from a primary mantle cell lymphoma were stained for CB1 (E) resulting in a specific granular staining (green).

Figure 4. Reduced cell survival in mantle cell lymphoma in response to anandamide (A) or WIN 55,212-2 (B) or 2-AG (C). Rec-1 mantle cell lymphoma cell line was incubated with 10 μ M of one of the CB1 binding substances anandamide, WIN 55,212-2 or 2-AG or with solvent alone in the various indicated concentrations of FCS. Cell viability was assayed at 24 hours by cell counting using trypan blue staining.

Figure 5. Reduced cell survival in primary MCL in response to anandamide or WIN 55,212-2. Primary MCL cells, freshly isolated from a diagnostic tumor biopsy, were incubated with 5 μ M anandamide or 5 μ M WIN 55,212-2 or solvent alone (control) in the indicated concentrations of FCS. Cell viability was assayed at 24 hours by cell counting using trypan blue staining.

Figure 6. Expression of CB1 protein. A) Western blotting of proteins from whole cell extracts of the indicated cell lines. B) Immunofluorescent staining against the CB1 receptor on the surface of Jurkat (left) and Rec-1 (right) cells.

Figure 7. A) MTT assay showing viability of Rec-1 (top) and SK-MM-2 (bottom) cells 48 hours after treatment with the CB1 agonist anandamide in medium containing 10% serum. B) MTT assay showing viability of Rec-1 cells 48 hours after treatment with the CB2 agonist JWH 015 in medium containing 10% serum. C) MTT assay showing viability of AtT20 (top) and AtT20[↑]CB1 (bottom) cells 48 hours after treatment with the CB1 agonist anandamide in medium containing 10% serum.

Figure 8. MTT assay showing viability of A) Rec-1 and B) Molt4 cells 24 hours after treatment with the CB1 antagonist AM 251 (left) or with the CB1 agonist anandamide (right) in medium containing 1% serum.

Figure 9. A) MTT assay showing viability and B) Caspase-3 fluorimetric assay showing caspase activity of Rec-1 cells 24 hours after treatment with 10 μ M of the CB1 agonist WIN 55,212-2 without (bar 2) or with (bar 3) 30 minutes of preincubation with 10 μ M of the VR antagonist capsazepin (Cz). Control (bar 1), as well as effects of VR antagonist alone (bar 4) or of 100 mM of the topoisomerase I inhibitor camptothecin (bar 5) are also shown.

Figure 10. Caspase-3 fluorimetric assay showing caspase activity of Rec-1 cells 24 hours after treatment with 0 μ M (bar 1) 2.5 μ M (bar 2) or 10 μ M (bar 3) of the CB1 antagonist AM 251, or with 10 μ M of camptothecin (bar 4).

Examples

Materials and methods

Tissue biopsies

Lymph node (n = 19), spleen (n = 5), tonsil (n = 2) or a biopsy from tongue base (n = 1) from 25 patients with MCL was investigated. From one patient, two biopsies, taken at two different time points, were analyzed.

All biopsies were taken for diagnostic purposes. Approximately 5 mm³ fragments were snap frozen upon arrival and subsequently stored at -70 °C. All diagnoses were established by morphology, immunophenotyping by flow cytometry and by immunohistochemical staining for cyclin D1 (using antibody clone D2D11F11 from Novocastra, Newcastle upon Tyne, UK). The t(11;14) translocation was confirmed by FISH analysis using the Vysis LSI IGH/CCND1 dual fusion probe (Vysis, Richmond, UK).

Control tissue: Biopsies from reactive lymph nodes (n=5) and tonsils (n=4) were used as controls.

RNA isolation and oligonucleotide array hybridisation

Total RNA was prepared using the TRIzol method as directed by the supplier (Invitrogen Life Technologies, Carlsbad, CA) followed by Trizol purification (Quiagen) and quality control on an Agilent Bioanalyzer (Agilent Technologies, Inc. Palo Alto, CA). The cRNA synthesis for micro array experiments and the hybridisations were performed using Affymetrix GeneChipTM high-density oligonucleotide human U133A arrays (Affymetrix, Inc, Santa Clara, CA) containing more than 22 000 genes, or the human U95Av.2 chip containing 12 500 genes, according to standard Affymetrix protocols at the core facility at the Department of Biosciences, Karolinska Institutet, Novum, Huddinge, Sweden.

Affymetrix data analysis

The data was analysed using Affymetrix Microarray Suite version 5.0, MicroDB 3.0 and DMT 3.0 as well as a beta test version of Geneweaver, provided by Affibody/Inforsense.

Comparative analyses were performed on the expression data using Affymetrix Datamining Tool version 3.0.

Cell lines and freshly isolated, viable MCL tumor cells

Two MCL cell lines were used, the Granta 519 (Jadayel DM et al (1997), Leukemia 11(1):64-72) and the Rec-1 (Rimokh R et al (1994), Blood 83(12):3689-96). The Granta 519, an EBV transformed MCL cell line, was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen and cultured in Dulbecco's MEM (DMEM) (Gibco, UK) (4.5% glucose) with 2 mM L-glutamine and 0.11 g/l Na Pyr with pyridoxine and 50 µg/ml of gentamycin. The Rec-1 cell line was kindly donated by Dr C Bastard, Centre Henri Becquerel, Rouen, France and cultured in RPMI-1640 with GlutaMax and 25 mM HEPES (RPMI) (Gibco) and 50 µg/ml of gentamycin. For propagation, both cell lines were cultured in medium with 10% fetal calf serum. In both MCL cell lines, the translocation t(11;14) was confirmed by FISH analysis and up-regulation of Cyclin D1 protein by immunocytochemistry.

The AtT20 cell line transfected with cDNA for the rat CB1 receptor (AtT20[↑]CB1) (Hsieh C et al (1999), J Neurochem 73(2):493-501) or with low endogenous levels of CB1 (AtT20) was kindly provided by Dr K Mackie (Dept of Anaesthesiology, University of Washington, Seattle, WA.) and cultured in Dulbecco's MEM with 10% fetal calf serum and 50 µg/ml of gentamycin. Selection was done with 400 µg G418. These cells are adherent, and trypsinization was used to bring the cells into suspension to allow cytospin preparation.

Molt4 and Jurkat, both of T-lymphocyte origin, were cultured in RPMI 1640 (Gibco) containing 10% calf serum and 50 µg/ml gentamycin.

Immunofluorescent staining for CB1

Cells on cytospin preparations or imprints from lymph nodes were fixed in 3.7% formaldehyde (Sigma, St Louis, MA) during 4 minutes, rinsed three times in PBS and incubated for 30 minutes in PBS containing 0.1% Triton X-100 (Sigma) and 0.1% BSA (Sigma). Rabbit polyclonal

antiserum against CB1 was kindly provided by Dr K Mackie and diluted 1:400 in PBS-Tween-BSA before being added to the cell preparation for 1 hour at room temperature in a moisture chamber. Thereafter, cells were rinsed three
5 times in PBS and incubated with Cy3 or FITC conjugated donkey anti-rabbit antiserum (cat no 711-165-152 (Cy3) and cat no 711-095-132 (FITC), Jackson ImmunoResearch, West Grove, PA) diluted 1:250 in PBS-Tween-BSA. DAPI (4',6-diamidino-2-phenylindole, Sigma) was used to stain
10 cell nuclei.

Western blotting

Cell extracts were prepared by lysing the cells in ice-cold sample buffer (50 mM HEPES, 500 mM NaCl, 0.05%
15 Tween 20, 0.1% Triton x-100) to which 1000 x diluted protease inhibitor cocktail (p8340, Sigma) had been added. Protein concentration was measured with BCA protein assay reagent kit (Pierce Biotechnology) as described by the manufacturer. Proteins (40 µg/lane) were resolved by 10%
20 SDS/PAGE and transferred onto PVDF membrane (Bio-Rad). After incubation in 5% BSA (Sigma) for 1 hour, the membrane was incubated with primary antibody (polyclonal anti-CB1 antibody, Chemicon Hampshire, UK, at 1:1000 dilution for the experiments shown in Figure 3; rabbit
25 polyclonal antibody against CB1, ab3559 from Abcam, UK, at 1:1000 for the experiments shown in Figure 6) overnight, washed in TBS-Tween and incubated with horseradish peroxidase conjugated sheep anti-mouse IgG (1:10000) or donkey anti-rabbit IgG (1:20000) (both from Amersham) for
30 1 hour. Following washes in TBS-Tween, antibody binding was detected by enhanced chemiluminescence using Super-signal® West Pico chemiluminescent substrate (Pierce Biotechnology).

35 *Caspase-3 assay*

DEVD-dependent caspase activity was measured with the Caspase-3/CPP32 Fluorimetric Assay (Nordic Biosite)

according to the manufacturer's instructions. The assay is based on fluorimetric detection of cleavage of the substrate DEVD-AFC. Uncleaved substrate emits blue light ($\lambda_{\text{max}} = 400 \text{ nm}$), whereas free AFC emits a yellow-green fluorescence ($\lambda_{\text{max}} = 505 \text{ nm}$). Briefly, 300 μl (6×10^5) treated cells were seeded in triplicates in 96-well plates and incubated at 37 °C for 4 or 24 hours. Cells were pelleted and resuspended in 25 μl of chilled lysis buffer for 10 min. 25 μl of 2x reaction buffer and 2.5 μl of 1 mM DEVD-AFC substrate were added. Following incubation for 1 hour, samples were read in a fluorometer at 400 nm excitation and 505 nm emission. Fold increase in caspase activity was determined by comparing values from treated sample/control.

15

MTT assay

Cell viability was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay (Roche Diagnostics) according to the manufacturer's instructions. The assay is based on cleavage of the yellow tetrazolium salt MTT to purple formazan crystals by metabolically active cells. The solubilized crystals can be quantified with a spectrophotometer. Briefly, 200 μl of treated cells were seeded in triplicates in 96-well plates and incubated at 37 °C for 24 or 48 hours. Following addition of MTT (5 mg/ml, 10 μl /well), the cells were incubated further for 4 hours. Cells were then lysed by adding 100 μl of solubilization solution per well and incubator overnight at 37 °C. Absorbance was measured at 570 nm by using a microplate reader and reported as optical density (OD). Percent viability is calculated as $100 * (\text{OD treated cells} / \text{OD untreated cells})$.

30

Cannabinoid reagents

(R)-(+)-WIN 55,212-2 mesylate salt (WIN 55,212-2) (Sigma-Aldrich), 2-Arachidonyl glycerol (2-AG) (Sigma-Aldrich), AM 251 (Tocris, USA), JWH 015 (Tocris, USA) and

35

Capsazepine (Tocris, USA) were initially dissolved to 10 mM in DMSO. These solutions were further diluted in two steps; 1:10 in RPMI and then to the final concentration directly in the culture medium. Arachidonic acid N-

5 (hydroxyethyl)amide (Anandamide) (Sigma-Aldrich) was initially dissolved to 10 mM in 70% ethanol, and then further diluted in two steps as described above.

Cell viability experiments

10 At the day of the experiment, Rec-1 cells were cultured at 1×10^6 /ml in fresh RPMI and Granta cells at 1×10^6 /ml in DMEM. Serum was added as indicated in the figure legends. Viable tumor cells from MCL tumor biopsies were cultured at 1×10^6 /ml in RPMI with 0.5% fetal
15 calf serum. Cells were cultured in flat-bottomed 96 well plates (Costar).

After 24 hours, the cell viability was determined by cell counting using trypan blue exclusion.

20 Results

High expression of CB1 and CB2 in MCL as assayed by gene expression analysis

Results from global gene expression screening using
25 the Affymetrix U95Av.2 chip on 9 MCL or the Affymetrix U133A chip on 11 MCL as described in the "Materials and methods" section showed that expression of CB1 and of CB2 was higher in MCL compared to reactive lymphoid tissue (Figure 1). The results have been further expanded and
30 the mean expression in 27 MCL tumors compared to 9 reactive tissues is shown in Figure 2.

Expression of CB1 protein in MCL as assayed by immunoblot and immunofluorescent staining

35 In order to confirm the microarray data, we performed immunofluorescent stainings on the MCL cell lines Rec-1 (Figure 6B) and Granta 519 (Figure 3B), which were

both found to express CB1. As a positive control, the cell line AtT20 stably expressing CB1 (AtT20[↑]CB1) was used (Figure 3C). In all three cell lines, a strong granular intracellular staining was detected, demonstrating presence of CB1 at the protein level. The CB1 protein expression was further confirmed by immunoblotting, which detected a specific band in Granta 519. In contrast, a Cyclin D1 positive plasma cell leukemia cell line, SK-MM-2, lacked expression of CB1 (Figure 3A). A similar type of granular intracellular stainings as in MCL cell lines was also detected in fresh MCL cells isolated from newly diagnosed MCL cases (Figure 3E).

Effect on cell-survival - cell viability experiments demonstrate reduced cell viability in MCL cell lines and freshly isolated MCL tumor cells from patients in response to anandamide, 2-AG, and WIN 55,212-2

The MCL cell lines Rec-1 and Granta 519 were cultured in the presence of 10 μ M of the endocannabinoids anandamide and 2-AG, respectively, or in the presence of 10 μ M of the CB1/CB2 specific agonist WIN 55,212-2. These substances reduced cell viability as measured by trypan blue exclusion (Figure 4). The effect was more pronounced at lower serum concentrations, and anandamide and WIN 55,212-2 were more potent than 2-AG (Figure 4). The effect on cell survival in MCL cells freshly isolated from patients is shown in Figure 5.

Incubation with CB1 ligands in CB1 expressing cells leads to decreased cell viability.

We have used cell lines expressing high or low levels of CB1 according to Western blotting and immunofluorescent studies (Figure 6A and 6B) or earlier reports (Hsieh C *et al* (1999), *supra*) in order to study the effects of CB1 modulating substances on cell viability. As a measurement of cell viability, the MTT assay was performed on cells expressing low (SK-MM-2) or high (Rec-1)

levels of the receptor. 48 hours after treatment with 10 μ m anandamide, cell viability was reduced to less than 20% in Rec-1 cells, whereas SK-MM-2 cells remained unaffected at the same dose (Figure 7A). In a comparative experiment, different doses of the CB2 agonist JWH 015 had little effect on viability of Rec-1 cells (Figure 7B). The experiment was repeated in AtT20 cells with low endogenous expression of CB1 and in AtT20 $\uparrow$ CB1 cells with constitutive overexpression of CB1. The decreased viability of cells with high CB1 expression after treatment with anandamide was confirmed in this system (Figure 7C). These studies have shown that ligation with a CB1 agonist caused decreased viability in cells overexpressing the CB1 receptor, whereas ligation with a CB2 agonist did not.

Since earlier studies have shown that the effect of CB1 agonists and antagonists can be hampered by high serum concentrations, further experiments were carried out with 0.5% or 1% serum. In order to study the effect of the CB1 antagonist AM 251, an MTT assay was performed after 24 hours of treatment in medium containing 1% serum. In Rec-1 cells, the viability was less than 20% after treatment with 5 μ m AM 251 as compared to approximately 60% after treatment with the CB1 agonist anandamide (Figure 8A). The experiment was repeated in Molt4 cells, which express low levels of CB1. At 5 μ m, anandamide had no effect on viability in Molt4 cells, and the decrease in viability caused by the same concentration of AM 251 was less than half the decrease observed in Rec-1 (Figure 8B). Thus, a substantial part of the effect on cell viability exerted by the CB1 modulating substances tested in this experiment is attributed to the CB1 receptor.

In order to elucidate a potential role for the vanilloid receptor (VR) in viability after treatment with CB1 agonists, WIN 55,212-2 was used together with the VR inhibitor capsazepin. The VR inhibitor did not abrogate the decreased viability caused by 10 μ m WIN 55,212-2

(Figure 9A). This fortifies the role of the CB1 receptor as the mediator of the decrease in viability.

5 *Cell-death by apoptosis induced by CB1 ligation in CB1
expressing MCL cells.*

To investigate the mechanism behind the reduction in viability, a caspase-3 assay was employed using the same experimental setup as in Figure 9A. The results mirror those obtained with the MTT assay (Figure 9B), indicating
10 that treatment with a CB1 agonist induces apoptosis in a VR independent manner. The CB1 antagonist AM 251 caused a similar induction of caspase activity (Figure 10) at 10 μ m. Thus, both agonists and antagonists of CB1 can induce apoptosis in CB1 expressing MCL cells.

15

CLAIMS

1. Use of a compound capable of modulating CB1 receptor signaling for the preparation of a medicament for treatment of a lymphoproliferative disorder involving cells with abnormal CB1 receptor function.

2. Use according to claim 1, wherein said treatment results in the death of said cells.

3. Use according to claim 1, wherein said treatment results in a reduced viability of said cells as compared with cells with normal CB1 receptor function.

4. Use according to any one of the preceding claims, wherein said compound is selected from the group consisting of CB1 agonists, CB1 inverse agonists and CB 1 antagonists.

5. Use according to any one of the preceding claims, wherein said compound has been identified using a method according to any one of claims 16-19.

6. Use according to any one of the preceding claims, wherein said compound is selected from the group consisting of WIN 55,212-2, anandamide and AM-251, and mixtures thereof.

7. Use according to any one of claims 1-5, wherein said compound is a polypeptide with a biospecific affinity for the CB1 receptor.

8. Use of a polypeptide with a biospecific affinity for the CB1 receptor for the preparation of a medicament for treatment of a lymphoproliferative disorder involving cells with abnormal CB1 receptor function.

9. Use according to any one of claims 7-8, wherein said polypeptide is selected from an antibody against the CB1 receptor and a functional fragment thereof.

5 10. Use according to any one of claims 7-8, wherein said polypeptide is a variant of a scaffold protein, which variant has been affinity selected from a library of many different such variants, the selection having been performed using the CB1 receptor as target.

10

11. Use according to any one of the preceding claims, wherein said lymphoproliferative disorder involving cells with abnormal CB1 receptor function is selected from the group consisting of lymphomas, posttransplanta-
15 tional lymphoproliferative disorders, Castleman's disease, atypical EBV infection, HTLV1 infection, infection with Herpes virus type 8, angioimmunoblastic lymphadenopathy and lymphomatoid granulomatosis.

20 12. Use according to claim 11, wherein said disorder is a B cell lymphoma.

13. Use according to claim 12, wherein said disorder is mantle cell lymphoma.

25

14. Use according to any one of the preceding claims, wherein said treatment is combined with at least one other treatment for said disorder.

30 15. Use according to any one of the preceding claims, wherein said cells with abnormal CB1 receptor function are B lymphocytes.

16. Method for identification of a compound capable
35 of modulating CB1 receptor signaling, which method comprises:

- providing a compound suspected of being capable of modulating CB1 receptor signaling;
- applying said compound to a test population of cells with abnormal CB1 receptor function;
- 5 - applying said compound to a control population of cells with normal CB1 receptor function;
- analyzing the viability of cells in said test population and in said control population;
- whereby reduced viability in the test population
- 10 relative to the control population identifies said compound as capable of modulating CB1 receptor signaling.

17. Method according to claim 16, in which said test population comprises transformed B lymphocytes and said

15 control population comprises non-transformed B lymphocytes.

18. Method for identification of a compound capable of modulating CB1 receptor signaling, which method comprises:
- 20 - providing a compound suspected of being capable of modulating CB1 receptor signaling;
- providing a test population and a control population of cells with abnormal CB1 receptor function;
- 25 - applying said compound to said test population but not to said control population; and
- analyzing the viability of cells in said test population and in said control population;
- whereby reduced viability in the test population
- 30 relative to the control population identifies said compound as capable of modulating CB1 receptor signaling.

19. Method according to claim 18, in which said cells comprise transformed B lymphocytes.

35

20. Method for the manufacture of a medicament for treatment of a lymphoproliferative disorder involving

cells with abnormal CB1 receptor function, comprising admixing a compound capable of modulating CB1 receptor signaling with at least one pharmaceutically acceptable excipient.

5

21. Method according to claim 20, in which said compound is as defined in any one of claims 2-7 and 9-10.

22. Method according to any one of claims 20-21, in which said disorder is as defined in any one of claims 11-13.

23. Method for treatment of a lymphoproliferative disorder involving cells with abnormal CB1 receptor function, which method comprises administering, to a subject in need thereof, a pharmaceutically active amount of a compound capable of modulating CB1 receptor signaling.

24. Method according to claim 23, in which said compound is as defined in any one of claims 2-7 and 9-10.

25. Method for treatment of a lymphoproliferative disorder involving cells with abnormal CB1 receptor function, which method comprises administering, to a subject in need thereof, a pharmaceutically active amount of a polypeptide with a biospecific affinity for the CB1 receptor

26. Method according to any one of claims 23-25, in which said disorder is as defined in any one of claims 11-13.

27. Method according to any one of claims 23-26, in which said treatment is combined with at least one other treatment for said disorder.

28. Method for the diagnosis of a lymphoproliferative disorder involving cells with abnormal CB1 receptor function in a subject, which method comprises

- taking cells from said subject;
- 5 - culturing said cells to obtain a first cell population;
- dividing said first cell population into a test population and a control population;
- applying a compound capable of modulating CB1 receptor signaling to said test population but not to said control population; and
- 10 - analyzing the viability of cells in said test and control populations;
- whereby reduced viability in the test population
- 15 relative to the control population implies the presence of said disorder.

29. Method according to claim 27, in which said compound is as defined in any one of claims 2-7 and 9-10.

20

30. Method according to any one of claims 27-28, in which said disorder is as defined in any one of claims 11-13.

25 31. Method for elimination of malignant lymphocytes from a cell population comprising malignant lymphocytes and non-malignant cells, comprising treating said cell population with a compound capable of modulating CB1 receptor signaling.

30

32. Method according to claim 31, in which said compound is as defined in any one of claims 2-7 and 9-10.

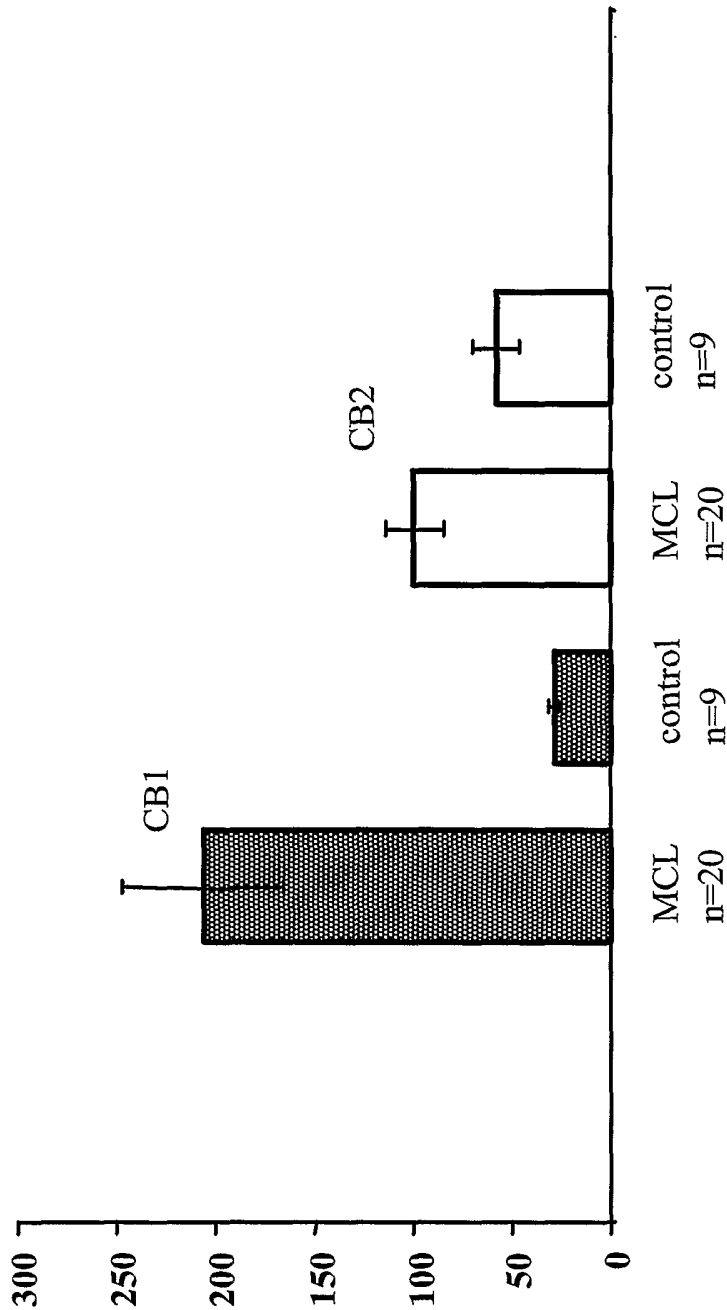


FIGURE 1

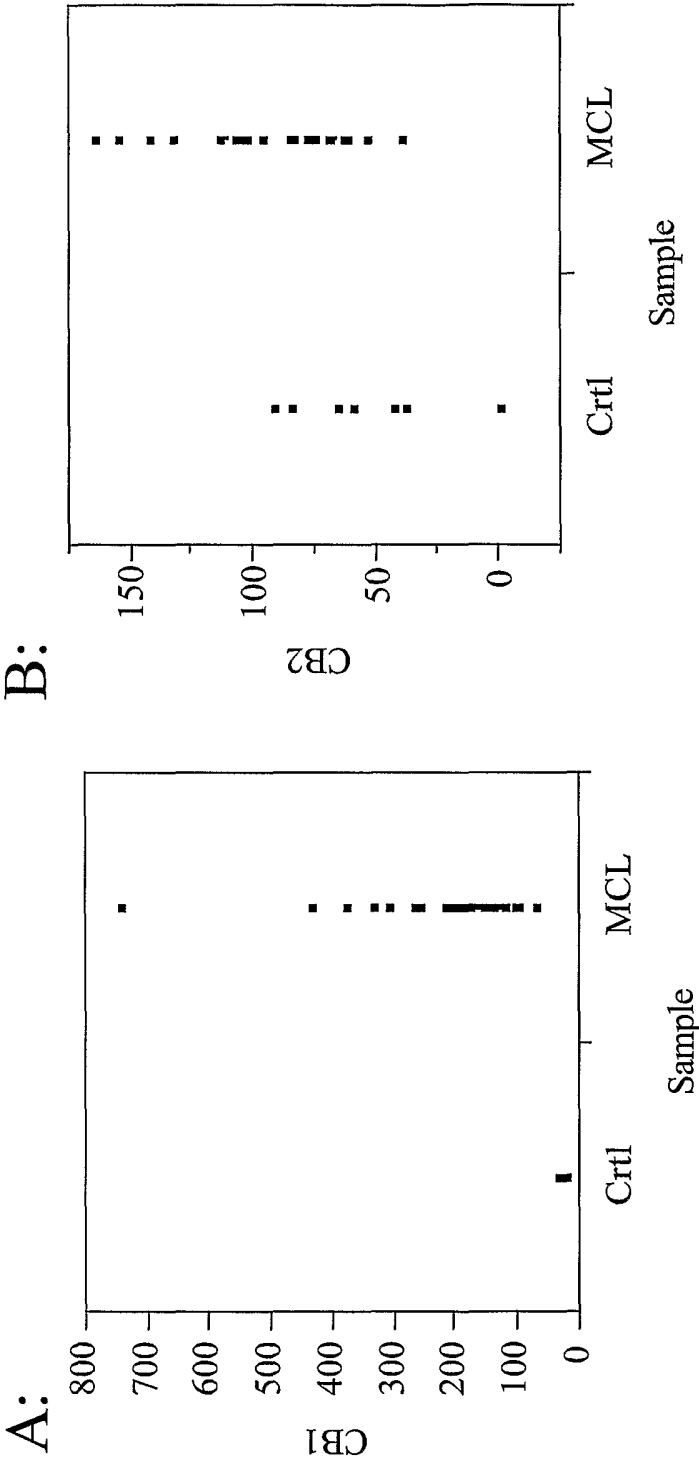


FIGURE 2

3/13

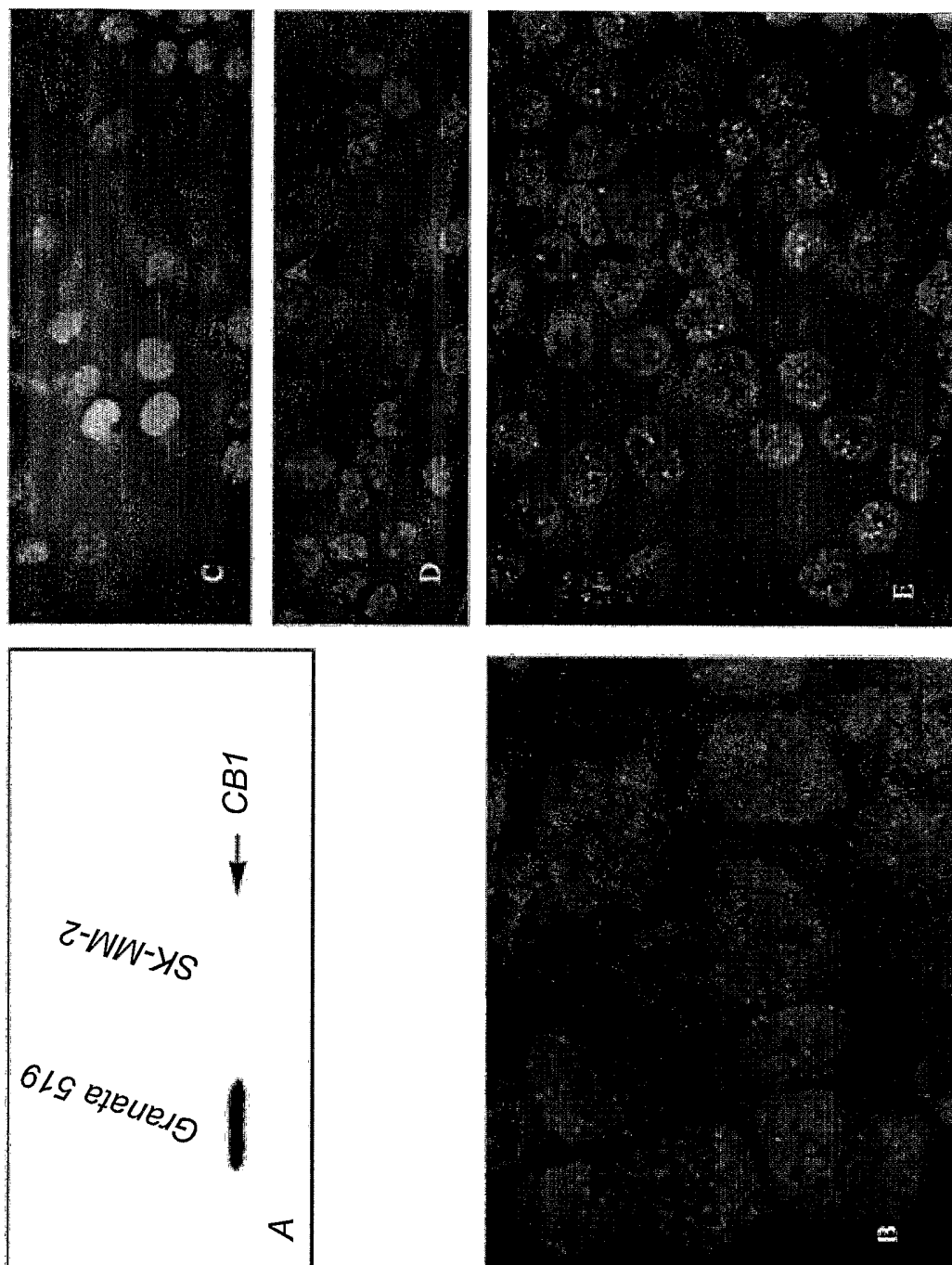
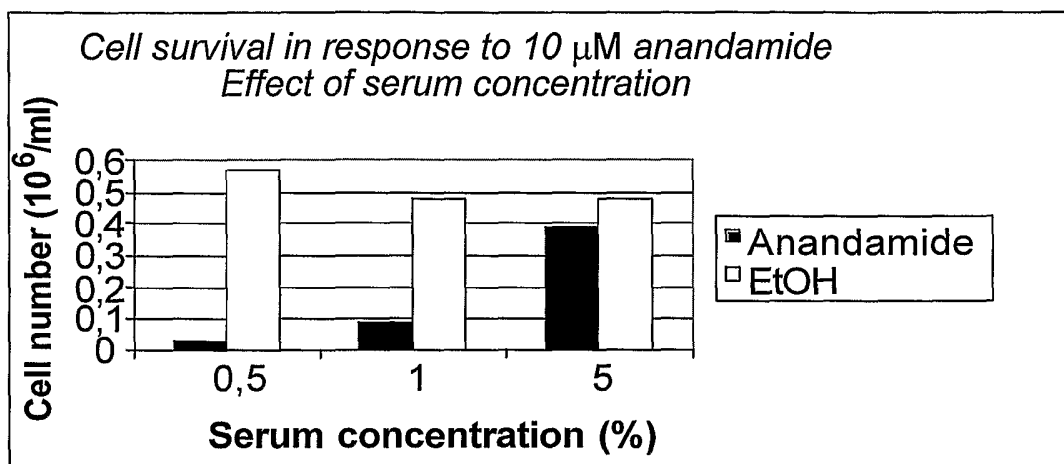


FIGURE 3

4/13

A:



B:

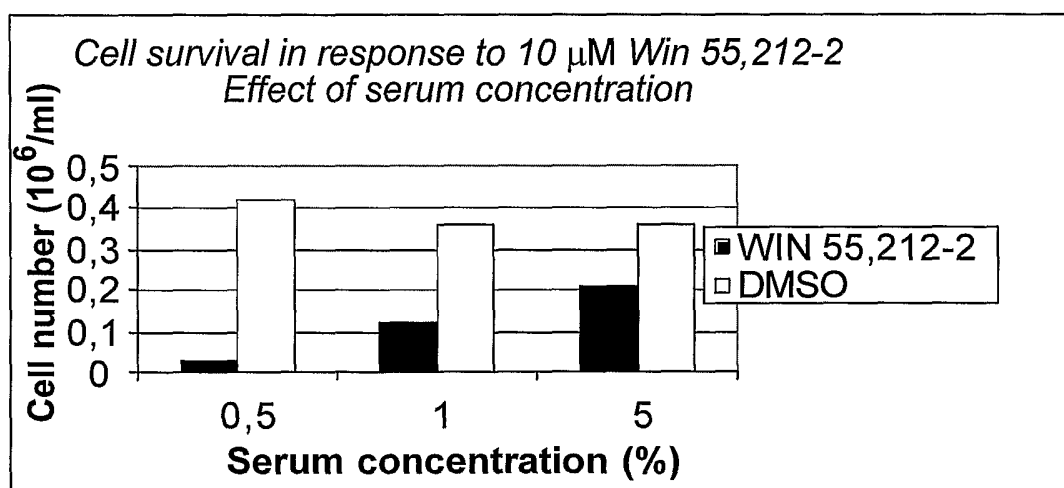
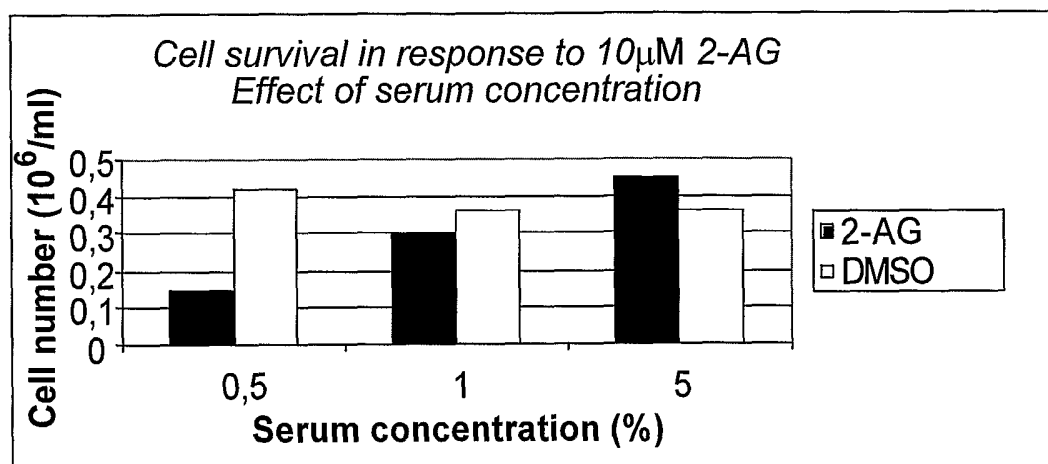


FIGURE 4

5/13

C:

**FIGURE 4 (cont.)**

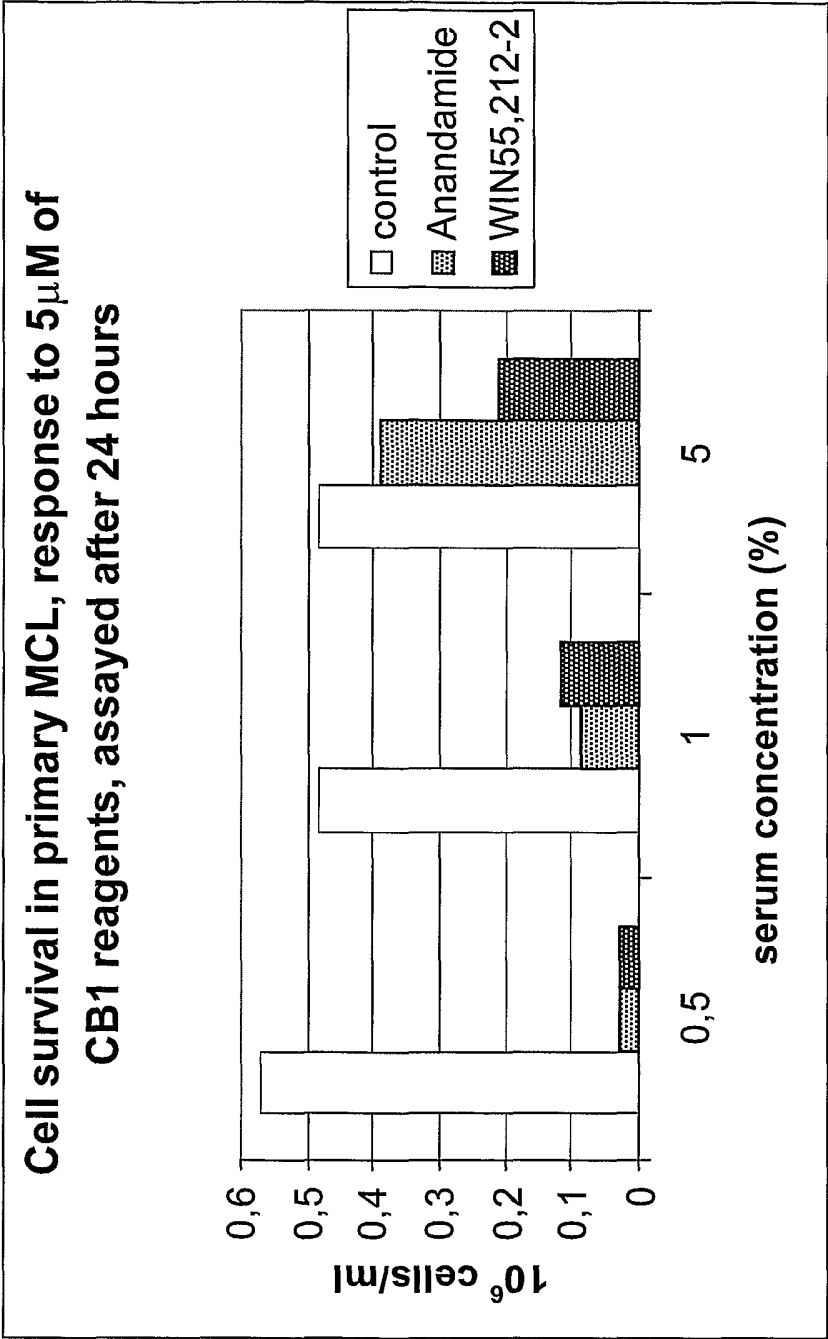
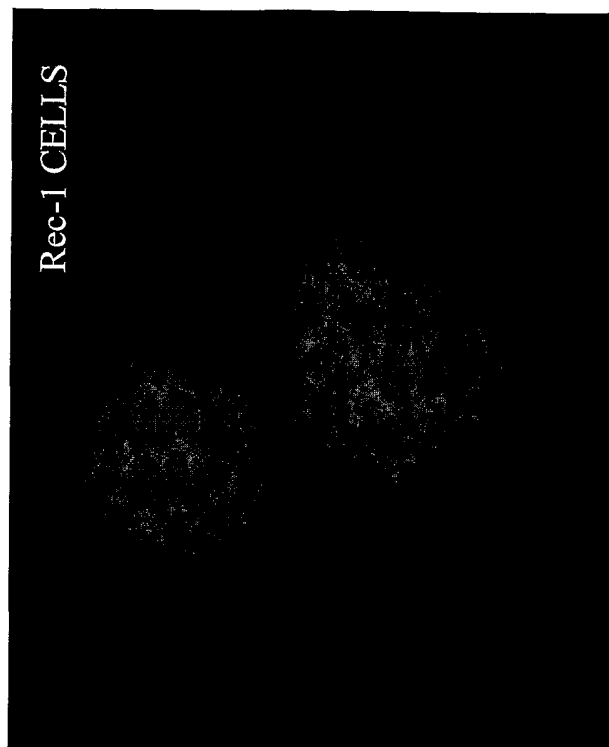


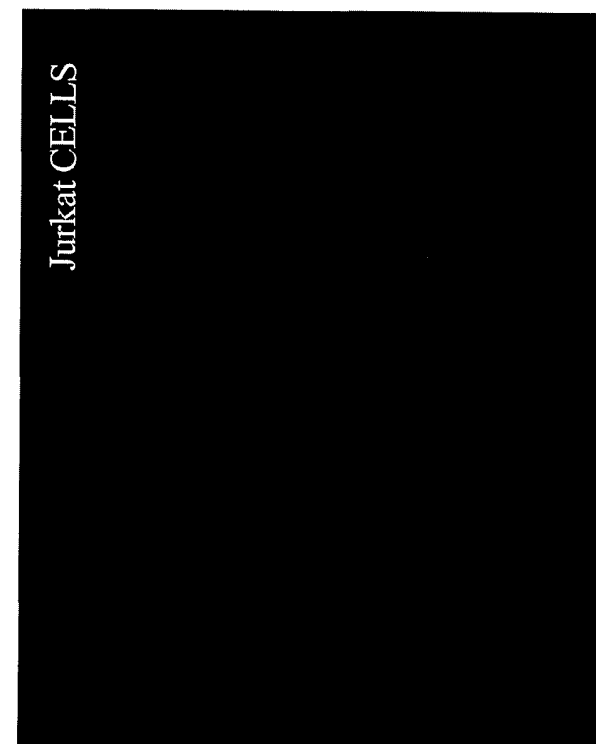
FIGURE 5

7/13



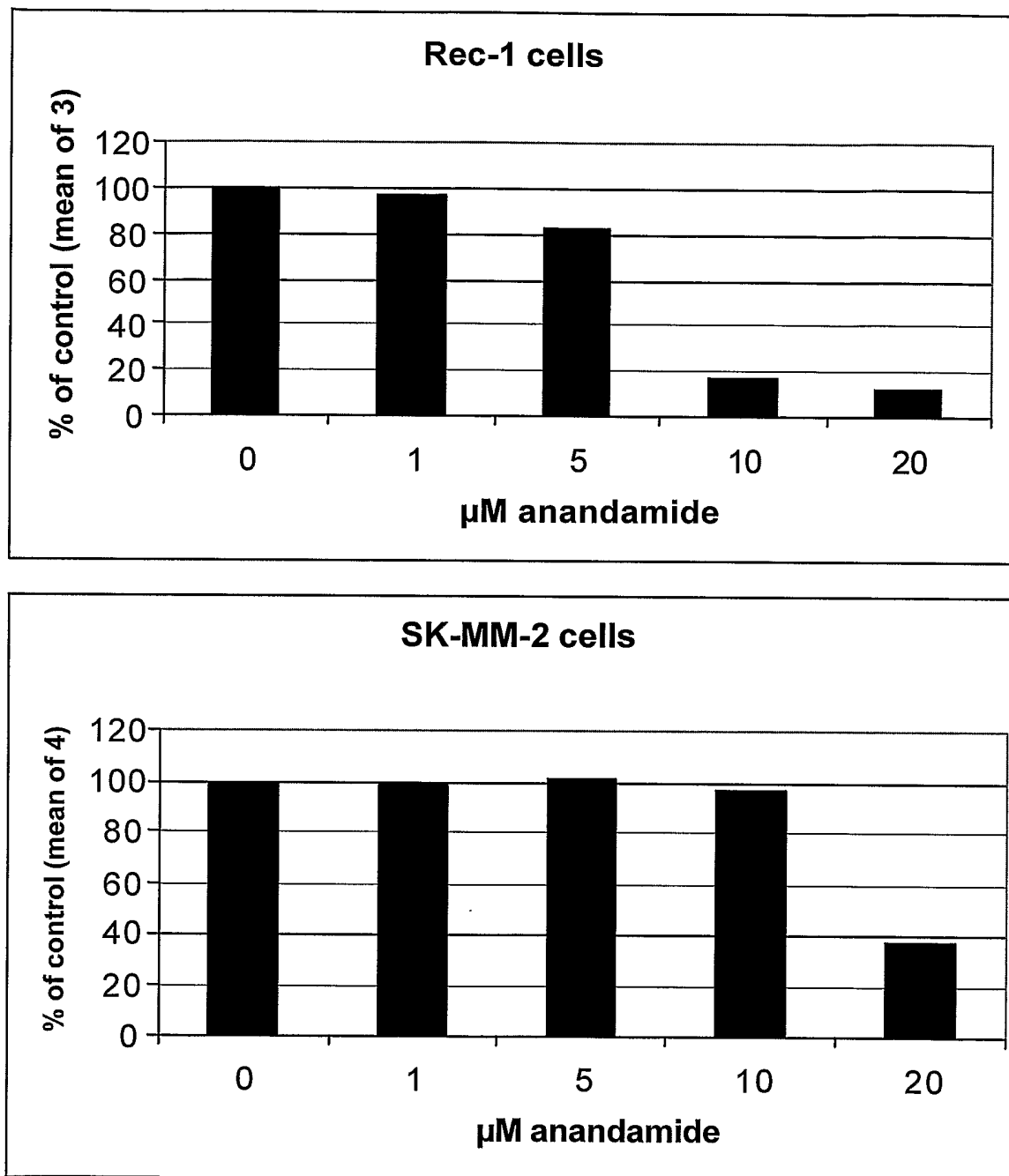
SKMM
Molt-4
Rec-1
Granta

A:

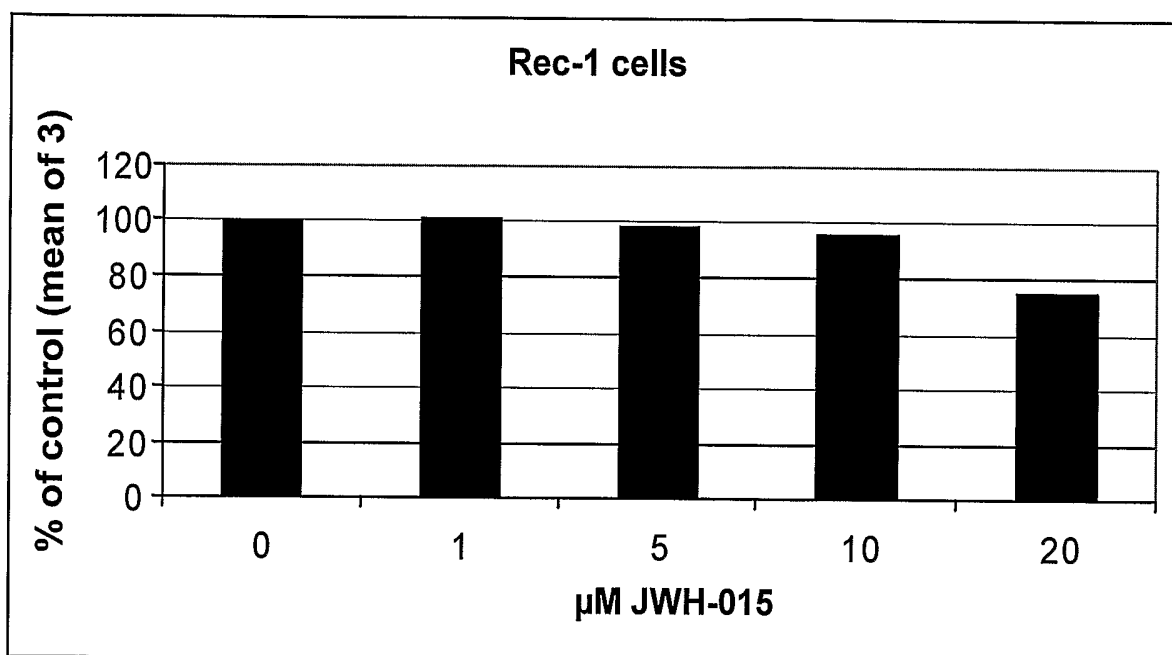


B:

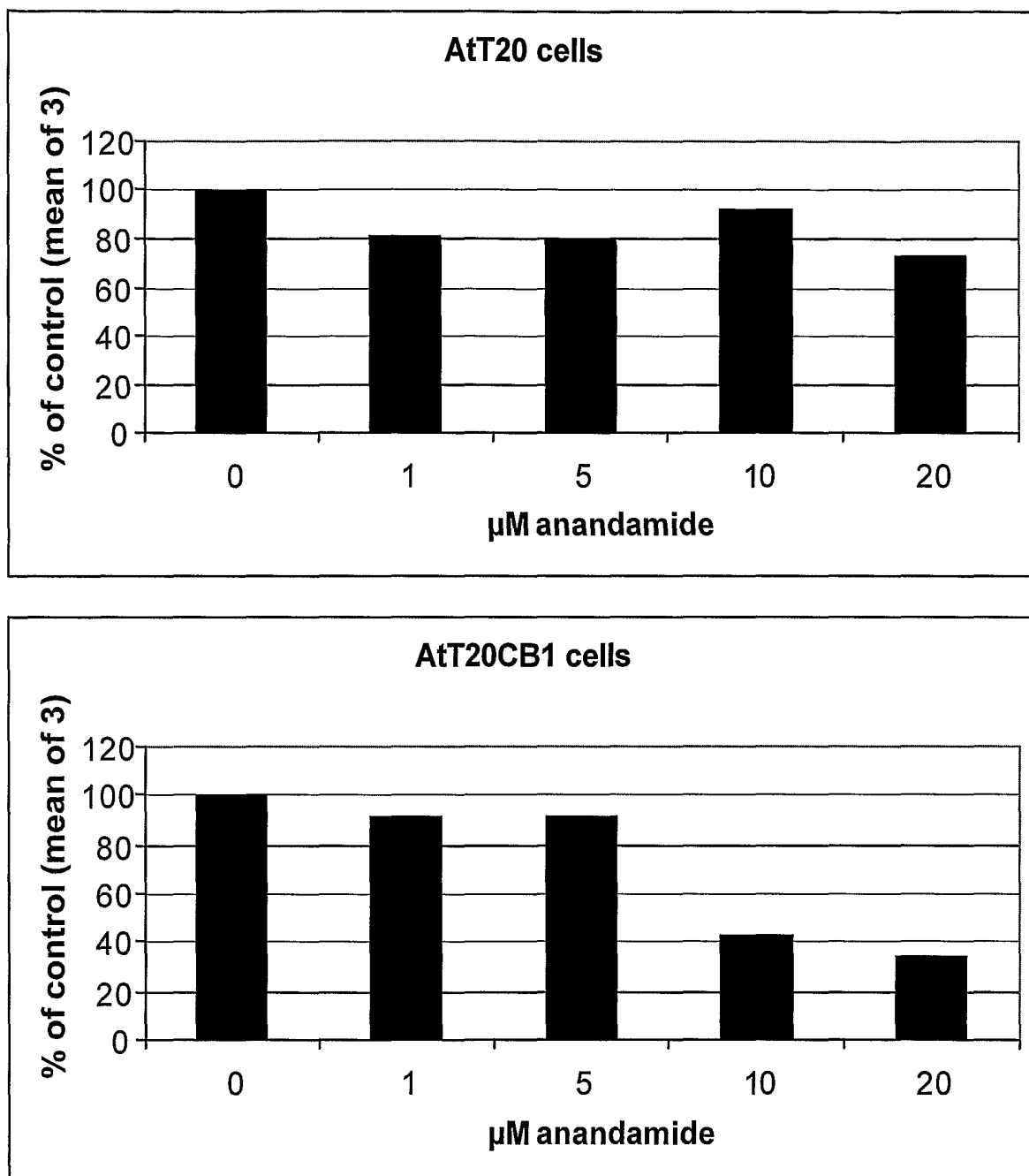
8/13

**FIGURE 7A**

9/13

**FIGURE 7B**

10/13

**FIGURE 7C**

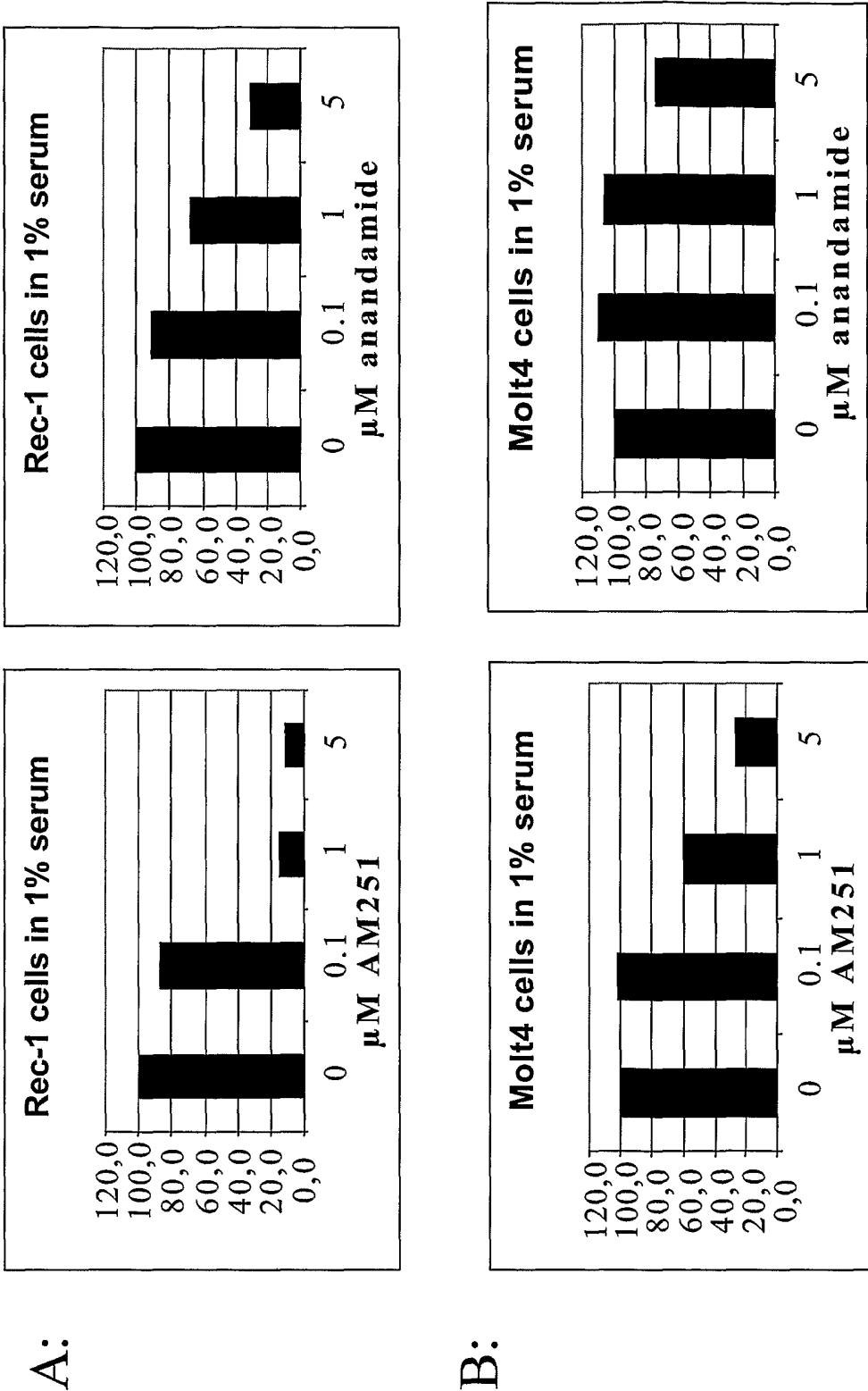
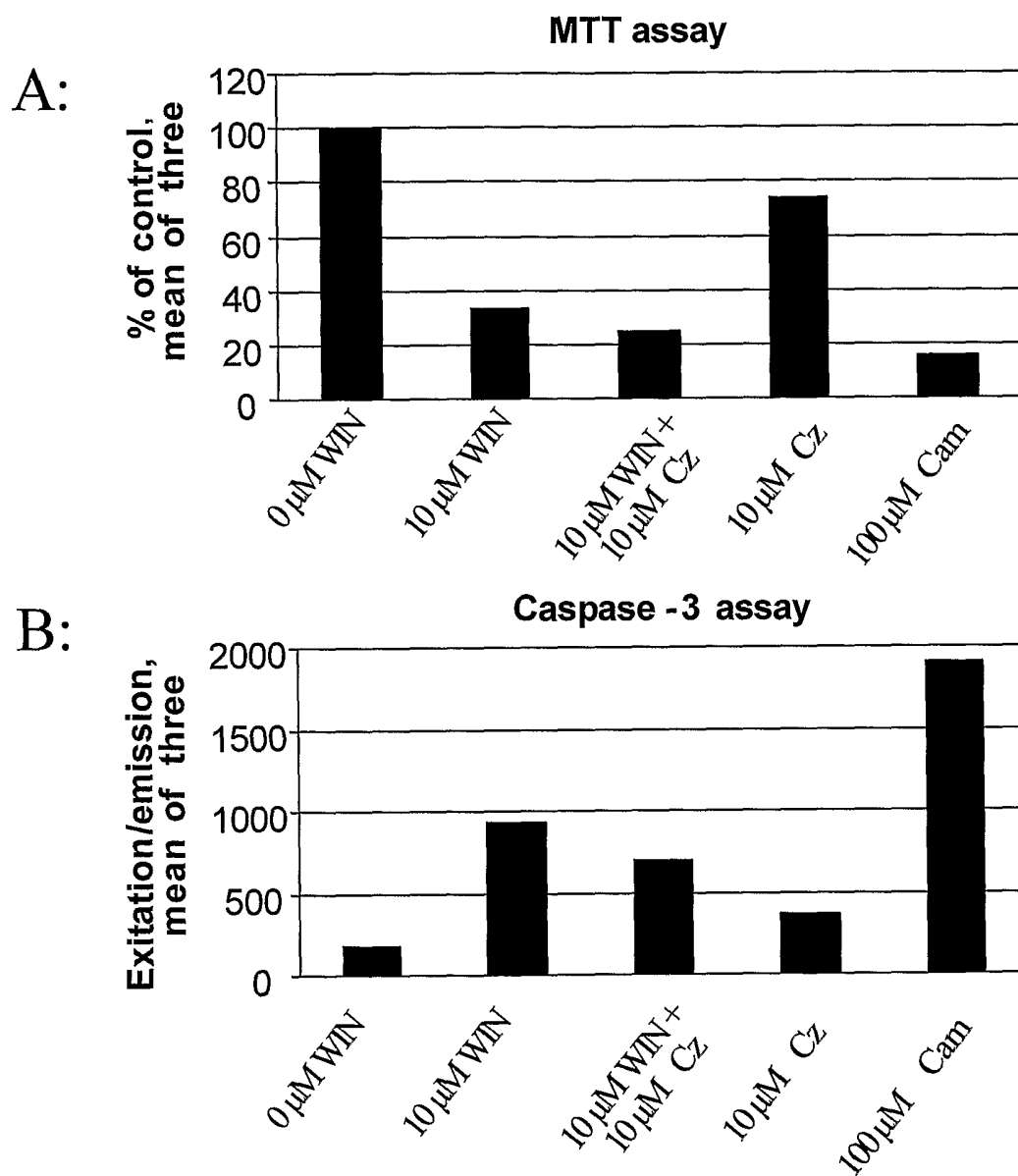
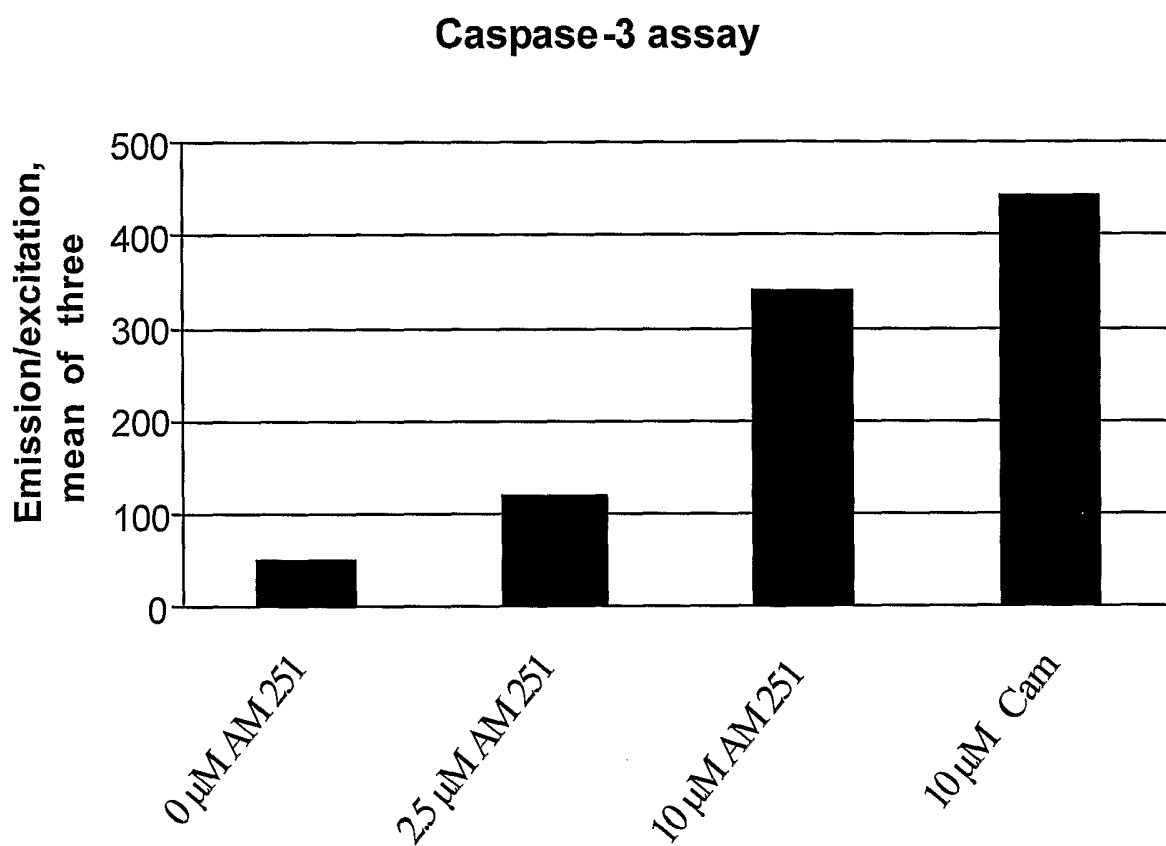


FIGURE 8

12/13

**FIGURE 9**

13/13

**FIGURE 10**