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(57) **Abrégé/Abstract:**

The present disclosure provides methods for the treatment of cell proliferative disorders by administration of a Syk kinase or Syk/Flt-3 kinase inhibitor. Cell proliferative disorders treatable by the methods include, hematopoietic neoplasms and virally associated tumors. The compounds are also directed to therapeutic or prophylactic inhibition of tumor metastasis.



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(54) Title: METHODS OF TREATING CELL PROLIFERATIVE DISORDERS BY USING PYRIMIDINEDIAMINE COMPOUNDS

(57) Abstract: The present disclosure provides methods for the treatment of cell proliferative disorders by administration of a Syk kinase or Syk/Flt-3 kinase inhibitor. Cell proliferative disorders treatable by the methods include, hematopoietic neoplasms and virally associated tumors. The compounds are also directed to therapeutic or prophylactic inhibition of tumor metastasis.

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METHODS OF TREATING CELL PROLIFERATIVE DISORDERS BY USING PYRIMIDINEDIAMINE COMPOUNDS

1.

[0001]

2. **TECHNICAL FIELD**

[0002] The present disclosure relates to methods and compositions for treating cell proliferative disorders, where the compositions comprise inhibitors that target kinase activities affecting the proliferative potential of cells.

3. **BACKGROUND**

[0003] Unregulated cell growth is the hallmark of tumors and cancers and other cell proliferative disorders. The cellular processes controlling cell division and cell proliferation are complex, involving an intricate interplay between gene products that promote cell division and growth and those that hold such processes in check. Positive regulators of growth and proliferation are generally described as proto-oncogenes, which are the normal counterparts of altered genes and their gene products known to promote tumor and cancer formation. Proto-oncogenes promote cell division and negatively control cell apoptosis. Uncoupling the activity of these gene products from their normal regulated state converts the proto-oncogenes to oncogenes. Normal function of proto-oncogenes includes growth factors, growth factor receptors, cellular signal transduction molecules, and nuclear factors. Activation of the proto-oncogenes into oncogenic forms can occur in a variety of ways, including gene mutation, amplification, gene translocation, and viral activation.

[0004] Tumor suppressors, as opposed to the proto-oncogenes, generally exert a negative effect on cell growth, promote apoptosis of cells, inhibit cell cycle progression, and affect invasive and metastatic potential. In some instances, tumor suppressors can counter the activity of oncogenes even in their altered forms. Upon loss or inhibition of tumor suppressor function, the unregulated activity of proto-oncogenes or their corresponding oncogenic forms leads to cell transformation and carcinogenesis. Gene mutation or deletion, suppressed transcription, increased degradation, or abnormalities of associated proteins that work in concert with the tumor suppressors may compromise tumor suppressor activity. Tumor suppressor genes act as recessive alleles such that a cell with a normal allele along with a mutant allele still behaves normally. Thus, loss of the normal allele, also called loss of heterozygosity (LOH), characterizes some types of abnormal cell growth and proliferation. Genomic instability arising as a consequence of oncogene activity and disruption of normal cell division controls can increase the probability of LOH and thus the occurrence of the transformed phenotype by oncogenes.

[0005] Treatment of cell proliferative disorders can target the oncogenes and/or the tumor suppressors affected in the transformed cells. However, a disorder arising from a loss-of-function, such as a tumor suppressor, is typically more problematic when attempting to treat the underlying molecular defect than treating the underlying molecular defect in a disorder arising from a gain-of-function change, such as activation of an oncogene. Altering cellular processes to provide the lost cellular function is not practicable in many cases. Thus, even for cell proliferative disorders arising from loss of tumor suppressor activity, therapy is typically directed at the dysregulated molecules (*e.g.*, proto-oncogenes) that act as a consequence of the lost tumor suppressor function. Although many molecular targets have been identified, such as non-receptor and receptor based protein kinases, the complex nature of the cellular regulatory mechanisms at play in cell proliferation and growth would indicate that other molecules that could be targets of therapy remain to be identified. Some of these will be unknown while others may be known but not linked to cell proliferative disorders.

[0006] Thus it is desirable to identify other cellular molecules that act in an oncogenic manner in cell proliferative disorders, either as a consequence of alteration of its own activity or as a result of loss of a cellular function that act to regulate its activity. Upon identification of such molecules, compounds specifically directed to that cellular molecule can be identified

and used, either independently or in combination with other known therapies, to treat the cell proliferative disorder.

4. SUMMARY

[0007] The present disclosure provides method of treating cell proliferative disorders by administration to subjects an amount of a Syk kinase inhibitory compound effective to treat the cell proliferative disorder. In some embodiments, the Syk kinase inhibitor is selective for Syk kinase, thereby specifically targeting the aberrant Syk kinase activity present in the proliferative disorder. Any cell proliferative disorder in which Syk plays a role in some aspect of abnormal cell division or cell growth can be treated with the inhibitor compounds. In some embodiments, the cell proliferative disorders treatable with the inhibitor compounds are hematopoietic neoplasms, which are abnormal growth involving cells of the hematopoietic lineage. Hematopoietic neoplasms treatable with the Syk inhibitory compounds include, among others, various myeloid and lymphoid neoplasms, such as chronic myelogenous leukemia, Burkitt's lymphoma, and acute myelogenous leukemia.

[0008] In some embodiments, the Syk kinase inhibitory compound comprises a Syk/Flt-3 kinase inhibitory compound, capable of inhibiting the activity of both Syk kinase and Flt-3 kinase. These compounds can be used to treat cell proliferative disorders that are associated with aberrant Flt-3 kinase activity. Various hematopoietic neoplasms in which Flt-3 activity is abnormal include, among others, acute myelogenous leukemia, B-precursor cell acute lymphoblastic leukemia, T-cell acute lymphoblastic leukemia, myelodysplastic syndrome, and chronic myelogenous leukemia. These and other such disorders involving Flt-3 can be treated with the Syk/Flt-3 inhibitory compounds, such as members of the 2,4-pyrimidinediamine compounds described herein.

[0009] In other aspects, the inhibitor compounds can be used to treat tumors mediated by viral genes that transform a normal cell into a tumor cell. In these embodiments, tumors in which the activity of a viral oncogene disrupts Syk kinase function as part of its transformation mechanism can be targeted for treatment. Thus, in some embodiments, virally mediated tumors are associated with infection by viruses carrying genes encoding proteins with immunoreceptor tyrosine-based activation motifs (ITAM). Normally, these conserved sequences modulate Syk kinase activity during development and function of immune system

cells (*e.g.*, B cells, T cells, neutrophils, etc.). However, persistent expression of viral proteins with ITAM sequences can lead to aberrant Syk kinase activity and consequent tumor formation and/or maintenance. In various embodiments, the virally mediated tumors can be associated with Kaposi's sarcoma-associated herpes virus, Epstein Barr virus, human T-cell lymphotropic virus (HTLV-1), or mammary tumor virus (MTV). Presence of these viruses are correlated with a number of cell proliferative disorders, including, Kaposi's sarcoma, Burkitt's lymphoma, Hodgkin's lymphoma, adult T cell leukemia, and certain forms of breast cancer.

[0010] In some aspects, the disclosure provides a method of inhibiting tumor metastasis by administration to a subject an amount of a Syk inhibitory compound effective to inhibit tumor metastasis. Any tumor with metastatic potential can be treated with the inhibitor compounds. In some embodiments, the tumor metastasis is associated with the activity of integrins, which can act through Syk kinase in modulating cell adhesive properties, such as that observed in metastasis of tumor cells. Syk kinase inhibitors can be used to inhibit tumor metastasis by affecting cell signaling events mediated by integrins, such as $\beta 1$, $\beta 2$, and/or $\beta 3$ integrins.

[0011] In some aspects, the inhibitor compounds can be used in combination with other cancer treatments. In some embodiments, Syk inhibitory compounds are used in combination with other chemotherapeutic agents, including, among others, antimetabolites, alkylating agents, coordination compounds, transcription inhibitors, topoisomerase inhibitors, DNA minor-groove binding compounds, vinca alkyloids, antitumor antibiotics, hormones, and antitumor enzymes.

5. BRIEF DESCRIPTION OF THE FIGURES

[0012] FIG. 1A, FIG. 1B, and FIG. 1C show the ability of Syk inhibitor compound VI to block proliferation and induce differentiation of TEL-Syk-transformed cells. DNA content of TEL-Syk- or BCRAbl-transformed cells treated for 36 hours with either DMSO, the Syk inhibitor compound VI (2 μ M) or Abl kinase inhibitor STI-571 (2 μ M) are shown in FIG. 1A. The ability of TEL-Syk to blocks pre-B cell differentiation is shown in FIG. 1B. Cells were cultured for 3 days in the absence of IL-7 and analyzed for the expression of kappa LC by FACS. Inhibition of TEL-Syk activity with compound VI is shown in FIG. 1C.

[0013] FIG. 2A and FIG. 2B show the ability of Syk inhibitor **VI** to block the proliferation of Myc-transformed pre-B cells. FIG. 2A is a FACS profile of pre-B cells that were transduced with Myc expression vectors and cultured in the absence of IL-7 for 1 week. Lower panel of FIG. 2B shows spleens of RAG/ γ C^{-/-} mice 5 weeks after injection with the indicated cells, illustrating the ability of the Myc transformed cells to cause splenomegaly and leukemia. FIG. 2B shows the DNA content of Myc-transformed cells that were treated for 36 hours with either DMSO, **VI** (2 μ M) or STI-571 (2 μ M).

[0014] FIG. 3A and FIG. 3B show the ability of Syk inhibitor **VI** to block the proliferation of tumorigenic SLP-65^{-/-} pre-B cell lines. FIG. 3A shows that SLP-65^{-/-} pre-B cell lines can cause splenomegaly and leukemia, as evidenced by the state of spleens of RAG/ γ C^{-/-} mice 5 weeks after injection with the indicated cells. FIG. 3B shows the DNA content of tumorigenic SLP-65^{-/-} pre-B cell lines that were treated for 36 hours with either DMSO, **VI** (2 μ M) or STI-571 (2 μ M).

[0015] FIG. 4 shows survival curves for NOD-SCID mice inoculated intravenously (i.v.) with MV4-11 human acute myeloid leukemia cells and treated with compound **VII** at 40 mg/kg twice daily, PO, from day 17 post tumor cell inoculation for the duration of the study. Calculations for % ILS for 40mg/kg **VII** group (10⁶ cells) assumes median day of death of 83 days, due to greater than 50% survival in this group at study termination (Day 83).

[0016] FIG. 5 shows days to death for individual animals. The survival data is for NOD-SCID mice injected intravenously with MV4-11 tumor cells. Mice were treated with vehicle or varying doses of **VII** twice daily, PO, from day 17 post-tumor cell injection until the end of the study (Day 83). Days to death for sacrificed animals are shown in the graph with median day of death denoted by blue line. The survivors in each group at study termination are shown at the top of the graph denoted as # survivors/total # mice.

[0017] FIG. 6A and FIG. 6B show frequency of tumors in MV4-11 tumor bearing NOD-SCID mice at necropsy. The severity of disease was evaluated by quantitating the frequency of mice having palpable tumors upon necropsy at termination, irrespective of reason for sacrifice (FIG. 6A). The total number of tumors per group is shown in FIG. 6B. Mice were inoculated i.v. with 5 or 10 million MV4-11 human AML cells (denoted as 5E6 or 10E6, respectively). At Day 17 post cell injection, mice were treated with vehicle or 40mg/kg **VII**

(PO, bid) until sacrifice or for the duration of the study, up to Day 83 for surviving animals. Mice were sacrificed and a comprehensive necropsy performed. The total number of necropsies performed per group ranged from 8-13 animals.

[0018] FIG. 7 shows the tumor distribution profile of experimental animals. Percent of total tumors found in various anatomical locations were noted upon necropsy at sacrifice. Mice were injected i.v. with 5 or 10 million MV4-11 cells and dosed orally with either vehicle or 40 mg/kg of **VII** twice daily for the duration of the study. Animals showing enlarged salivary glands are included in the graph, although no palpable tumor was detected. The total number of necropsies performed per group ranged from 8-13 animals.

[0019] FIG. 8 shows the engraftment profile of MV4-11 tumor cells in bone marrow (BM) and peripheral blood (PB) of NOD-SCID mice. The data is from engraftment of MV4-11 human tumor cells in NOD-SCID mice treated with vehicle or 40mg/kg **VII** twice daily, PO, from day 17 post i.v. tumor cell injection until the end of the study. Bone marrow (BM) and peripheral blood (PB) tumor cell engraftment were detected by flow cytometric analysis using CD33 and HLA cell surface staining for detection of MV4-11 tumor cells. Data were normalized, and percent human tumor cells out of total cells were calculated. Total cells were defined as the number of human CD33 + HLA + positive events and murine CD45 positive staining events. Graph includes all samples.

[0020] FIG. 9 shows detection of Flt-3 in MV4-11 xenografts by immunoprecipitation and Western Blot analysis. Panel A is detection of phosphorylated Flt-3 in MV4-11 tumor lysates from mice approximately two hours after the final dose of **VII** or vehicle using anti-phosphotyrosine antibody on the left and anti-phospho-Flt-3 specific antibody on the right side of the blot. Additionally, a reprobe of the blot for the total Flt-3 levels are shown in Panel B.

[0021] FIG. 10 shows phosphorylated histone H3 analysis of MV4-11 tumor xenografts. Proliferation was assessed *ex vivo* in formalin fixed tumor sections from three randomly selected mice from this study using immunohistochemical staining of human-specific phosphorylated histone H3 (phH3) as a marker for tumor cell proliferation. Human phH3 expression was reduced in tumor sections in a dose-dependent manner following treatment with **VII**. When compared to MV4-11 tumor xenografts from vehicle treated mice, treatment

with 20 and 40mg/kg **VII** resulted in a 53% and 71% inhibition of pH3 staining, respectively. These data indicate that **VII** mediated inhibition reduced the proliferative capacity of MV4-11 tumors *in vivo*, correlating with the reduced tumor volumes observed during the in-life portion of the study. The reduced proliferation is likely to be due to reduced constitutive Flt-3 phosphorylation, as this activity has been shown to be required for survival of MV4-11 cells *in vitro*.

[0022] FIG. 11 shows a representative data illustrating immunohistochemical staining for pErk1/2 and pStat5 in tumor sections from MV4-11 tumor bearing mice treated orally twice per day for 26 days. Tumors were harvested from mice ~2 hours following final dose of vehicle or 40mg/kg **VII**.

[0023] FIG. 12 shows the effect of treating AML cells or 32D transfectants with 1 uM **VI** in inducing apoptosis as determined by Annexin V and PI staining.

6. DETAILED DESCRIPTION OF EMBODIMENTS

6.1 Methods of Treatment

[0024] The present disclosure provides method of treating cell proliferative disorders by administration of Syk kinase or Syk/Flt-3 kinase inhibitory compounds. Syk kinase is one of the two known members of the Syk family (Syk and ZAP-70) non-receptor tyrosine kinases. Syk is activated upon the binding of its src homology 2 (SH2) domains to immunoreceptor tyrosine-based activation motifs (ITAM). Syk kinase plays an essential role in lymphocyte development and activation of immune cells and is best characterized for its role in B cell receptor signaling and Fc receptor mediated release of mast cell granules. Although expressed ubiquitously in hematopoietic cells, Syk is also expressed in other tissues, such as breast epithelial cells and hepatocytes. It is believed by some in the field that Syk is a tumor suppressor and acts as a negative regulator of metastatic potential. Loss of Syk activity is suggested as being associated with formation of invasive breast cancer, and chromosomal loss of the Syk gene is indicated in certain types of lymph node metastasis of primary breast cancer. Thus, use of Syk inhibitors to treat cell proliferative disorders or as treatment to reduce the metastatic potential of tumor cells would be contraindicated if Syk functions as a tumor suppressor.

[0025] Flt-3 is also a tyrosine kinase, but unlike Syk, it belongs to the family of tyrosine kinase receptor proteins. Flt-3 is a member of the class III receptor tyrosine kinases, which are related by amino acid sequence and structural characteristics. Flt-3 is activated by binding to its cognate ligand, Flt-3 L. Flt-3 kinase is expressed in early hematopoietic stem cells of normal bone marrow, and appears to function in the development of multipotent stem cells and B cells by controlling the activity of various downstream cellular targets that include, among others, phospholipase C- (PLC), the p85 subunit of phosphatidylinositol 3'-kinase (PI3K), Shc, Shp-2, Ship, Grb2, Vav, Fyn kinase, Src kinase, Stat5 signal transducing protein, and Erk. Although Flt-3 kinase is normally expressed in progenitor cells in the bone marrow, high levels of expression are also observed in a spectrum of hematologic cell proliferative disorders, such as acute myelogenous leukemia (AML), B-precursor cell acute lymphoblastic leukemia, T-cell acute lymphoblastic leukemia, and chronic myelogenous leukemia (CML) in lymphoid blast crisis (see. *e.g.*, Rosnet et al., 1996, *Leukemia* 10:238-248; Carow et al., 1996, *Blood* 87:1089-1096). Presence of Flt-3 mutations in hematological cell proliferative disorders correlates with decreased remission rates, increases in relapse rates, and generally lower overall survival rates (Rombouts et al., 2000, *Leukemia* 14:675-683; Thiede et al., 2002, *Blood* 99:4326-4335; Frohling et al., 2002, *Blood* 100:4372-4380).

[0026] Although the art suggests that Syk may act as a tumor suppressor, the present disclosure is based on indications that Syk functions contrary to that posited role. For instance, forced expression of Syk kinase in tumor cells does not appear to reverse the transformed phenotype of tumor cells. To the contrary, it is suggested herein that Syk acts in an oncogenic capacity to promote and/or maintain cell proliferation. With this perspective on the role of Syk, the disclosure provides methods of treating cell proliferative disorders by administering to a subject an amount of a Syk kinase inhibitory compound effective to treat a cell proliferative disorder.

[0027] Moreover, it is shown herein that some Syk inhibitory compounds, such as members of the 2,4-pyrimidinediamine compounds described in further detail below, can also act as inhibitors of the Flt-3 kinase. Consequently, in some embodiments, Syk inhibitory compounds with this dual- or multi-acting inhibitory profile of inhibiting Flt-3 kinase as well as Syk kinase (*i.e.*, Syk/Flt-3 kinase inhibitory activities) find applications for treatment of cell proliferative disorders associated with aberrant Flt-3 receptor kinase activity. The

association of both Syk kinase and Flt-3 kinase with the development of hematopoietic cells supports the use of such Syk/Flt-3 kinase inhibitory compounds for treatment of hematopoietic neoplasms.

[0028] In the descriptions of the methods herein, the terms used will have their ordinary and common meaning, unless specifically defined otherwise herein.

[0029] "Syk" or "Syk kinase" refers to the 72kDa non-receptor (cytoplasmic) spleen protein tyrosine kinase expressed in B-cells and other hematopoietic cells. Syk kinase is characterized by two consensus Src-homology 2 (SH2) domains in tandem that bind to phosphorylated immunoreceptor tyrosine-based activation motifs ("ITAMs"), a "linker" domain and a catalytic domain (for a review, see Sada et al., 2001, *J. Biochem. (Tokyo)* 130:177-186 and also Turner et al., 2000, *Immunology Today* 21:148-154 and Wong et al., 2004, *Expert Opin Investig Drugs* 13(7):743-62.). Syk kinase is also critical for tyrosine phosphorylation of multiple proteins which regulate important pathways leading from immunoreceptors, such as Ca^{2+} mobilization and mitogen-activated protein kinase (MAPK) cascades and degranulation. Syk kinase also plays a critical role in integrin signaling in neutrophils (see, *e.g.*, Mocsai et al. 2002, *Immunity* 16:547-558). Syk kinase includes kinases from any species of animal, including but not limited to, homo sapiens, simian, bovine, porcine, rodent, etc., recognized as belonging to the Syk family. Specifically included are isoforms, splice variants, allelic variants, mutants, both naturally occurring and man-made. The amino acid sequences of such Syk kinases are available from GENBANK. Specific examples of mRNAs encoding different isoforms of human Syk kinase are available at GENBANK accession no. gi|21361552|ref|NM__003177.2, gi|496899|emb|Z29630.1|HSSYKPTK[496899] and gi|15030258|gb|BC011399.1|BC011399[15030258].

[0030] "Flt-3" or "Flt-3 receptor tyrosine kinase" refers to a receptor tyrosine kinase that binds the flt-3 ligand (Flt-3 L or FL). Flt-3 is also known as Fms-like tyrosine kinase 3, FLK-2 (fetal liver kinase-2) and STK-1 (human stem cell kinase-11) (see, *e.g.*, Mathews et al., 1991, *Cell*. 65:1143-1152; Rosnet et al., 1991, *Oncogene*. 6:1641-1650). Flt-3 has sequence similarity to members of the class III receptor tyrosine kinase (RTKIII) family, a

subset of which includes, among others, FMS, platelet-derived growth factor receptor (PDGFR), and Kit (Rosnet et al., 1993, *Crit Rev Oncog.* 4:595-613. The Flt-3 polypeptide in mouse and human is a 1000- and 993-amino acid protein, respectively, and is known to be expressed in immature hematopoietic cells, placenta, gonads, and brain. As noted above, Flt-3 has a postulated role in the development of multipotent stem cells and B cells. Similar to other members of the class III receptor tyrosine kinases, Flt-3 is characterized by 5 immunoglobulin-like repeats in the extracellular domain, a transmembrane (TM) domain, a juxtamembrane (JM) domain, two intracellular tyrosine kinase (TK1 and TK2) domains separated by a kinase insert (KI) domain, and a C-terminus domain (Agnes et al., 1994, *Gene* 145: 283-288; 2004, Griffith et al., *Molecular Cell* 13:169-178). Flt-3 kinase includes kinases from any species of animal, including but not limited to, homo sapiens, simian, bovine, porcine, rodent, etc., recognized as belonging to the Flt-3 receptor family. Specifically included are isoforms, splice variants, allelic variants, mutants, both naturally occurring and man-made. The amino acid sequences of such Syk kinases are available from GENBANK. Specific examples of nucleic acid sequences and corresponding amino acid sequences of different Flt-3 kinases include, among others, human (Accession Nos. NM_004119.1), chimpanzee (Accession Nos. 452508; XM_509601.1; XP_509601.1), dog (NM_001020811.1; NP_001018647.1), and mouse (Accession Nos. 142551; NM_010229.11; NP_034359.11; AK0458654; AK1492924; AK1636404; BC1090034; BC1090044; L361634; M646894; X593984).

[0031] Some embodiments of Flt-3 variants are associated with certain hematopoietic cell proliferative disorders. “Flt-3 ITD” refers to a variant of Flt-3 having internal tandem duplications (ITDs) in the juxtamembrane (JM) domain (Nakao et al., 1996, *Leukemia* 10:1911-1918; Griffith et al., *supra*). The number of JM domains duplicated in Flt-3 ITD varies from subject to subject but are in-frame and generally results in protein with an aberrant (*i.e.*, increased) tyrosine kinase activity. Variants of the Flt-3 ITD type are often associated with acute myelogenous leukemia and myelodysplastic syndrome. Another type of variant is “Flt-3 activation loop mutations,” which refer to variants having an amino sequence change (as compared to wild-type) in the activation loop of the second tyrosine kinase (TK2) domain. Without being bound by theory, the activation loop appears to function in blocking access of adenosine triphosphate (ATP) and substrate to the kinase domain, and thereby exerts an inhibitory effect on the kinase. As a consequences, activation

loop mutations can give rise to Flt-3 kinase forms with constitutive activity. An exemplary activation loop variant is D835A, which is observed in acute myelogenous leukemia, myelodysplastic syndrome, and acute lymphoblastic leukemia (Yamamoto et al., 2001, *Blood* 97:2434-2439; Griffin, J.D., 2001, *Blood* 97:2193a). Other exemplary activation loop variations associated with cell proliferative disorders in humans included, among others, D835Y, D835A, D835E, D835H, D835N, D835V, D835del, and I836del

[0032] “Cell proliferative disorder” refers to a disorder characterized by abnormal proliferation of cells. A proliferative disorder does not imply any limitation with respect to the rate of cell growth, but merely indicates loss of normal controls that affect growth and cell division. Thus, in some embodiments, cells of a proliferative disorder can have the same cell division rates as normal cells but do not respond to signals that limit such growth. Within the ambit of “cell proliferative disorder” is neoplasm or tumor, which is an abnormal growth of tissue. Cancer refers to any of various malignant neoplasms characterized by the proliferation of cells that have the capability to invade surrounding tissue and/or metastasize to new colonization sites.

[0033] “Hematopoietic neoplasm” refers to a cell proliferative disorder arising from cells of the hematopoietic lineage. Generally, hematopoiesis is the physiological process whereby undifferentiated cells or stem cells develop into various cells found in the peripheral blood. In the initial phase of development, hematopoietic stem cells, typically found in the bone marrow, undergo a series of cell divisions to form multipotent progenitor cells that commit to two main developmental pathways: the lymphoid lineage and the myeloid lineage. The committed progenitor cells of the myeloid lineage differentiate into three major sub-branches comprised of the erythroid, megakaryocyte, and granulocyte/monocyte developmental pathways. An additional pathway leads to formation of dendritic cells, which are involved in antigen presentation. The erythroid lineage gives rise to red blood cells while the megakaryocytic lineage gives rise to blood platelets. Committed cells of the granulocyte/monocyte lineage split into granulocyte or monocyte developmental pathways, the former pathway leading to formation of neutrophils, eosinophils, and basophils and the latter pathway giving rise to blood monocytes and macrophages.

[0034] Committed progenitor cells of the lymphoid lineage develop into the B cell pathway, T cell pathway, or the non-T/B cell pathway. Similar to the myeloid lineage, an additional lymphoid pathway appears to give rise to dendritic cells involved in antigen presentation. The B cell progenitor cell develops into a precursor B cell (pre-B), which differentiates into B cells responsible for producing immunoglobulins. Progenitor cells of the T cell lineage differentiate into precursor T cells (pre-T) that, based on the influence of certain cytokines, develop into cytotoxic or helper/suppressor T cells involved in cell mediated immunity. Non-T/B cell pathway leads to generation of natural killer (NK) cells. Neoplasms of hematopoietic cells can involve cells of any phase of hematopoiesis, including hematopoietic stem cells, multipotent progenitor cells, oligopotent committed progenitor cells, precursor cells, and mature differentiated cells. The categories of hematopoietic neoplasms can generally follow the descriptions and diagnostic criteria employed by those of skill in the art (see, *e.g.*, International Classification of Disease and Related Health Problems (ICD 10), World Health Organization (2003)). Hematopoietic neoplasms can also be characterized based on the molecular features, such as cell surface markers and gene expression profiles, cell phenotype exhibited by the aberrant cells, and/or chromosomal aberrations (*e.g.*, deletions, translocations, insertions, etc.) characteristic of certain hematopoietic neoplasms, such as the Philadelphia chromosome found in chronic myelogenous leukemia. Other classifications include National Cancer Institute Working Formulation (Cancer, 1982, 49:2112-2135) and Revised European-American Lymphoma Classification (REAL).

[0035] “Lymphoid neoplasm” refers a proliferative disorder involving cells of the lymphoid lineage of hematopoiesis. Lymphoid neoplasms can arise from hematopoietic stem cells as well as lymphoid committed progenitor cells, precursor cells, and terminally differentiated cells. These neoplasms can be subdivided based on the phenotypic attributes of the aberrant cells or the differentiated state from which the abnormal cells arise. Subdivisions include, among others, B cell neoplasms, T cell neoplasms, NK cell neoplasms, and Hodgkin’s lymphoma.

[0036] “Myeloid neoplasm” refers to proliferative disorder of cells of the myeloid lineage of hematopoiesis. Neoplasms can arise from hematopoietic stem cells, myeloid committed progenitor cells, precursor cells, and terminally differentiated cells. Myeloid neoplasms can be subdivided based on the phenotypic attributes of the aberrant cells or the differentiated

state from which the abnormal cells arise. Subdivisions include, among others, myeloproliferative diseases, myelodysplastic/myeloproliferative diseases, myelodysplastic syndromes, acute myeloid leukemia, and acute biphenotypic leukemia.

[0037] “Virally mediated tumor” refers to a neoplasm or tumor associated with viral infection or an activity of a virally encoded product. The neoplasm can arise from presence of a latent virus integrated into the cellular genome or arise from activity of a virally associated gene product. Infection with the virus need not be tightly correlated in time with tumor formation in that incubation periods can extend from months to years before development of a tumor phenotype. Because the treatments herein are directed to use of Syk inhibitors, the applicable virally associated tumors are those in which viral modulation of Syk activity is correlated with aberrant cell proliferation. Any virus, including RNA and DNA viruses and viruses that reside episomally or integrate into the cellular genome, in which activation of Syk is a consequence of virus infection can be targeted using the methods herein.

[0038] “Tumor metastasis” refers to the capability of tumor cells to migrate from the original tumor site and colonize in other tissues. Tumors formed from cells that have spread are referred to as “secondary tumors” and contain cells that are similar to those in the original “primary” tumor. Metastatic tumors typically form by migration of tumor cells from the original tumor site through the blood and lymph system to other tissues.

[0039] “Syk mediated integrin signaling” refers to signal transduction of cell surface integrins that occur via interaction with Syk kinase. Integrins comprise an extended family of cell surface adhesion receptors that bind extracellular matrix and cell surface ligands. Structurally, integrins are heterodimeric proteins composed of an α and β chain, where each subunit has an extracellular domain, a single transmembrane domain, and a cytoplasmic domain. The α subunit generally composed of about seven tandem repeats, where a subset of the repeats contain putative metal binding sequences of the general structure DxDxDGxxD, where x is any amino acid. Two groups of integrins can be characterize by the α subunits: those that contain an “A” domain and those having a proteolytic cleavage site. The β subunit comprises a conserved region of about 200 amino acids in the extracellular domain, which is characterized by a region having structural similarity to the “A” domain of the α subunit and another region with epidermal growth factor (EGF) like repeats, similar to those found in

laminin (see, *e.g.*, Xiong et al., 2003, *Blood*, 102(4):1155-1159). Integrin activity may modulate intracellular Syk, or conversely, the integrin function can be modulated via the activity of Syk. It is generally understood that in some instances, integrins require activation within the cell to bind its cognate ligands (inside-out activation). Integrins that either modulate or is modulated by Syk include, among others, β 1-integrins (Lin et al., *J Biol. Chem.* 1995, 270(27):16189-97) such as $\alpha_2\beta_1$ (Keely et al., 1996, *J Biol Chem.* 271(43):26668-76), β 2 integrins, and β 3 integrins (Woodside et al., 2001, *Curr Biol.* 11(22):1799-804) such as $\alpha_{IIb}\beta_3$ (Clark et al., *J. Biol. Chem.* 1994, 269(46):28859-64). For instance, it is believed that Syk binds directly to the integrin β_3 cytoplasmic tail through the SH2 domains. However, unlike Syk binding to ITAMs, the interaction with β_3 integrin appears independent of the phosphotyrosine binding function of the tandem SH2 domains.

[0040] Generally, cell proliferative disorders treatable with the compounds disclosed herein relate to any disorder characterized by aberrant cell proliferation. These include various tumors and cancers, benign or malignant, metastatic or non-metastatic. Specific properties of cancers, such as tissue invasiveness or metastasis, can be targeted using the methods described herein. Cell proliferative disorders include a variety of cancers, including, among others, breast cancer, ovarian cancer, renal cancer, gastrointestinal cancer, kidney cancer, bladder cancer, pancreatic cancer, lung squamous carcinoma, and adenocarcinoma.

[0041] In some embodiments, the cell proliferative disorder treated is a hematopoietic neoplasm, which is aberrant growth of cells of the hematopoietic system. Hematopoietic malignancies can have its origins in pluripotent stem cells, multipotent progenitor cells, oligopotent committed progenitor cells, precursor cells, and terminally differentiated cells involved in hematopoiesis. Some hematological malignancies are believed to arise from hematopoietic stem cells, which have the ability for self renewal. For instance, cells capable of developing specific subtypes of acute myeloid leukemia (AML) upon transplantation display the cell surface markers of hematopoietic stem cells, implicating hematopoietic stem cells as the source of leukemic cells. Blast cells that do not have a cell marker characteristic of hematopoietic stem cells appear to be incapable of establishing tumors upon transplantation (Blair et al., 1997, *Blood* 89:3104-3112). The stem cell origin of certain hematological malignancies also finds support in the observation that specific chromosomal abnormalities associated with particular types of leukemia can be found in normal cells of

hematopoietic lineage as well as leukemic blast cells. For instance, the reciprocal translocation t(9q34;22q11) associated with approximately 95% of chronic myelogenous leukemia appears to be present in cells of the myeloid, erythroid, and lymphoid lineage, suggesting that the chromosomal aberration originates in hematopoietic stem cells. A subgroup of cells in certain types of CML displays the cell marker phenotype of hematopoietic stem cells.

[0042] Although hematopoietic neoplasms often originate from stem cells, committed progenitor cells or more terminally differentiated cells of a developmental lineage can also be the source of some leukemias. For example, forced expression of the fusion protein Bcr/Abl (associated with chronic myelogenous leukemia) in common myeloid progenitor or granulocyte/macrophage progenitor cells produces a leukemic-like condition. Moreover, some chromosomal aberrations associated with subtypes of leukemia are not found in the cell population with a marker phenotype of hematopoietic stem cells, but are found in a cell population displaying markers of a more differentiated state of the hematopoietic pathway (Turhan et al., 1995, *Blood* 85:2154-2161). Thus, while committed progenitor cells and other differentiated cells may have only a limited potential for cell division, leukemic cells may have acquired the ability to grow unregulated, in some instances mimicking the self-renewal characteristics of hematopoietic stem cells (Passegue et al., *Proc. Natl. Acad. Sci. USA*, 2003, 100:11842-9).

[0043] In some embodiments, the hematopoietic neoplasm treated is a lymphoid neoplasm, where the abnormal cells are derived from and/or display the characteristic phenotype of cells of the lymphoid lineage. Lymphoid neoplasms can be subdivided into B-cell neoplasms, T and NK -cell neoplasms, and Hodgkin's lymphoma. B-cell neoplasms can be further subdivided into precursor B-cell neoplasm and mature/peripheral B-cell neoplasm. Exemplary B-cell neoplasms are precursor B-lymphoblastic leukemia/lymphoma (precursor B-cell acute lymphoblastic leukemia) while exemplary mature/peripheral B-cell neoplasms are B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma, B-cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, splenic marginal zone B-cell lymphoma, hairy cell leukemia, plasma cell myeloma/plasmacytoma, extranodal marginal zone B-cell lymphoma of MALT type, nodal marginal zone B-cell lymphoma, follicular lymphoma, mantle-cell lymphoma, diffuse large B-cell lymphoma, mediastinal large B-cell

lymphoma, primary effusion lymphoma, and Burkitt's lymphoma/Burkitt cell leukemia. T-cell and Nk-cell neoplasms are further subdivided into precursor T-cell neoplasm and mature (peripheral) T-cell neoplasms. Exemplary precursor T-cell neoplasm is precursor T-lymphoblastic lymphoma/leukemia (precursor T-cell acute lymphoblastic leukemia) while exemplary mature (peripheral) T-cell neoplasms are T-cell prolymphocytic leukemia T-cell granular lymphocytic leukemia, aggressive NK-cell leukemia, adult T-cell lymphoma/leukemia (HTLV-1), extranodal NK/T-cell lymphoma, nasal type, enteropathy-type T-cell lymphoma, hepatosplenic gamma-delta T-cell lymphoma, subcutaneous panniculitis-like T-cell lymphoma, Mycosis fungoides/Sezary syndrome, Anaplastic large-cell lymphoma, T/null cell, primary cutaneous type, Peripheral T-cell lymphoma, not otherwise characterized, Angioimmunoblastic T-cell lymphoma, Anaplastic large-cell lymphoma, T/null cell, primary systemic type. The third member of lymphoid neoplasms is Hodgkin's lymphoma, also referred to as Hodgkin's disease. Exemplary diagnosis of this class that can be treated with the compounds include, among others, nodular lymphocyte-predominant Hodgkin's lymphoma, and various classical forms of Hodgkin's disease, exemplary members of which are Nodular sclerosis Hodgkin's lymphoma (grades 1 and 2), Lymphocyte-rich classical Hodgkin's lymphoma, Mixed cellularity Hodgkin's lymphoma, and Lymphocyte depletion Hodgkin's lymphoma. In various embodiments, any of the lymphoid neoplasms that are associated with aberrant Syk activity can be treated with the Syk inhibitory compounds.

[0044] In some embodiments, the hematopoietic neoplasm treated is a myeloid neoplasm. This group comprises a large class of cell proliferative disorders involving or displaying the characteristic phenotype of the cells of the myeloid lineage. Myeloid neoplasms can be subdivided into myeloproliferative diseases, myelodysplastic/myeloproliferative diseases, myelodysplastic syndromes, and acute myeloid leukemias. Exemplary myeloproliferative diseases are chronic myelogenous leukemia (*e.g.*, Philadelphia chromosome positive (t(9;22)(qq34;q11)), chronic neutrophilic leukemia, chronic eosinophilic leukemia/hypereosinophilic syndrome, chronic idiopathic myelofibrosis, polycythemia vera, and essential thrombocythemia. Exemplary myelodysplastic/myeloproliferative diseases are chronic myelomonocytic leukemia, atypical chronic myelogenous leukemia, and juvenile myelomonocytic leukemia. Exemplary myelodysplastic syndromes are refractory anemia, with ringed sideroblasts and without ringed sideroblasts, refractory cytopenia

(myelodysplastic syndrome) with multilineage dysplasia, refractory anemia (myelodysplastic syndrome) with excess blasts, 5q- syndrome, and myelodysplastic syndrome with t(9;12)(q22;p12) (TEL-Syk fusion; see, *e.g.*, Kuno et al., 2001, *Blood* 97:1050). In various embodiments, any of the myeloid neoplasms that are associated with aberrant Syk activity can be treated with the Syk inhibitory compounds.

[0045] In some embodiments, the Syk inhibitory compounds can be used to treat Acute myeloid leukemias (AML), which represent a large class of myeloid neoplasms having its own subdivision of disorders. These subdivisions include, among others, AMLs with recurrent cytogenetic translocations, AML with multilineage dysplasia, and other AML not otherwise categorized. Exemplary AMLs with recurrent cytogenetic translocations include, among others, AML with t(8;21)(q22;q22), AML1(CBF- α)/ETO, Acute promyelocytic leukemia (AML with t(15;17)(q22;q11-12) and variants, PML/RAR- α), AML with abnormal bone marrow eosinophils (inv(16)(p13q22) or t(16;16)(p13;q11), CBF β /MYH11X), and AML with 11q23 (MLL) abnormalities. Exemplary AML with multilineage dysplasia are those that are associated with or without prior myelodysplastic syndrome. Other acute myeloid leukemias not classified within any definable group include, AML minimally differentiated, AML without maturation, AML with maturation, Acute myelomonocytic leukemia, Acute monocytic leukemia, Acute erythroid leukemia, Acute megakaryocytic leukemia, Acute basophilic leukemia, and Acute panmyelosis with myelofibrosis.

[0046] In other aspects, cell proliferative disorders that can be targeted with the Syk kinase inhibitors comprise virally mediated tumors. These can arise from infection of cells by an oncogenic virus that has the capability of transforming a normal cell into a tumor cell. Because rates of viral infection far exceed the number of actual incidence of cell transformation, viral mediated transformation generally act together with other cellular factors to generate a transformed tumor cell. Thus, a virally mediated tumor does not require the virus to be the sole causative agent of the cell proliferative disorder, but rather that the viral infection or persistent presence of virus is associated with the generation of the tumor. Generally, tumors where the causative agent is a virus typically has continual expression of a limited number of viral genes and that viral these oncogenes, expressed as part of the viral infection or through persistence of the virus, disrupts the normal cellular gene expression and

signal transduction pathways. Without being bound by theory, viral oncogenes involved in cell transformation appear to disrupt four main cellular processes: cell surface receptors that interact with growth factors and extracellular matrix, transmembrane signaling networks, cytosolic elements such as soluble proteins and second messengers, and nuclear proteins including DNA binding proteins and factors which function directly and indirectly in gene regulation and replication. Because certain virally encoded proteins target specific cellular components as part of the transformation process, application of Syk inhibitors can be appropriate where the viral component targets the activity of Syk kinase.

[0047] In some embodiments, the virally mediated tumor treatable with the compounds disclosed herein is associated with any virus that encodes an immunoreceptor tyrosine-based activation motif (ITAM) capable of modulating Syk activity. This motif, as noted above, refers to a conserved amino acid sequence motif that functions by interacting with and activating nonreceptor tyrosine kinases. ITAM motifs are found in, among others, the β and γ chains of Fc ϵ RI, the ϵ subunit of the T cell receptor, and immunoglobulin β (Ig β) and Ig α of the B cell receptor. The canonical sequence motif is typically Yxx(L/I)_{x6-8}Yxx(L/I), where x represents any amino acid. Generally, the tyrosine residues in the motif are involved in ITAM signaling and are substrates for phosphorylation by Src family of kinases. The phosphorylated form of ITAMs function as interaction sites for SH2 (src homology domain) containing signaling proteins, such as Syk/ZAP-70 kinases. In addition to its presence in a variety of cellular cell surface molecules, the ITAM sequences have been identified in virally encoded proteins. In view of the descriptions herein indicating function of Syk kinase as an oncogene, tumors associated with viruses carrying genes encoding proteins with ITAM sequences can be treated with Syk inhibitor compounds.

[0048] Accordingly, in some embodiments, the virally mediated tumor treatable with the inhibitor compounds is associated with Kaposi's sarcoma (KS) associated herpes virus, a lymphotropic virus implicated in Kaposi's sarcoma, a rare malignancy found at higher incidence among HIV infected population. The KS associated herpes virus encodes a transmembrane protein termed KI having an immunoreceptor tyrosine-based activation motif (ITAM)-like sequence. The KI gene product is thought to act in a constitutive manner through its cysteine-rich ectodomain to activate Syk and its related kinase Zap-70 (Lagunoff, M. et al., 1999, *Proc. Natl. Acad. Sci. USA* 96(10):5704-5709). In further support of the

methods herein, transgenic mice bearing the KI gene appears to increase the incidence of certain sarcomas and lymphomas in an infected animal, indicating a role for KI activity in oncogenesis (Prakash et al., 2002, *J. Natl. Cancer Inst.* 94:926-35).

[0049] In some embodiments, the virally mediated tumor is associated with Epstein Barr Virus (EBV). Epstein Barr Virus is a member of the Herpesviridae family that, following primary infection, replicates in the epithelial cells of the oropharynx and infect recirculating B lymphocytes. Infection can lead to acute infectious mononucleosis, also known as glandular fever. Infectious mononucleosis is a benign lymphoproliferative disease characterized by transient immunosuppression and an expansion of atypical lymphocytes, the majority of which are CD8⁺ T cells. In these T cells, EBV establishes a latent but persistent infection during which a select set of viral genes are expressed. The entire genome can persist in the proliferating lymphocytes as episomal DNA. EBV infection is associated with Burkitt's lymphoma, Hodgkin's lymphoma, and adult T cell leukemia.

[0050] The LMP2A protein encoded by the EBV genome is a transmembrane protein thought to play a role in maintaining the latency of the EBV virus following infection. It consists of an extended amino terminal tail, 12 membrane spanning domains, and a cytoplasmic domain. The amino terminal region contains the ITAM motif, which allows interaction of LMP2A with Syk kinase (Fruehling et al., 1997, *Virology*, 235:241-251). LMP2A appears to regulate Syk kinase in lymphoid cells to promote B-cell survival and maintain latency. Because Syk plays a critical role in the signal transduction pathways that regulate other signaling pathways, such as PI-3K, BLNK, and phospholipase γ 2 and is involved in enhancing lymphoid cell survival, improper Syk activation through LMP2A protein or other virally mediated effectors may play a role in inducing aberrant lymphoproliferation (Caldwell et al., 2000, *J Virol* 74(19):9115; Caldwell et al., 1998, *Immunity* 9:405)). Thus, inhibition of Syk activity can provide a therapeutic benefit for cell proliferative disorders associated with EBV viral infection.

[0051] In some embodiments, the virally mediated tumor to be treated with the Syk inhibitor is associated with Human T-cell Lymphotropic Virus (HTLV-1 virus), a retrovirus in the same class of virus as the AIDS virus, HIV-1. The virus is tropic for CD4⁺ T-cells although CD8⁺ T-cells can also serve as a viral reservoir. HTLV-1 infection is associated with, among

others, adult T-cell Leukemia/lymphoma (ATLL) and a number of other lymphocyte disorders. During HTLV-1 infection, Syk is expressed in infected cells while expression of the Syk related kinase, ZAP-70, is absent (Weil et al., 1999, *J. Virol.* 73(5):3709-17). Dysregulation of a number of kinases, including Syk, is implicated in HTLV-1 mediated induction of adult T-cell leukemia.

[0052] In some embodiments, the virally mediated tumor is associated with mammary tumor virus (MTV). ITAM sequences are found within the Env gene of murine mammary tumor virus (MMTV), a B type retrovirus identified as an etiological agent for breast cancer in mice. Mouse mammary epithelial cells transfected with MMTV Env gene display characteristics of a transformed phenotype, such as colony formation in soft agar and invasiveness in basement membrane preparations (Katz et al., 2005, *J Exp Med.* 201(3):431-9). Murine mammary tumor virus-like sequences are also present in human cancers, such as breast cancer and T cell lymphomas (Wang et al., 2000, *Clinical Cancer Res.* 6:1273-1278), and correlated with tumorigenesis as these sequences are not observed in the majority of normal breast tissue. Thus, tumors associated with MTV can be treated with the Syk kinase inhibitors.

[0053] It is to be understood that use of Syk inhibitor compounds for treating virally mediated tumors is not limited to tumors associated with the viruses specified above. As noted, any tumors associated with an oncogenic virus in which Syk is activated as part of its oncogenic mechanism, whether or not it involves ITAM sequences, can be targeted using the Syk inhibitor compounds.

[0054] In some embodiments, the cell proliferative disorder that can be targeted with the inhibitors comprise cell proliferative disorders associated with aberrant Flt-3 kinase activity. The term “aberrant Flt-3 kinase activity” refers to activity that is abnormal from what the normal level of Flt-3 kinase activity would be for a wild type Flt-3 kinase or in a normal Flt-3 expressing cell, tissue, organ, or organism. Aberrant Flt-3 kinase activity can arise from mislocalization of the protein (spatial expression), increases or decrease in activity of the enzyme (directly or indirectly), or changes in temporal expression (*i.e.*, developmental expression).

[0055] In some embodiments, the aberrant kinase activity is associated with variants of Flt-3. In some embodiments, these variants are characterized by increased Flt-3 receptor kinase activity. As used herein, “increased kinase activity” refers to kinase activity that is higher than observed for a wild type Flt-3 kinase or in a normal Flt-3 expressing cell, tissue, organ, or organism. Exemplary increased kinase activity are found in cells with certain Flt-3 variants, such as for example, Flt-3 ITD and Flt-3 activation loop mutations.

[0056] Because aberrant Flt-3 kinase activity is observed in many different types of hematological neoplasms, in some embodiments, the Syk/Flt-3 inhibitory compounds can be used to treat hematopoietic neoplasms characterized by the presence of an aberrant Flt-3 kinase. Thus, in some embodiments, the hematological neoplasms treatable with the Syk/Flt-3 inhibitory compound can include, among others, acute myelogenous leukemia, B-precursor cell acute lymphoblastic leukemia, T-cell acute lymphoblastic leukemia, and chronic myelogenous leukemia (CML) in which the presence of an aberrant Flt-3 has been diagnosed or is suspected in the subject. However, it is to be understood that the skilled artisan can apply the treatments without such information and that the inhibitory compounds can be given prophylactically to increase the probability of survival. Moreover, the skilled artisan can apply the Syk/Flt-3 inhibitory compounds herein to other cell proliferative disorders in which an aberrant Flt-3 kinase activity has been detected or is suspected.

[0057] In other aspects, the present disclosure is directed to the treatment of tumor metastasis by use of the Syk kinase inhibitors. Metastasis is a characteristic of malignant tumor cells whereby tumor cells detach from its site of origin and then spread to colonize at other sites. These secondary tumors can form in tissues unrelated to the cells from which the tumor cells originate. It is the formation of these secondary tumors by metastasis that appears to be the primary cause of mortality from malignant forms of cancer. Metastasis begins when malignant cells break off from the primary tumor and enter the blood or lymphatic system, and then migrate to other colonization sites. Generally, normal cells do not detach and invade other tissues because of various signals that inhibit dissimilar cells from adhering to each other, as well as signals between cells that inhibit cell growth. Cell transformation, however, alters these normal regulatory programs such that tumor cells interact with local tissue cells to modify the local extracellular matrix, stimulate migration, and promote proliferation and survival. Alterations of cell adhesion molecule (CAMs), such as those

members of the immunoglobulin and calcium-dependent cadherin families and integrins, appear to play critical role in invasion and metastasis. For instance, alteration of N-CAM from a highly adhesive isoform to a poorly adhesive form, which along with its down regulation, may lead to invasive pancreatic cancer.

[0058] Another class of adhesion proteins implicated in tissue invasiveness and metastasis are integrins. As noted above, integrins form a diverse class of cell surface molecules. Permutations in the spectrum of the more than 22 integrin subtypes are able to create a flexible system for changing interaction of cells in response to different cell signaling states or changing external environments. Integrins interact dynamically with extracellular ligands depending on activation state of the integrin, which is controlled by intracellular signals (*i.e.*, inside out signaling) that modifies integrin affinity and avidity. Conversely, interaction of integrin with extracellular ligands can trigger signal transduction cascades that affect cell adhesive properties and cellular responses to changing extracellular environments (*i.e.*, outside-in signaling). Changes in expression of integrin subunits induce or inhibit invasive and metastatic growth, implicating these proteins as critical determinants of these processes (Guo et al., 2004, *Nat. Rev. Mol. Cell Biol.* 5(10):816-26; Jin et al., 2004, *Br. J. Cancer*, 90:561-565). For instance, integrin $\alpha_v\beta_3$ is required for angiogenesis, and its upregulation is correlated with tumor invasiveness and metastatic potential (Liapis et al., 1996, *Diag. Mol. Pathol.* 23:127-135). Cultured metastatic breast cancer cells show constitutive expression of $\alpha_v\beta_3$ (Pecher et al., 2002, *FASEB J.* 16:1266-1268) while inhibiting α_v subunit containing integrin activity, such as by use of RGD peptidomimetic agents that compete for binding of the integrin with its natural substrate, can reduce the metastatic potential of tumors without affecting cell proliferation properties (Kristen et al., 2004, *Clin. Exp. Metastasis* 21(2):129-38; Harms et al., 2004, *Clin. Exp. Metastasis* 21(2):119-28). Similarly, overexpression of β_1 integrins can disrupt adherens junctions that function to keep cells attached to the basement membrane.

[0059] Syk kinase activity is associated with various integrins expressed on cells of the hematopoietic lineage, but also in non-hematopoietic cells. Syk kinase is implicated in β_1 integrin signaling of lung epithelial cells (Ulanova et al., 2004, *Am. J. Physiol. Lung Cell Mol. Physiol.* 288:L497-L507) and monocytes (Lin et al., 1995, *J. Biol. Chem.* 270(27):16189-97), β_2 integrin signaling in granulocytes/neutrophils (Miura et al., 2000,

Blood 96(5):1733-9; Kusumoto et al., 2001, *Microbiol. Immunol.* 45(3):241-8), and $\beta 3$ integrin signaling in platelet activation and cell adhesion (Gao et al., 1997, *EMBO J.* 16(21):6414-25). Given the connection provided herein between Syk kinase activity and tumorigenesis, the use of Syk kinase inhibitors in attenuating the invasiveness and metastatic properties of tumors is indicated through the link between Syk kinase activity and certain integrins (Mocsai et al., 2002, *Immunity* 16(4):547-58). Thus, in some embodiments, inhibitors of Syk kinase can be used to modulate metastatic properties of tumors mediated via integrin activity. In some embodiments, the Syk kinase inhibitors can be used to attenuate tumor cell tissue invasiveness and metastatic potential affected by $\beta 1$ integrins (Lin et al., 1995, *J. Biol. Chem.* 270:16189-16197; Kusumoto et al., *Microbiol Immunol.*, 2001, 45(3):241-8; Ortiz-Stern et al., 2005, *J Leukoc Biol.* (Epub)). An exemplary integrin of this type is integrin $\alpha_2\beta_1$.

[0060] In some embodiments, the Syk kinase inhibitors can be used to attenuate tumor cell tissue invasiveness and metastatic potential affected by the activity of $\beta 2$ integrins (CD18) (Willeke et al., 2003, *J Leukoc. Biol.* 74(2):260-9). These include, among others, CD11a/CD18, CD11b/CD18, CD11c/CD18, and CD11d/CD18. In further embodiments, the Syk kinase inhibitors can be used to attenuate tumor cell tissue invasiveness and metastatic potential affected by the activity of $\beta 3$ integrins. Exemplary integrins of this type are $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$.

[0061] Various tumor types capable of metastasis can be treated with the Syk inhibitor compounds. Such tumors include, by way of example and not limitation, breast cancer, ovarian cancer, renal cancer, gastrointestinal cancer, kidney cancer, bladder cancer, pancreatic cancer, lung squamous carcinoma, and adenocarcinoma (see, e.g., Felding-Habermann et al., 2001, *Proc Natl Acad Sci USA* 98(4):1853-8). Therapeutic treatment to attenuate the metastasis of established tumors can follow a diagnosis of metastasis. If no diagnosis of metastasis has been made, the inhibitor compound can be administered prophylactically to reduce the probability of metastasis.

[0062] It is to be understood that the Syk inhibitor compounds can be used independently of any other treatment, or used in combination with other cancer treatment regimens, including surgery, radiology, or other chemotherapies. Accordingly, in some embodiments, the Syk

kinase inhibitors can be used in combination with other chemotherapeutic agents. Combination treatments with Syk inhibitors can target different cellular components by appropriate choice of the second chemotherapeutic agent. For instance, Syk inhibitors can be used in some embodiments to limit the metastatic potential of tumor cells while another chemotherapeutic agent can be used to eliminate or kill aberrant cells.

[0063] Various chemotherapeutic agents can be used in combination with Syk kinase inhibitors to treat cell proliferative disorders. These chemotherapeutic agents can be general cytotoxic agents or target a specific cellular molecule. Various classes of cancer chemotherapeutic agents include, among others, antimetabolites, agents that react with DNA (*e.g.*, alkylating agents, coordination compounds, etc.), inhibitors of transcription enzymes, topoisomerase inhibitors, DNA minor-groove binding compounds, antimitotic agents (*e.g.*, vinca alkyloids), antitumor antibiotics, hormones, and enzymes. Exemplary alkylating agents include, by way of example and not limitation, mechlorothamine, cyclophosphamide, ifosfamide, melphalan, chlorambucil, ethyleneimines, methylmelamines, alkyl sulfonates (*e.g.*, busulfan), and carmustine. Exemplary antimetabolites include, by way of example and not limitation, folic acid analog methotrexate; pyrimidine analogs fluorouracil, cytosine arabinoside; and purine analogs mecaptopurine, thioguanine, and azathioprine. Exemplary vinca alkyloids include, by way of example and not limitation, vinblastine, vincristine, paclitaxel, and colchicine. Exemplary antitumor antibiotics include, by way of example and not limitation, actinomycin D, daunorubicin, and bleomycin. An exemplary enzyme effective as anti-neoplastic agent is L-asparaginase. Exemplary coordination compounds include, by way of example and not limitation, cisplatin and carboplatin. Exemplary hormones and hormone related compounds include, by way of example and not limitation, adrenocorticosteroids prednisone, and dexamethasone; aromatase inhibitors amino glutethimide, formestane, and anastrozole; progestin compounds hydroxyprogesteron caproate, medroxyprogesterone; and anti-estrogen compound tamoxifen. Exemplary topoisomerase inhibitors include, by way of example and not limitation, amsacrine (m-AMSA); mitoxantrone, topotecan, irinotecan, and camptothecin.

[0064] These and other useful anti-cancer compounds are described in *Merck Index*, 13th Ed. (O'Neil, M.J. et al., ed) Merck Publishing Group (2001) and *Goodman and Gilman's The*

Pharmacological Basis of Therapeutics, 10th Edition, Hardman, J.G. and Limbird, L.E. eds., pg. 1381-1287, McGraw Hill, (1996).

[0065] Other anti-proliferative compounds useful in combination with the Syk inhibitor compounds include, by way of example and not limitation, antibodies directed against growth factor receptors (*e.g.*, anti-Her2); cytokines such as interferon- α and interferon- γ , interleukin-2, and GM-CSF; and antibodies for cell surface markers (*e.g.*, anti-CTLA-4. anti-CD20 (rituximab); anti-CD33). When antibodies against cell surface markers are used, a chemotherapeutic agent can be conjugated to it for specific targeting to the tumor cell. Suitable conjugates include radioactive compounds (*e.g.*, radioactive metal bound to an antibody conjugated chelator), cytotoxic compounds, and drug activating enzymes (*e.g.*, allinase, peptidases, esterases, catalytic antibodies, etc.) (see, *e.g.*, Arditti et al., 2005, *Mol. Cancer Therap.* 4(2):325-331; U.S. Patent No. 6,258,360).

[0066] In some embodiments, the Syk inhibitors can be used with a second kinase inhibitor that targets an oncogenic kinase different from Syk or Syk and Flt-3. Given that Syk inhibitors are disclosed herein for the treatment of hematopoietic neoplasms, other compatible kinase inhibitors used for treating hematopoietic neoplasms can also be used. In some embodiments, the second kinase inhibitor is an inhibitor of Abl kinase. Chronic myelogenous leukemia is a myeloid neoplasm characterized by malignant proliferation of leukemic stem cells in the bone marrow. The majority of chronic myelogenous leukemia are associated with a cytogenetic abnormality defined by a reciprocal translocation t(9;22)(q34;q11). This chromosomal aberration results in generation of a BCR/ABL fusion protein with activated kinase activity. Inhibitors of the fusion protein kinase activity can be effective in treating chronic myelogenous leukemia although resistant forms can develop upon continued treatment. Use of Syk kinase inhibitor in combination of Abl kinase inhibitors can lessen the chances of resistant cells by targeting a different cellular process than targeted by the second kinase inhibitor. An exemplary Abl kinase inhibitor is 2-phenylaminopyrimidine, also known as imatinib mesylate and Gleevec®. Thus, in some embodiments, the Syk kinase inhibitors can be used in combination with Abl kinase inhibitor 2-phenylaminopyrimidine and its derivatives. In other embodiments, the second kinase inhibitor can be pyridol[2-3-d]pyrimidine and its derivatives, which was originally identified as inhibitors of Src kinase. In still other embodiments, the second kinase inhibitor can be

tyrphostins and its derivatives (*e.g.*, adaphostin), which can affect the association of the kinase with its substrates. Other kinase inhibitor compounds will be apparent to the skilled artisan.

[0067] As further described herein, the administration of other chemotherapeutic agents can be done in the form of a composition, or administered adjunctively in combination with the Syk inhibitor. When provided adjunctively, the chemotherapeutic agents can be administered simultaneously with or sequentially with administration of the Syk inhibitor.

6.2 Syk Kinase and Syk/Flt-3 Kinase Inhibitors

[0068] In reference to various inhibitors, the terms used to describe the compounds will have their ordinary and common meaning as used by those in the art unless a different definition is provided herein or is provided in the references describing the specific inhibitor compounds.

[0069] "Alkyl" by itself or as part of another substituent refers to a saturated or unsaturated, branched, straight-chain or cyclic monovalent hydrocarbon group having the stated number of carbon atoms (*i.e.*, C₁-C₆ means from one to six carbon atoms) derived by the removal of one hydrogen atom from a single carbon atom of a parent alkane, alkene or alkyne. Typical alkyl groups include, but are not limited to, methyl; ethyls such as ethanyl, ethenyl, ethynyl; propyls such as propan-1-yl, propan-2-yl, cyclopropan-1-yl, prop-1-en-1-yl, prop-1-en-2-yl, prop-2-en-1-yl (allyl), cycloprop-1-en-1-yl; cycloprop-2-en-1-yl, prop-1-yn-1-yl, prop-2-yn-1-yl, etc.; butyls such as butan-1-yl, butan-2-yl, 2-methyl-propan-1-yl, 2-methyl-propan-2-yl, cyclobutan-1-yl, but-1-en-1-yl, but-1-en-2-yl, 2-methyl-prop-1-en-1-yl, but-2-en-1-yl, but-2-en-2-yl, buta-1,3-dien-1-yl, buta-1,3-dien-2-yl, cyclobut-1-en-1-yl, cyclobut-1-en-3-yl, cyclobuta-1,3-dien-1-yl, but-1-yn-1-yl, but-1-yn-3-yl, but-3-yn-1-yl, etc.; and the like. The term "alkyl" is specifically intended to include groups having any degree or level of saturation, *i.e.*, groups having exclusively single carbon-carbon bonds, groups having one or more double carbon-carbon bonds, groups having one or more triple carbon-carbon bonds and groups having mixtures of single, double and triple carbon-carbon bonds. Where a specific level of saturation is intended, the expressions "alkanyl," "alkenyl," and "alkynyl" are used. The expression "lower alkyl" refers to alkyl groups composed of from 1 to 6 carbon atoms.

[0070] "Alkanyl" by itself or as part of another substituent refers to a saturated branched, straight-chain or cyclic alkyl group. Typical alkanyl groups include, but are not limited to, methanyl; ethanyl; propanyls such as propan-1-yl, propan-2-yl (isopropyl), cyclopropan-1-yl, etc.; butanyls such as butan-1-yl, butan-2-yl (sec-butyl), 2-methyl-propan-1-yl (isobutyl), 2-methyl-propan-2-yl (t-butyl), cyclobutan-1-yl, etc..

[0071] "Alkenyl" by itself or as part of another substituent refers to an unsaturated branched, straight-chain or cyclic alkyl group having at least one carbon-carbon double bond derived by the removal of one hydrogen atom from a single carbon atom of a parent alkene. The group can be in either the cis or trans conformation about the double bond(s). Typical alkenyl groups include, but are not limited to, ethenyl; propenyls such as prop-1-en-1-yl, prop-1-en-2-yl, prop-2-en-1-yl (allyl), prop-2-en-2-yl, cycloprop-1-en-1-yl; cycloprop-2-en-1-yl; butenyls such as but-1-en-1-yl, but-1-en-2-yl, 2-methyl-prop-1-en-1-yl, but-2-en-1-yl, but-2-en-1-yl, but-2-en-2-yl, buta-1,3-dien-1-yl, buta-1,3-dien-2-yl, cyclobut-1-en-1-yl, cyclobut-1-en-3-yl, cyclobuta-1,3-dien-1-yl, etc.

[0072] "Alkynyl" by itself or as part of another substituent refers to an unsaturated branched, straight-chain or cyclic alkyl group having at least one carbon-carbon triple bond derived by the removal of one hydrogen atom from a single carbon atom of a parent alkyne. Typical alkynyl groups include, but are not limited to, ethynyl; propynyls such as prop-1-yn-1-yl, prop-2-yn-1-yl, etc.; butynyls such as but-1-yn-1-yl, but-1-yn-3-yl, but-3-yn-1-yl, etc..

[0073] "Parent Aromatic Ring System" refers to an unsaturated cyclic or polycyclic ring system having a conjugated π electron system. Specifically included within the definition of "parent aromatic ring system" are fused ring systems in which one or more of the rings are aromatic and one or more of the rings are saturated or unsaturated, such as, for example, fluorene, indane, indene, phenalene, etc. Typical parent aromatic ring systems include, but are not limited to, aceanthrylene, acenaphthylene, acephenanthrylene, anthracene, azulene, benzene, chrysene, coronene, fluoranthene, fluorene, hexacene, hexaphene, hexalene, as-indacene, s-indacene, indane, indene, naphthalene, octacene, octaphene, octalene, ovalene, penta-2,4-diene, pentacene, pentalene, pentaphene, perylene, phenalene, phenanthrene, picene, pleiadene, pyrene, pyranthrene, rubicene, triphenylene, trinaphthalene, and the like.

[0074] "Aryl" by itself or as part of another substituent refers to a monovalent aromatic hydrocarbon group having the stated number of carbon ring atoms (i.e., C₅-C₁₄ means from 5 to 14 carbon ring atoms) derived by the removal of one hydrogen atom from a single carbon atom of a parent aromatic ring system. Typical aryl groups include, but are not limited to, groups derived from aceanthrylene, acenaphthylene, acephenanthrylene, anthracene, azulene, benzene, chrysene, coronene, fluoranthene, fluorene, hexacene, hexaphene, hexalene, as-indacene, s-indacene, indane, indene, naphthalene, octacene, octaphene, octalene, ovalene, penta-2,4-diene, pentacene, pentalene, pentaphene, perylene, phenalene, phenanthrene, picene, pleiadene, pyrene, pyranthrene, rubicene, triphenylene, trinaphthalene, and the like. In preferred embodiments, the aryl group is (C₅-C₁₄) aryl, with (C₅-C₁₀) being even more preferred. Particularly preferred aryls are cyclopentadienyl, phenyl and naphthyl.

[0075] "Arylalkyl" by itself or as part of another substituent refers to an acyclic alkyl group in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp₃ carbon atom, is replaced with an aryl group. Typical arylalkyl groups include, but are not limited to, benzyl, 2-phenylethan-1-yl, 2-phenylethen-1-yl, naphthylmethyl, 2-naphthylethan-1-yl, 2-naphthylethen-1-yl, naphthobenzyl, 2-naphthophenylethan-1-yl and the like. Where specific alkyl moieties are intended, the nomenclature arylalkanyl, arylalkenyl and/or arylalkynyl is used. In preferred embodiments, the arylalkyl group is (C₆-C₁₆) arylalkyl, *e.g.*, the alkanyl, alkenyl or alkynyl moiety of the arylalkyl group is (C₁-C₆) and the aryl moiety is (C₅-C₁₀). In particularly preferred embodiments the arylalkyl group is (C₆-C₁₃), *e.g.*, the alkanyl, alkenyl or alkynyl moiety of the arylalkyl group is (C₁-C₃) and the aryl moiety is (C₅-C₁₀).

[0076] "Parent Heteroaromatic Ring System" refers to a parent aromatic ring system in which one or more carbon atoms are each independently replaced with the same or different heteroatoms or heteroatomic groups. Typical heteroatoms or heteroatomic groups to replace the carbon atoms include, but are not limited to, N, NH, P, O, S, Si, etc. Specifically included within the definition of "parent heteroaromatic ring systems" are fused ring systems in which one or more of the rings are aromatic and one or more of the rings are saturated or unsaturated, such as, for example, arindole, benzodioxan, benzofuran, chromane, chromene, indole, indoline, xanthene, etc. Also included in the definition of "parent heteroaromatic ring system" are those recognized rings that include substituents, such as benzopyrone. Typical

parent heteroaromatic ring systems include, but are not limited to, arsindole, benzodioxan, benzofuiran, benzopyrone, carbazole, β -carboline, chromane, chromene, cinnoline, furan, imidazole, indazole, indole, indoline, indolizine, isobenzofuran, isochromene, isoindole, isoindoline, isoquinoline, isothiazole, isoxazole, naphthyridine, oxadiazole, oxazole, perimidine, phenanthridine, phenanthroline, phenazine, phthalazine, pteridine, purine, pyran, pyrazine, pyrazole, pyridazine, pyridine, pyrimidine, pyrrole, pyrrolizine, quinazoline, quinoline, quinolizine, quinoxaline, tetrazole, thiadiazole, thiazole, thiophene, triazole, xanthene, and the like.

[0077] "Heteroaryl" by itself or as part of another substituent refers to a monovalent heteroaromatic group having the stated number of ring atoms (*i.e.*, "5-14 membered" means from 5 to 14 ring atoms) derived by the removal of one hydrogen atom from a single atom of a parent heteroaromatic ring system. Typical heteroaryl groups include, but are not limited to, groups derived from acridine, arsindole, carbazole, .beta.-carboline, chromane, chromene, cinnoline, furan, imidazole, indazole, indole, indoline, indolizine, isobenzofuran, isochromene, isoindole, isoindoline, isoquinoline, isothiazole, isoxazole, naphthyridine, oxadiazole, oxazole, perimidine, phenanthridine, phenanthroline, phenazine, phthalazine, pteridine, purine, pyran, pyrazine, pyrazole, pyridazine, pyridine, pyrimidine, pyrrole, pyrrolizine, quinazoline, quinoline, quinolizine, quinoxaline, tetrazole, thiadiazole, thiazole, thiophene, triazole, xanthene, and the like. In some embodiments, the heteroaryl group is a 5-14 membered heteroaryl or a 5-10 membered heteroaryl.

[0078] "Heteroarylalkyl" by itself or as part of another substituent refers to an acyclic alkyl group in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp_3 carbon atom, is replaced with a heteroaryl group. Where specific alkyl moieties are intended, the nomenclature heteroarylalkanyl, heteroarylalkenyl and/or heteroarylalkynyl is used. In some embodiments, the heteroarylalkyl group is a 6-20 membered heteroarylalkyl, *e.g.*, the alkanyl, alkenyl or alkynyl moiety of the heteroarylalkyl is 1-6 membered and the heteroaryl moiety is a 5-14-membered heteroaryl. In particularly preferred embodiments, the heteroarylalkyl is a 6-13 membered heteroarylalkyl, *e.g.*, the alkanyl, alkenyl or alkynyl moiety is 1-3 membered and the heteroaryl moiety is a 5-10 membered heteroaryl.

[0079] "Substituted Alkyl, Aryl, Arylalkyl, Heteroaryl or Heteroarylalkyl" refers to an alkyl, aryl, arylalkyl, heteroaryl or heteroarylalkyl group in which one or more hydrogen atoms is replaced with another substituent group. Exemplary substituent groups include, but are not limited to, -OR', -SR', -NR'R', -NO₂, -NO, -CN, -CF₃, halogen (*e.g.*, -F, -Cl, -Br and -I), -C(O)R', -C(O)OR', -C(O)NR', -S(O)₂R', -S(O)₂NR'R', where each R' is independently selected from the group consisting of hydrogen and (C₁-C₆) alkyl.

[0080] "Prodrug" refers to a derivative of an active compound (drug) that requires a transformation under the conditions of use, such as within the body, to release the active drug. Prodrugs are frequently, but not necessarily, pharmacologically inactive until converted into the active drug. Prodrugs are typically obtained by masking a functional group in the drug believed to be in part required for activity with a progroup (defined below) to form a promoiety which undergoes a transformation, such as cleavage, under the specified conditions of use to release the functional group, and hence the active drug. The cleavage of the promoiety can proceed spontaneously, such as by way of a hydrolysis reaction, or it can be catalyzed or induced by another agent, such as by an enzyme, by light, by acid, or by a change of or exposure to a physical or environmental parameter, such as a change of temperature. The agent can be endogenous to the conditions of use, such as an enzyme present in the cells to which the prodrug is administered or the acidic conditions of the stomach, or it can be supplied exogenously.

[0081] A wide variety of progroups, as well as the resultant promoieties, suitable for masking functional groups in the active drugs to yield prodrugs are well-known in the art. For example, a hydroxyl functional group can be masked as a sulfonate, ester or carbonate promoiety, which can be hydrolyzed *in vivo* to provide the hydroxyl group. An amino functional group can be masked as an amide, carbamate, imine, urea, phosphenyl, phosphoryl or sulfenyl promoiety, which can be hydrolyzed *in vivo* to provide the amino group. A carboxyl group can be masked as an ester (including silyl esters and thioesters), amide or hydrazide promoiety, which can be hydrolyzed *in vivo* to provide the carboxyl group. Other specific examples of suitable progroups and their respective promoieties will be apparent to those of skill in the art.

[0082] Various compounds that inhibit Syk kinase or Syk/Flt-3 kinase activity can be used in the methods described herein. These include, among others, small organic molecules, peptides or proteins, or nucleic acids. As used herein, a “Syk inhibitor” or “Syk kinase inhibitory compound” refers to any compound that directly inhibits the activity of Syk kinase itself or inhibits Syk interaction with other cellular targets needed for proper Syk function in the IC₅₀ range described herein. Inhibitors as used herein include the classical description of enzyme inhibitors, such as competitive, noncompetitive and uncompetitive inhibitors. Compounds that are Syk inhibitors are generally those that display an IC₅₀ with respect to a Syk kinase activity, such as the ability of Syk kinase to phosphorylate a synthetic or endogenous substrate, in an *in vitro* or cellular assay, in the range of about 5uM or lower, about 1uM or lower, about 500nm or lower, about 100nM or lower, about 50nM or lower, about 10nM or lower, or about 1nM or lower. For instance, exemplary Syk inhibitor compounds are disclosed in U.S. application Serial No. 10/631,029 and PCT publication WO 2004/014382. Skilled artisans will appreciate that compounds exhibiting lower IC₅₀s, such as in the range of about 100nM, 10nM, 1nM, or even lower, are useful for the methods herein.

[0083] In some embodiments, the inhibitor compound can be selective for Syk kinase. A “Syk kinase selective inhibitory compound” refers to a compound displaying selectivity for Syk, which is defined as the ratio of an IC₅₀ for a reference kinase over an IC₅₀ for Syk kinase in a defined set of assays. Generally the Syk kinase selective inhibitory compound can have a selectivity for Syk kinase that is greater than about 10, greater than about 50, greater than about 100, greater than about 1000, or higher. The reference kinase can be any kinase activity associated with cell proliferative disorders, including kinases such as, by way of example and not limitation, Aurora-A, AKT, CDK1/cyclinB, CDK2/cyclinA, CDK3/cyclinE, CDK5/p35, CDK6/cyclinD3, CDK7/cyclinH/MAT1, CHK1, CHK2, EGFR, c-RAF, RAS, cSRC, Yes, Fyn, Lck, Fes, Lyn, Bmx, FGFR3, GSK3 α , GSK3 β , P13, IGF-1R, MAPK2, MAPKAP-K2, JNK, MEK1, p70S6K, PAK2, PDGFR α , PDGFR β , PDK1, PKA, PKC ϵ , PKC, PKD2, VEGF, PRAK, PRK2, ROCK-II, Rsk1, Rsk2, Rsk3, SGK. Various assays for each of the kinases will be apparent to the skilled artisan. For example, Aurora kinase activities can use natural or synthetic substrates (*e.g.*, fluorescent peptides, Histone H3) in *in vitro* assays, or measurement of phosphorylated products in cells (Walter et al., 2000, *Oncogene* 19(42):4906-16). Kinase activities can be detected using various approaches,

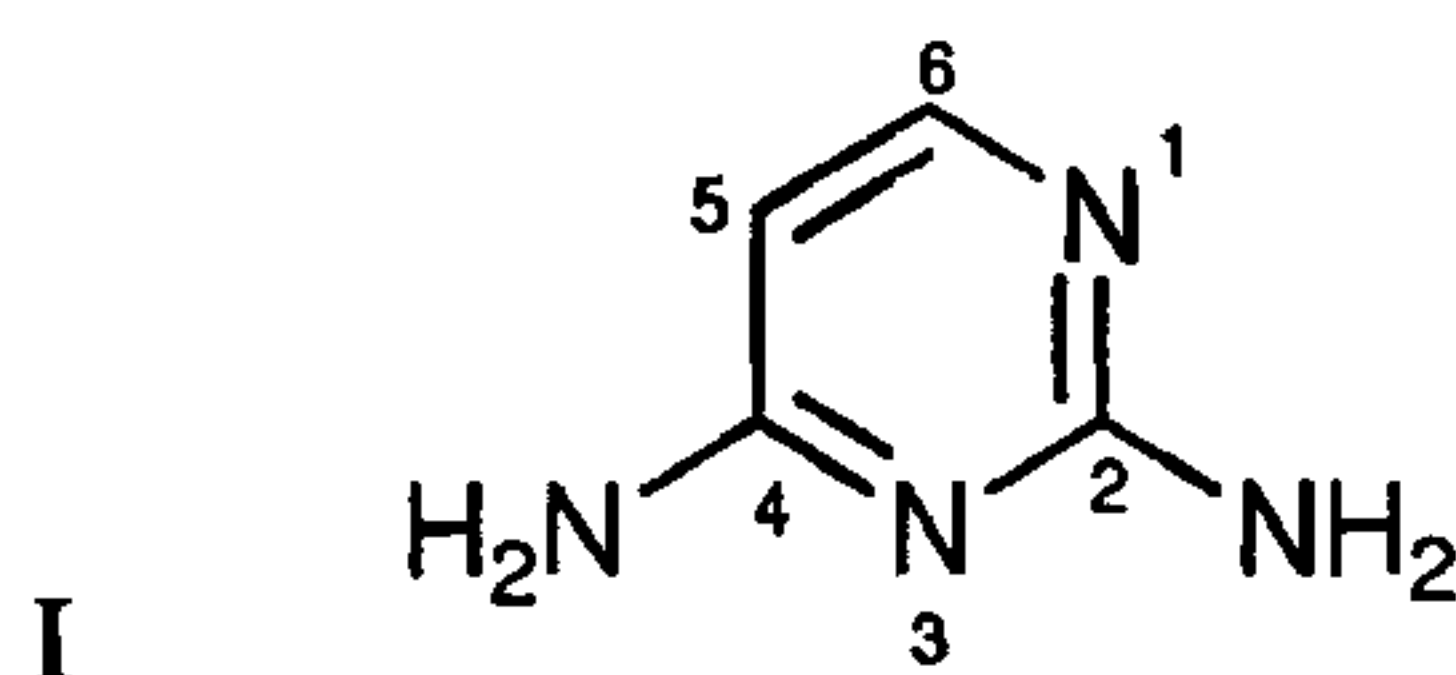
including, by way of example and not limitation, immunoprecipitation (*e.g.*, Cyclex Aurora A kinase Assay; MBL Corp, Woburn, MA, USA) mobility shift (*e.g.*, Caliper Technologies, Mountain View, CA, USA), autofluorescent fusion protein substrates (*e.g.*, U.S. Patent No. 6,248,550), and FRET based assays (Z-LYTE®; Invitrogen, CA, USA). As will be appreciated by the skilled artisan, other active kinases involved in aberrant cell proliferation can be used to determine the selectivity of a kinase inhibitor for Syk.

[0084] In some embodiments, the inhibitor compound used for treating the cell proliferative disorder comprises a Syk/Flt-3 kinase inhibitory compound. A “Syk/Flt-3 kinase inhibitory compound” or “Syk/Flt-3 kinase inhibitor” refers to a Syk inhibitory compound that can also inhibit Flt-3 kinase, such as by directly inhibiting the activity of Flt-3 kinase itself or by inhibiting the interaction with other cellular targets needed for proper Flt-3 function in the IC_{50} range described herein. Compounds that are Syk/Flt-3 inhibitors are generally those that display an IC_{50} with respect to a Flt-3 kinase activity, such as the ability of Flt-3 kinase to phosphorylate a synthetic or endogenous substrate, in an *in vitro* or cellular assay, in the range of about 5uM or lower, about 1uM or lower, about 500nm or lower, about 100nM or lower, about 50nM or lower, about 10nM or lower, or about 1nM or lower. For instance, exemplary Syk/Flt-3 inhibitor compounds can be found in the genus of compounds disclosed in U.S. Application No. 2007/0060603 and PCT publication WO 2004/014382. Skilled artisans will appreciate that compounds exhibiting lower IC_{50} s, such as in the range of about 100nM, 10nM, 1nM, or even lower, are useful for the methods herein.

[0085] Various kinase inhibitors can be used in the methods herein, and is meant to include, where applicable, the salts, hydrates, solvates, and N-oxides of the corresponding inhibitor compounds. In some embodiments, the Syk kinase or Syk/Flt-3 kinase inhibitor comprises 2,4-pyrimidinediamine compounds and its various derivatives, as described in U.S. Application No. 2007/0060603 and published PCT application No. WO 2004/014382.

These compounds generally comprise a

2,4-pyrimidinediamine “core” having the following structure and numbering convention:



[0086] The compounds are substituted at the C2 nitrogen (N2) to form a secondary amine and are optionally further substituted at one or more of the following positions: the C4 nitrogen (N4), the C5 position and/or the C6 position. When substituted at N4, the substituent forms a secondary amine. The substituent at N2, as well as the optional substituents at the other positions, can range broadly in character and physico-chemical properties. For example, the substituent(s) can be a branched, straight-chained or cyclic alkyl, a branched, straight-chained or cyclic heteroalkyl, a mono- or polycyclic aryl a mono- or polycyclic heteroaryl or combinations of these groups. These substituent groups can be further substituted, as is described in U.S. application No. 2007/0060603 and PCT publication WO 2004/014382.

[0087] The N2 and/or N4 substituents can be attached directly to their respective nitrogen atoms, or they can be spaced away from their respective nitrogen atoms *via* linkers, which can be the same or different. The nature of the linkers can vary widely, and can include virtually any combination of atoms or groups useful for spacing one molecular moiety from another. For example, the linker can be an acyclic hydrocarbon bridge (*e.g.*, a saturated or unsaturated alkylene such as methano, ethano, etheno, propano, prop[1]eno, butano, but[1]eno, but[2]eno, buta[1,3]dieno, and the like), a monocyclic or polycyclic hydrocarbon bridge (*e.g.*, [1,2]benzeno, [2,3]naphthaleno, and the like), a simple acyclic heteroatomic or heteroalkyldiyl bridge (*e.g.*, -O-, -S-, -S-O-, -NH-, -PH-, -C(O)-, -C(O)NH-, -S(O)-, -S(O)₂-, -S(O)NH-, -S(O)₂NH-, -O-CH₂-, -CH₂-O-CH₂-, -O-CH=CH-CH₂-, and the like), a monocyclic or polycyclic heteroaryl bridge (*e.g.*, [3,4]furano, pyridino, thiopheno, piperidino, piperazino, pyrazidino, pyrrolidino, and the like) or combinations of such bridges.

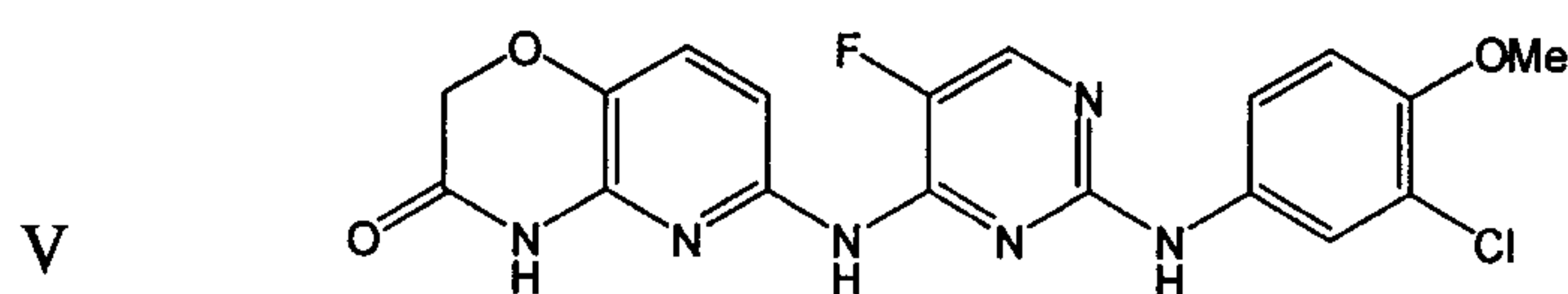
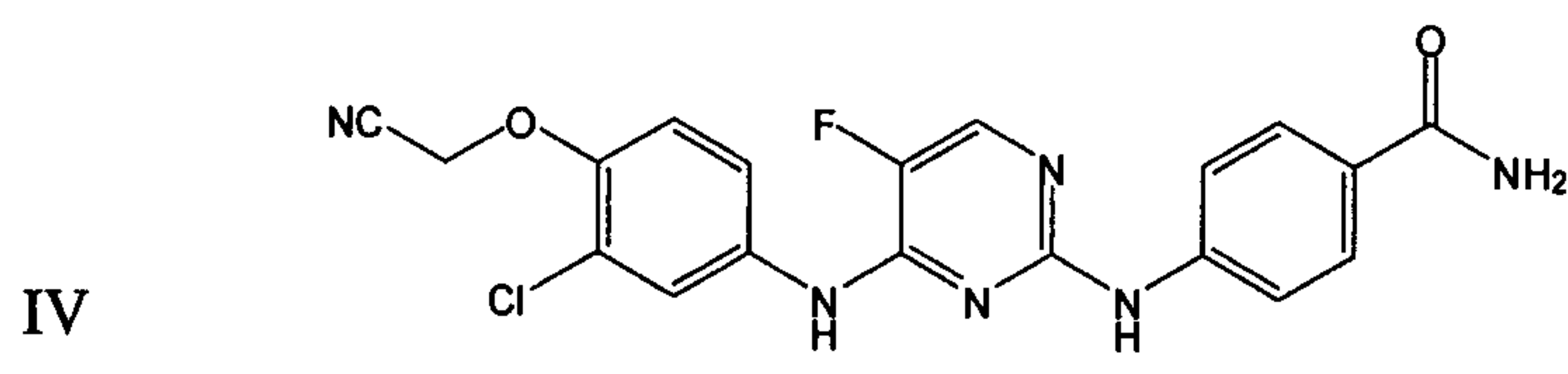
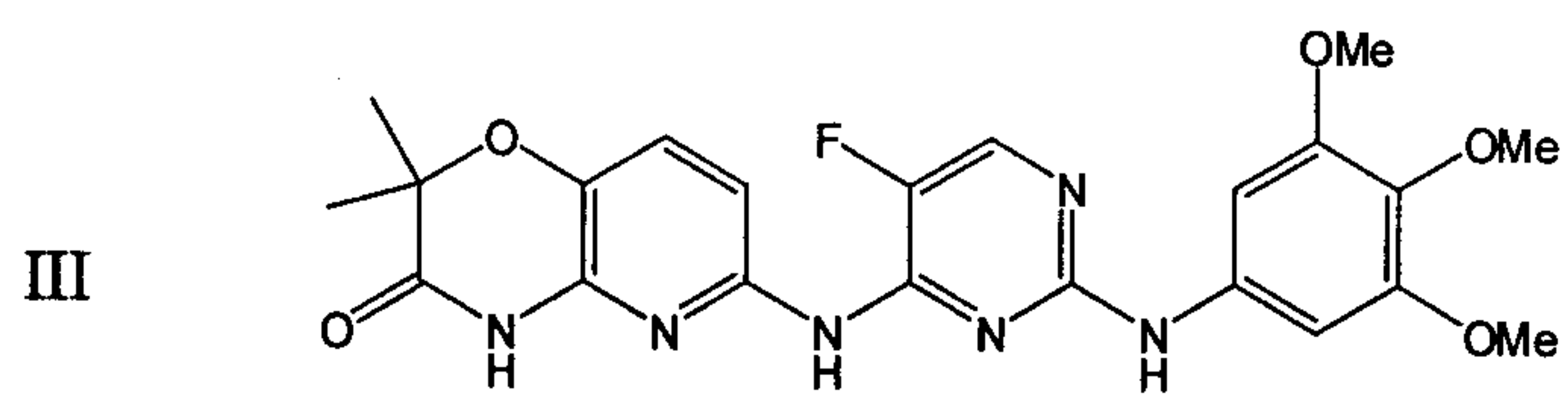
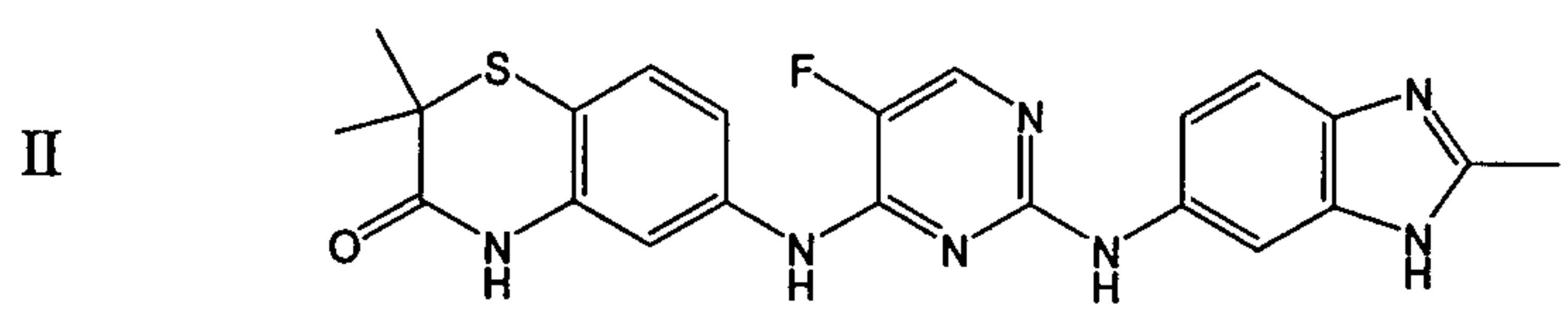
[0088] The substituents at the N2, N4, C5 and/or C6 positions, as well as the optional linkers, can be further substituted with one or more of the same or different substituent groups. The nature of these substituent groups can vary broadly. Non-limiting examples of suitable substituent groups include branched, straight-chain or cyclic alkyls, mono- or polycyclic aryls, branched, straight-chain or cyclic heteroalkyls, mono- or polycyclic heteroaryls, halos, branched, straight-chain or cyclic haloalkyls, hydroxyls, oxos, thioxos, branched, straight-

chain or cyclic alkoxys, branched, straight-chain or cyclic haloalkoxys, trifluoromethoxys, mono- or polycyclic aryloxys, mono- or polycyclic heteroaryloxys, ethers, alcohols, sulfides, thioethers, sulfanyls (thiols), imines, azos, azides, amines (primary, secondary and tertiary), nitriles (any isomer), cyanates (any isomer), thiocyanates (any isomer), nitrosos, nitros, diazos, sulfoxides, sulfonyls, sulfonic acids, sulfamides, sulfonamides, sulfamic esters, aldehydes, ketones, carboxylic acids, esters, amides, amidines, formadines, amino acids, acetylenes, carbamates, lactones, lactams, glucosides, gluconurides, sulfones, ketals, acetals, thioketals, oximes, oxamic acids, oxamic esters, etc., and combinations of these groups. Substituent groups bearing reactive functionalities can be protected or unprotected, as is well-known in the art.

[0089] Specific embodiments of Syk kinase inhibitory compounds are also described in U.S. Application Publication No. 2007/0060603, published March 15, 2007; U.S. application Publication No. 2005/0234049 ; U.S. application Publication No. 2005/0209224 ; and PCT publication WO 2004/014382.

[0090] In some embodiments, the Syk inhibitor compounds do not include specific embodiments described in U.S. application Publication No. 2005/0113398.

[0091] Exemplary embodiments of Syk or Syk/Flt-3 kinase inhibitors based on 2,4-pyrimidinediamines for the treatment of the conditions and disorders described herein include, among others, compounds having the following structures:



and various salts, hydrates, solvates, and N-oxides thereof.

[0092] Other exemplary embodiments of Syk or Syk/Flt-3 kinase inhibitors based on 2,4-pyrimidinediamines include, among others, N4-(2,2-Dimethyl-3-oxo-4H-5-pyrid[1,4]oxazin-6-yl)-5-fluoro-N2-(3,4,5-trimethoxyphenyl)-2,4-pyrimidinediamine (Compound VI); N4-(2,2-dimethyl-4-[(dihydrogen phosphonoxy)methyl]-3-oxo-5-pyrido[1,4]oxazin-6-yl)-5-fluoro-N2-(3,4,5-trimethoxyphenyl)-2,4-pyrimidinediamine (Compound VII); and various the salts (*e.g.*, calcium salts, etc.), hydrates, solvates, and N-oxides thereof.

[0093] In some embodiments, the Syk kinase inhibitors can comprise compounds based on pyridine, pyrimidine, or triazine rings, as described in published U.S. application No. 2004/0106615 and PCT publication WO 2004/016597.

Generally, the pyridine, pyrimidine, or triazine ring is directly attached to a 6-membered aryl or heteroaryl ring having 0-3 nitrogen atoms.

[0094] In some embodiments, the Syk kinase inhibitor comprises compounds based on amino- or diaminotriazoles, as described in PCT publications WO 2005/013982 and WO 2004/046120, and published U.S. Application No. 20040214817.

The aminotriazole compounds typically have substituents on the nitrogen atoms at the 3 or 4 position of the triazole ring, or the amino substituent on the ring. Exemplary aminotriazoles include, among others, aminotriazole pyridines and aminotriazole pyrimidines (see, *e.g.*, WO 2005/013982). Similarly, diaminotriazole compounds that inhibit kinases have substituents on one of the amino groups, and a substituent on the nitrogen atom at the 3 or 4 position of the triazole ring. Exemplary kinase inhibitors based on diaminotriazoles are described in WO2004/046120 and US 20040214817.

[0095] In some embodiments, the Syk kinase inhibitor comprises compounds based on azaindoles, as described in U.S. Patent No. 6,849,641, published U.S. Patent Application No. 2004/0053931, and PCT publication WO 03/000688.

U.S. Patent No. 6,849,641 describes 3-heteroarylideneazaindolin-2-one compounds. Similarly, U.S. Patent Application No. 2004/0053931 and PCT Publication No. WO 03/000688 describes azaindole compounds, among others, in which the pyrrolopyridine has aromatic or heterocyclic substituents (*e.g.*, benzyl or indolyl) at the 2 or 3 position.

[0096] In other embodiments, the Syk kinase inhibitor comprises compounds based on benzimidazoles, as described in published U.S. Patent Application No. 2004/0048868 and PCT publication WO 03/020698.

These compounds typically have substituents at the 1 and 2 positions of the imidazolyl along with additional substitutions on the benzyl ring. Exemplary substituent at the 2 position is an aryl or heteroaryl, such as a pyrazolyl, triazolyl, imidazolyl, indolyl, indazolyl, thienopyrazolyl, tetrahydroindazolyl, tetrahydrocyclopentapyrazolyl, dihydrofuropyrazolyl, oxodihydropyridazinyl, tetrahydropyrrolopyrazolyl, oxotetrahydropyrrolopyrazolyl, tetrahydropyranopyrazolyl, tetrahydropyridinopyrazolyl, or oxodihydropyridinopyrazolyl group.

[0097] In some embodiments, the Syk kinase inhibitor comprises compounds based on thiazoles, as described in U.S. Patent No. 6,762,179, published U.S. Patent Application Nos. 2003/0119856 and 2005/0004152, and PCT Publication No. WO 02/096905.

Exemplary thiazole based inhibitors are 4-thiazolylpyrimidines in which the pyrimidine has substituents at the 2 and 4 positions. Typically, the group at the 2 position is a unsubstituted or substituted amine. Substituents on the amine are generally monocyclic and heterocyclic rings, such as substituted phenyl, indanyl, naphthyl, pyrimidinyl, or pyridyl rings.

[0098] In some embodiments, the Syk kinase inhibitor comprises compounds based on pyrrolopyrimidines, as described in published U.S. Patent Application No. 2004/0142947 and PCT Publication Nos. WO 03/000695 and WO 2004/016597.

In some embodiments, the pyrrolopyrimidine is attached to the 3 position of an indole ring. Generally, the indole has substituents on the 1 and/or 5 positions. An additional substituent can be present at the 4 position of the pyrrolopyrimidine, including, among others, cyano, halo, hydroxy, nitro, aryl, heteroaryl, alkenyl, or alkynyl.

[0099] In some embodiments, the Syk kinase inhibitor comprises compounds based on indazoles, as described in published U.S. Patent Application No. 2005/0009876 and U.S. Patent No. 6,534,524. U.S. Patent No. 6,534,524 discloses inhibitor compounds in which the indazole has substituents at the 3 and/or 5 positions. Substituents at the 3 position are, among others, an unsubstituted aryl or substituted or unsubstituted heteroaryl, or $\text{CH}=\text{CH}-\text{R}$ or $\text{CH}=\text{N}-\text{R}$, where R is a substituted or unsubstituted alkyl, alkenyl, cycloalkyl, heterocycloalkyl, aryl or heteroaryl. Substituents at the 5 position are, among others, substituted or unsubstituted aryl, heteroaryl, or a Y-X, where Y is O, S, $\text{C}=\text{CH}_2$, $\text{C}=\text{O}$, $\text{S}=\text{O}$, SO_2 , alkylidene, NH, N-alkyl, where R^1 is a substituted or an unsubstituted aryl, heteroaryl, or $\text{N}-\text{R}'$, where R' is alkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, alkoxy, or dialkylamide. Similarly, U.S. Patent Application No. 2005/0009876 discloses compounds in which the indazole has substituents at the 3 and/or 5 positions of the indazole. At the 3 position, a substituted or unsubstituted aryl, or a heteroaryl or heterocycle fused to a phenyl is attached via an alkyl, such as an alkanyl, alkenyl, or alkynyl. Substituents at the 5 position are, among others, halogen, hydroxy, carboxy, alkyl, alkoxy, haloalkyl, acyloxy, thioalkyl, sulfinylalkyl, sulfonylalkyl, hydroxyalkyl, aryl,

substituted aryl, arylalkyl, substituted arylalkyl, heterocycle, substituted heterocycle, substituted or unsubstituted heterocycloalkyl, ester, amide, cyano, or substituted or unsubstituted amine.

[0100] In some embodiments, the Syk kinase inhibitor comprises bicyclic compounds, as described in U.S. Patent No. 6,573,295, published U.S. Patent Application No. 2002/0062031, and PCT publication WO 00/27802.

In some of these embodiments, an unsubstituted or substituted benzyl is fused to a cycloalkyl, which is also substituted or unsubstituted. An exemplary cycloalkyl is a heptenyl. Exemplary bicyclic inhibitor compounds are {4-[2-(7-carbamoyl-8-cyclohexylmethoxy-2,3,4,5-tetrahydro-benzo[b]oxepin-(S)-5-ylcarbamoyl)-2-phenylacetyl-amino-ethyl]-2-phosphono-phenyl}-phosphonic acid; {4-[(S)-2-Acetyl-amino-2-(3-carbamoyl-2-cycyclooxylmethoxy-6,7,8,9-tetrahydro-5H-benzocyclohepten-(S)-5-ylcarbamoyl)-ethyl]-phenoxy}-acetic acid; and (4-[(s)-2-Acetyl-amino-2-(3-carbamoyl-2-cycyclooxylmethoxy-6,7,8,9-tetrahydro-5H-benzocyclohepten-(S)-5-ylcarbamoyl)-ethyl]-2-carboxymethyl-phenoxy -acetic acid.

[0101] In other embodiments, the kinase inhibitors comprises chromenone oxime compounds as described in published U.S. Patent Application No. 2004/0198750 and PCT publication WO 2004/092154. Generally, substituents are on the 2 and 7 positions of the chromenone oxime nucleus.

[0102] Other kinase inhibitors compounds include, substituted heterocycles (*e.g.*, thiazole, oxazole, isoxazole, diazole, oxadiazole, dioxazole, furan, and pyridine) described in PCT publication WO99/47529, and substituted aryl or 5- or 6-membered heteraryl rings as described in PCT publication No. WO 2004/085388; and sulfonamides as described in Lai et al., 2003, *Bioorg Med Chem Lett.*, 13(18):3111-4. Other kinase inhibitor compounds will be apparent to the skilled artisan, and can be tested for Syk kinase inhibitory activity using the guidance provided herein.

[0103] Compounds can be tested in various biochemical and cellular assays for their inhibitory effect on Syk and/or Flt-3 kinase. Syk kinase phosphorylates LAT and PLC- γ , which leads to, among other things, degranulation in mast and/or basophil cells. Syk kinase activity is also observed in response to T-cell receptor stimulation. It is to be understood that

any of these activities can be used to confirm the activity of the Syk inhibitor compounds. In some embodiments, the Syk kinase assay is a degranulation assay based on measurement of granule content release following stimulation with anti-IgE. These assays include, for example, measurement of tryptase, histamine, leukotriene LTC₄, or hexosaminidase release. In other embodiments, the activity is determined by contacting an isolated Syk kinase, or an active fragment thereof with an inhibitor compound in the presence of a Syk kinase substrate (*e.g.*, a synthetic peptide or a protein that is known to be phosphorylated by Syk in a signaling cascade) and assessing whether the Syk kinase phosphorylates the substrate. Alternatively, the assay can be carried out with cells that express a Syk kinase. The cells can express the Syk kinase endogenously or they can be engineered to express a recombinant Syk kinase. The cells can optionally also express the Syk kinase substrate. Cells suitable for performing such confirmation assays, as well as methods of engineering suitable cells will be apparent to those of skill in the art. Suitable Syk kinase substrate include, by way of example and not limitation, human band 3 protein (Wang et al., 1999, *J Biol Chem.* 274(45), 32159-32166); protein kinase C (Kawakami et al., 2003, *Proc Natl Acad Sci USA*, 100(16):9470-5), tubulin (Peters et al., 1996, *J. Biol. Chem.* 271:4755), cortactin (Maruyama et al., 1996, *J. Biol. Chem.* 271:6631), and p50/HS1 (Ruzzene et al., 1996, *Biochemistry* 35:1527). Specific examples of biochemical and cellular assays suitable for confirming the activity of the Syk inhibitor compounds are described in Fox et al., 1998, *Protein Science*, 7:2249, U.S. application Serial No. 10/631,029, WO 2004/014382.

[0104] Activation of Flt-3 kinase leads to autophosphorylation as well as phosphorylation of a number of cellular substrates, including, among others, of Src homology 2 (SH2)-containing inositol-5-phosphatase (SHIP) and a 100-kD protein in monocytic THP-1 cells; phosphorylation of Shc and Cbl in myeloid cells; β -arrestin; SH2-containing tyrosine phosphatase, and Cbl-b in pro-B cells (see, *e.g.*, Rottapel et al., 1994, *Oncogene* 9:1755-1765; Zhang et al., 1999, *J. Leukoc. Biol.* 65:372-380). In some embodiments, the activity of Flt-3 kinase can be determined by used of antibodies to the phosphorylated form of Flt-3 kinase (*i.e.*, autophosphorylation assay; Kiyoi et al. 1998, *Leukemia* 12:1333-1337). In some embodiments, the activity can be determined by contacting an isolated Flt-3 kinase, or an active fragment thereof, with an inhibitor compound in the presence of an Flt-3 kinase substrate (*e.g.*, a synthetic peptide or a protein that is known to be phosphorylated by Flt-3 in a

signaling cascade). Alternatively, the assay can be carried out with cells that express an Flt-3 kinase, either endogenously or they being engineered to express a recombinant Flt-3 kinase (*e.g.*, Yamamoto et al., 2001, *Blood* 97(8):2434-2439). The cells can optionally also express the Flt-3 kinase substrate. In some embodiments, the Flt-3 expressing cells can be examined for activation of various downstream targets described above, such as, for example, phospholipase C- (PLC), the p85 subunit of phosphatidylinositol 3'-kinase (PI3K), SHC, SHP-2, SHIP, GRB2, VAV, Fyn kinase, Src kinase, Stat5 signal transducing protein, and ERK.

[0105] Determining the effect of the inhibitor compounds on cell proliferation can use any number of *in vitro* and *in vivo* assays. For example, proliferating cells can be suitably cultured *in vitro* and treated with the compounds of interest. Proliferative capacity in the cell populations can be determined use dye staining (*e.g.*, trypan blue dye-exclusion; 3-4,5-dimethylthiazol-2,5-diphenyltetrazolium (MTT); and annexin V), or cell sorting techniques (*e.g.*, fluorescence activated cell sorting with propidium iodide). *In vivo* assays for cell proliferation can be based on transplantation of tumor cells into experimental animals followed by administration of the inhibitor compounds. These and other methods of assessing cell proliferation will be apparent to the skilled artisan.

6.3 Dosages

[0106] The active compound(s), or compositions thereof, can be used in an amount effective to treat or prevent the particular disease being treated. The compound(s) can be administered therapeutically to achieve therapeutic benefit or prophylactically to achieve prophylactic benefit. By therapeutic benefit is meant eradication or amelioration of the underlying cell proliferative disorder being treated, *e.g.*, lymphoid neoplasm, myeloid neoplasm, viral associated tumors, and/or eradication or amelioration of one or more of the symptoms associated with the underlying disorder such that the patient reports an improvement in condition, notwithstanding that the patient can still be afflicted with the underlying disorder. Therapeutic benefit also includes halting or slowing the progression of the disease, regardless of whether improvement is realized.

[0107] For prophylactic administration, the active compound can be administered to a patient at risk of developing a disorder characterized by, caused by or associated with aberrant cell

proliferation, such as the various disorders previously described above. For instance, if a patient is diagnosed with a tumor but there is no indication of metastasis, the inhibitor compounds can be administered prophylactically to inhibit tumor metastasis.

[0108] The amount of inhibitor compound(s) administered will depend upon a variety of factors, including, for example, the particular indication being treated, the mode of administration, whether the desired benefit is prophylactic or therapeutic, the severity of the indication being treated and the age and weight of the patient, the bioavailability of the particular active compound, etc. Determination of an effective dosage is well within the capabilities of those skilled in the art.

[0109] Initial dosages can be estimated initially from *in vitro* assays. For example, an initial dosage for use in animals can be formulated to achieve a circulating blood or serum concentration of compound that inhibits Syk or Flt-3 sufficient to reduce the cell proliferation or invasiveness of the tumor cells. Alternatively, an initial dosage for use in animals can be formulated to achieve a circulating blood or serum concentration of active compound that is equal to or greater than the IC_{50} as measured in Syk kinase or Flt-3 inhibition assay. Calculating dosages to achieve such circulating blood or serum concentrations taking into account the bioavailability of the particular inhibitor compound is well within the capabilities of skilled artisans. For guidance, the reader is referred to Fingl and Woodbury, "General Principles," In: *The Pharmaceutical Basis of Therapeutics*, Chapter 1, pp. 1-46, 1975, and the references cited therein. Initial dosages can also be estimated from *in vivo* data, such as animal models. Animal models useful for testing the efficacy of compounds to treat or prevent diseases characterized by, caused by or associated with Syk kinase or Flt-3 kinase activity are described herein.

[0110] Dosage amounts will typically be in the range of from about 1 mg/kg/day to about 100 mg/kg/day, 200 mg/kg/day, 300 mg/kg/day, 400 mg/kg/day or 500 mg/kg/day, but can be higher or lower, depending upon, among other factors, the activity of the inhibitory compound, its bioavailability, the mode of administration and various factors discussed above. Dosage amount and interval can be adjusted individually to provide plasma levels of the active compound(s) which are sufficient to maintain therapeutic or prophylactic effect. In cases of local administration or selective uptake, such as local topical administration, the

effective local concentration of active compound(s) may not be related to plasma concentration. Skilled artisans will be able to optimize effective dosages without undue experimentation.

[0111] The compound(s) can be administered once per day, a few or several times per day, or even multiple times per day, depending upon, among other things, the indication being treated and the judgment of the prescribing physician.

[0112] Preferably, the active compound(s) will provide therapeutic or prophylactic benefit without causing substantial toxicity. Toxicity of the active compound(s) can be determined using standard pharmaceutical procedures. The dose ratio between toxic and therapeutic (or prophylactic) effect is the therapeutic index. Active compound(s) that exhibit high therapeutic indices are preferred.

6.4 Administration

[0113] When used to treat or prevent cell proliferative disorders, the Syk or Syl/Flt-3 inhibitor compounds can be administered singly, as mixtures of one or more active compounds or in mixture or combination with other agents useful for treating such diseases and/or symptoms associated with such diseases. The active compounds can be administered *per se* or as pharmaceutical compositions.

[0114] Pharmaceutical compositions comprising the active compounds of the invention can be manufactured by means of conventional mixing, dissolving, granulating, dragee-making levigating, emulsifying, encapsulating, entrapping or lyophilization processes. The compositions can be formulated in conventional manner using one or more physiologically acceptable carriers, diluents, excipients or auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. The actual pharmaceutical composition administered will depend upon the mode of administration. Virtually any mode of administration can be used, including, for example topical, oral, systemic, inhalation, injection, transdermal, etc.

[0115] The active compound can be formulated in the pharmaceutical compositions *per se*, or in the form of a pharmaceutically acceptable salt. As used herein, the expression “pharmaceutically acceptable salt” means those salts which retain substantially the biological

effectiveness and properties of the active compound and which is not biologically or otherwise undesirable. Such salts can be prepared from inorganic and organic acids and bases, as is well-known in the art. Typically, such salts are more soluble in aqueous solutions than the corresponding free acids and bases.

[0116] For topical administration, the active compound(s) can be formulated as solutions, gels, ointments, creams, suspensions, etc. as are well-known in the art.

[0117] Systemic formulations include those designed for administration by injection, e.g., subcutaneous, intravenous, intramuscular, intrathecal or intraperitoneal injection, as well as those designed for transdermal, transmucosal oral or pulmonary administration.

[0118] Useful injectable preparations include sterile suspensions, solutions or emulsions of the active compound(s) in aqueous or oily vehicles. The compositions can also contain formulating agents, such as suspending, stabilizing and/or dispersing agent. The formulations for injection can be presented in unit dosage form, *e.g.*, in ampules or in multidose containers, and can contain added preservatives.

[0119] Alternatively, the injectable formulation can be provided in powder form for reconstitution with a suitable vehicle, including but not limited to sterile pyrogen free water, buffer, dextrose solution, etc., before use. To this end, the active compound(s) can be dried by any art-known technique, such as lyophilization, and reconstituted prior to use.

[0120] For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are known in the art.

[0121] For oral administration, the pharmaceutical compositions can take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulfate). The tablets can be coated by methods well known in the art with, for example, sugars or enteric coatings.

[0122] Liquid preparations for oral administration can take the form of, for example, elixirs, solutions, syrups or suspensions, or they can be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-*p*-hydroxybenzoates or sorbic acid). The preparations can also contain buffer salts, flavoring, coloring and sweetening agents as appropriate. Preparations for oral administration can be suitably formulated to give controlled release of the active compound.

[0123] For buccal administration, the compositions can take the form of tablets or lozenges formulated in conventional manner.

[0124] For rectal and vaginal routes of administration, the active compound(s) can be formulated as solutions (for retention enemas) suppositories or ointments containing conventional suppository bases such as cocoa butter or other glycerides.

[0125] For administration by inhalation, the active compound(s) can be conveniently delivered in the form of an aerosol spray from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit can be determined by providing a valve to deliver a metered amount. Capsules and cartridges of *e.g.* gelatin for use in an inhaler or insufflator can be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[0126] For prolonged delivery, the active compound(s) can be formulated as a depot preparation, for administration by implantation; *e.g.*, subcutaneous, intradermal, or intramuscular injection. Thus, for example, the active ingredient can be formulated with suitable polymeric or hydrophobic materials (*e.g.*, as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives; *e.g.*, as a sparingly soluble salt.

[0127] Alternatively, transdermal delivery systems manufactured as an adhesive disc or patch which slowly releases the active compound(s) for percutaneous absorption can be used. To this end, permeation enhancers can be used to facilitate transdermal penetration of the active compound(s). Suitable transdermal patches are described in for example, U.S. Pat. No. 5,407,713.; U.S. Pat. No. 5,352,456; U.S. Pat. No. 5,332,213; U.S. Pat. No. 5,336,168; U.S. Pat. No. 5,290,561; U.S. Pat. No. 5,254,346; U.S. Pat. No. 5,164,189; U.S. Pat. No. 5,163,899; U.S. Pat. No. 5,088,977; U.S. Pat. No. 5,087,240; U.S. Pat. No. 5,008,110; and U.S. Pat. No. 4,921,475.

[0128] Alternatively, other pharmaceutical delivery systems can be employed. Liposomes and emulsions are well-known examples of delivery vehicles that can be used to deliver active compounds(s). Certain organic solvents such as dimethylsulfoxide (DMSO) can also be employed, although usually at the cost of greater toxicity.

[0129] The pharmaceutical compositions can, if desired, be presented in a pack or dispenser device which can contain one or more unit dosage forms containing the active compound(s). The pack can, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device can be accompanied by instructions for administration.

7. EXAMPLES

7.1 Example 1: Effect of Syk Inhibitory Compound on Syk Induced pre-B Cell Transformation

[0130] Experiments showing the inhibition of proliferation of Syk-transformed cells by a Syk inhibitory compound are described in Wossning, T., Herzog, S., Köhler, F., Meixlsperger, S., Kulathu, Y., Mittler, G., Abe, A., and Jumaa, H., "The protein tyrosine kinase Syk is involved in malignant transformation and leukemia development," (manuscript in preparation). The experiments described in the manuscript are briefly summarized below.

[0131] Aberrant Syk variants resulting from chromosomal translocation Tel-Syk were tested for its ability to transform pre-B cells by retrovirally transducing freshly isolated bone marrow pre-B cells with IRES-GFP vectors expressing Tel-Syk. Withdrawal of IL-7 from these cell cultures led to an enrichment of Tel-Syk-expressing cells and the generation of stable IL-7 independent cell lines, indicating that TEL-Syk expression can promote proliferation and transformation of pre-B cells. Transformation of the pre B cells by Tel-Syk activity was

confirmed by injecting the TEL-SYK transduced cells into RAG/ γ C^{-/-} mice, which led to animals presenting symptoms of myelodysplastic disease. The data show that Tel-Syk-expressing cells can proliferate rapidly *in vivo*, thereby inducing aggressive leukemia.

[0132] To test whether inhibition of Syk activity in the cells transformed with Tel-Syk block cell proliferation, the effect of Syk kinase inhibitor 2,4-pyrimidinediamine compound **IV** was tested *in vitro* by examining the DNA content of treated cells. Compound **IV** effectively blocked Syk-induced pre-B cell proliferation and permitted the pre B cells to differentiate, as indicated by expression of kappa light chain in these cells. The data show that Syk expression may be required for proliferation of leukemic cells and that block of Syk kinase activity can lead to inhibition of leukemic cell proliferation.

7.2 Example 2: Effect of Syk Inhibitors on Established Leukemias

[0133] To determine the effect of Syk inhibitor on proliferation of leukemic cells, several tumorigenic pre-B cell lines that induce leukemia after injection into mice were used. Because tumorigenic cells need the pre-B cell receptor (pre-BCR) for proliferation, and Syk is a critical protein for pre-BCR signaling cascade, it is expected that Syk inhibitors will inhibit proliferation of these tumorigenic pre-B cells. In one experiment, the ability of compound **VI** to block proliferation was examined using pre-B cells transduced with Myc expression vectors and cultured in the absence of IL-7. Cells were injected into RAG/ γ C^{-/+} mice to examine their ability to proliferate *in vivo*. The ability of **VI** to block proliferation was tested *in vitro* using the Myc transduced cell lines (FIG. 2A and FIG. 2B).

[0134] The ability of **VI** to block proliferation was also examined using tumorigenic SLP-65^{-/-} pre-B cell line. Cells were injected into RAG/ γ C^{-/+} mice to examine their ability to proliferate *in vivo*. The ability of **VI** to block proliferation of the tumorigenic SLP-65^{-/-} pre-B cell line was tested *in vitro* (FIG. 3A and FIG. 3B).

[0135] Other leukemic cell lines useful for examining the effect of Syk inhibitor compounds include, by way of example and not limitation, B-cell lymphoma cell line JM1 (ATCC No. CRL-10423), Hodgkin's lymphoma cell line RPMI-6666 (ATCC No. CCL-113), promyelocytic leukemia cell line HL-60 clone 15 (ATCC No. CRL-1964), Burkitt's lymphoma cell line GL-10 (ATCC No. CRL-2392), acute myelogenous leukemia cell line BDCM (ATCC No. CRL-2740), myelomonocytic leukemia (EBV) cell line CESS (ATCC

No. TIB-190), EBV transformed plasmacytoma/myeloma cell line MC-CAR (ATCC No. CRL-8083), and EBV transformed plasma cell leukemia cell line ARH-77 (ATCC No. CRL-1621).

7.3 Example 3: In Vivo Evaluation Of The Calcium Salt Form Of Compound **VII**, and Cell Titration In The MV4-11 Acute Myeloid Leukemia (AML) Intravenous Tumor Engraftment Model In NOD-SCID Immunocompromised Mice Pretreated With Cyclophosphamide

[0136] The Flt-3 ITD mutation has been associated with poor prognosis and reduced remission rates in affected AML patients, making it an attractive target for therapeutic intervention. Studies have shown that mutant Flt-3 ITD receptors dimerize in a Flt-3 ligand-independent manner and auto-phosphorylation of the receptor results in constitutive activation, increased proliferation, and growth factor independence of the mutant cell. Initial studies showed that the calcium salt form **VII** reduced subcutaneous tumor growth and prolonged survival and reduced the tumor burden of mice intravenously (i.v.) inoculated with 5 million MV4-11 human acute myeloid leukemia cells having the Flt-3 ITD mutation. This study was carried out to demonstrate the efficacy of administering **VII** in inhibiting tumor growth through twice daily oral treatment with 40 mg/kg **VII** or vehicle on disease progression, severity, and survival resulting from i.v. injection of either 5 or 10 million MV4-11 leukemia cells into NOD-SCID mice pretreated with cyclophosphamide.

7.3.1 Experimental Methods

[0137] Cell Lines.

[0138] Leukemia Cell Lines: MV4-11 human acute myelogenous leukemia (AML) (Supplier: American Type Culture Collection (ATCC)) were maintained and harvested by the Oncology group at Rigel. Cells were IMPACT tested on 11-30-04 and were negative for Ectromelia, EDIM, Hantaan, K virus, LCMV, LDEV, MAD, mCMV, MHV, MMV, MPV, MTV, Mycoplasma sp., Polyoma, PVM, REO3, Sendai, TMEV and GDVII.

[0139] Cell Line Maintenance: MV4-11 cells will be maintained in Iscove's modified Dulbecco's medium (ATCC® Number: 30-2005) with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 80%; fetal bovine serum, 20% (GIBCO BRL, Carlsbad, CA), 1% Penicillin/Streptomycin (10,000 IU/ml and 10,000 ug/ml) (CellGro/MediaTech, Cat# 30-

002-Cl). Cells were harvested in logarithmic growth phase, washed, and injected in fresh medium. MV4-11 cells were inoculated at 5×10^6 or 10×10^6 cells per animal.

[0140] Pharmaceuticals.

[0141] The specifications of the test articles and test formulations used in this study are listed on the formulations sheet below. Test article and vehicle formulations were made by the Pharmaceuticals department before the start of dosing (Vehicle (Lot: 1024-15-01); 8 mg/mL **VII** calcium salt (Lot: 1024-15-03)). Calcium salt form **VII** was prepared at equivalent concentration of **VI**. Formulations were stored at 4°C, light protected, and were vortexed prior to use.

***Vivo* Evaluation of the kinetics of engraftment of MV411 leukemia (AML) cells administered i.v. in NOD-S immunocompromised mice and effect of Compound VII on level of engraftment.**

Test Article Id. Number	Formulation Details	Conc. (mg/mL)	Calculations Based on Free Base or Salt?	Formulation Required* (mL)	Free Base Equivalent (mg)	Dose Volume (mL/kg)	Dilution Factor*	Dose (mg/kg)
Vehicle (0.1%CMC Na - 0.1% Methylparaben- .02%Propylparaben-H ₂ O)	Vehicle (0.1%CMC Na - 0.1% Methylparaben- 0.02%Propylparaben -H ₂ O)	0.00	406 Free Base	198.4	0.00	5.0	1	0
Ca salt of VII	Vehicle (0.1%CMC Na - 0.1% Methylparaben- 0.02%Propylparaben -H ₂ O)	16.00	406 Free Base	198.4	3,174.00	5.0	1	80

[0142] Animals and Housing.

[0143] Approximately 10 week old, female NOD.CB17-Prkdc scid/J (NOD/SCID) mice (n=60; DOB 05/30/05; rec'd 07/19/05 from Jackson Laboratories, Bar Harbor, ME) were used for the study. NOD-SCID mice (stock # 001303) are exclusively available from The Jackson Laboratory and the multiple defects in innate and adaptive immunity unique to the NOD-SCID mouse provide an excellent in vivo environment for reconstitution with human hematopoietic cells. Approximately 50 mice were given cyclophosphamide and used for the in-life portion of this study, while the additional mice were used for non-tumored controls for the assessment of background staining for flow cytometric analysis of engraftment levels. Slightly older mice (>19grams) were used (as compared to V050144) to try to avoid early deaths due to excessive weight loss following cyclophosphamide injection.

[0144] Animals were housed five per cage in Micro isolator cages in ventilated, HEPA-filtered cage racks (Alternative Design, Siloam Springs, AZ). Upon arrival, they were acclimated for at least four working days before use. Temperature was maintained at $72 \pm 5^{\circ}\text{F}$, relative humidity at 35-70%, and a 12-h light/dark cycle was used. The mice were fed certified rodent chow (Deans Feed, San Carlos, CA) ad libitum. Autoclaved R.O. drinking water was available ad libitum. Cages and food were autoclaved prior to use. All in-life procedures were approved by the Rigel IACUC.

[0145] NOD-SCID mice are both insulinitis- and diabetes-free throughout life despite the NOD autoimmune diabetes-prone background, but there is a high incidence of thymic lymphomas in this congenic stock limiting the mean lifespan to only 8.5 months under specific pathogen-free conditions. The Emv-30 provirus on chromosome 11 results in high frequency of thymomas beginning at 5-6 months of age in the NOD-SCID (3, 4), (5), limiting the duration of experiments performed using NOD-SCID mice as recipients. In this experiment, all surviving mice were terminated when mice reached approximately 5 months of age (day 83 of the study) in order to limit the affects of spontaneous thymomas on outcome variables in this study.

[0146] Animal Procedures.

[0147] Bone Marrow Depletion for Enhanced Engraftment. At the end of the acclimatization period for the animals (>3 weeks in this study), mice were pretreated with sterile filtered (using a 0.22 μ M syringe filter) Cyclophosphamide prepared in 0.9% sterile saline (Sigma, St Louis, MO) by intraperitoneal injection of 150 mg/kg/day for 2 days followed by a rest period of 72 hours prior to intravenous (i.v.) injection of leukemia cells via the lateral tail vein, as described below.

[0148] Tumor Inoculations: On day 0 of the study, mice were inoculated once by intravenous tail vein injection of 5 or 10 million human MV4-11 leukemia cells resuspended in 100 microliters of fresh media as outlined in Table 1.

[0149] Dosing: After mice were inoculated with human MV4-11 cells (day 0), dosing began on day 17 of the study. Mice were dosed orally (PO) twice daily (bid) for the duration of the study with either Vehicle or 40mg/kg **VII** at 5mL/kg dose volume with a minimum separation of 8 hours between doses

[0150] Experimental Procedures

[0151] Tumor Model: This study was designed to assess the effects of various doses of **VII** on tumor engraftment, disease progression, severity, and overall survival in a murine intravenous MV4-11 leukemia model using NOD-SCID immunocompromised mice as a host with cyclophosphamide pretreatment to enhance tumor engraftment. Historical data show variable tumor cell engraftment in bone marrow (BM) and peripheral blood mononuclear cells (PBMC), despite uniform lethality of tumor cell injection as determined by >20% weight loss, moribund status, severe loss of motility due to hindlimb paralysis or unexpected death as endpoints (Studies V050123 and V050144). In addition, cyclophosphamide was shown to be required for lethality in this model. Published studies showing median survival time of 51 days in vehicle control mice also demonstrate variable BM engraftment at sacrifice with a range of 2-19% human cells detected in the BM compartment.

[0152] This study was conducted in an effort to confirm a previous efficacy data from study, but also to determine if 5 or 10 million cell inoculations would yield similar inconsistencies

in tumor cell engraftment in the bone marrow and peripheral blood or alter the resultant mortality in these mice. Since a detectable level of engraftment in some animals was observed as early as 4 weeks post tumor cell injection in pilot studies and historical efficacy data had been determined with this regimen, animals were treated orally (PO) with vehicle or R945788 calcium salt at 40mg/kg on a twice per day (bid) schedule, every day beginning approximately 2 weeks after tumor cell injection. Animals were continued on this regimen until euthanized or until the end of the study. In this experiment, all surviving mice were terminated when mice reached approximately 5 months of age (day 83 of the study) in order to limit the affects of the spontaneous thymoma development, which can be seen in NOD-SCID mice beginning at approximately 5-6 months of age.

[0153] Study Design: On day 0 (8/12/05), mice were i.v. injected with human MV4-11 cells in fresh medium at 5 or 10 million cells per mouse via the lateral tail vein. By day 3 post MV4-11 cell injection (on 8/15/05), one mouse was excluded due to early death from excessive body weight loss prior to randomization or treatment, potentially due to cyclophosphamide treatment. Remaining animals were randomized into treatment groups using body weights and dosing began on day 17 post cell injection (8/29/05). Mean body weights of groups 1-4 were 20.66 ± 0.43 , 20.23 ± 0.41 , 20.18 ± 0.54 , and 20.88 ± 0.59 , respectively. Treatment groups are outlined in Table 1:

[0154] Table 1: Treatment Groups.

Group	Cell Line	Cells per inoculation	Treatment	N=
1	MV4-11	5.0×10^6	Vehicle	12
2	MV4-11	5.0×10^6	40mg/kg VII	12
3	MV4-11	10.0×10^6	Vehicle	13
4	MV4-11	10.0×10^6	40mg/kg VII	13
Approximately n=10 mice were obtained for nontumored control tissues, whole blood, or bone marrow				60

[0155]

[0156] Disease Progression and Disease Severity: Overall disease severity was determined from a combination of body weight loss, clinical observations, necropsy findings, and quantitation of tumor cell engraftment in selected tissues. Mice were weighed 2-3 times weekly and percent weight change calculated. At the first indication of morbidity, hind limb

paralysis with apparent wasting, severe cachexia or weight loss greater than 20%, involving lethargy, ruffled fur etc, as outlined in IACUC Protocol Rigel 6-2002 “Efficacy of Novel Chemotherapeutics in Human/Mouse Tumor Xenograft Models”, animals were removed from the study and euthanized. At sacrifice, animals were anesthetized with CO₂ gas and blood was collected by cardiocentesis. Blood from animal was transferred to K₂EDTA coated Microtainer (lavendar top) tubes. Whole blood was placed indirectly on ice and processed for flow cytometric staining, as described in section below. To further assess disease severity, the location and description of palpable or suspected tumors on gross examination was carefully noted upon necropsy.

[0157] Tumor Cell Engraftment: To assess tumor burden, the distribution of MV4-11 tumor cells in the bone marrow and peripheral blood was determined in some of the mice at sacrifice via flow cytometric analysis using human and mouse surface marker staining.

[0158] The extent of tumor engraftment was evaluated in some mice by isolation of femurs and/or tibias at sacrifice. The bone marrow (BM) was aspirated into clean ependorff tube with 1 ml of cold PBS and retained on ice prior to flow staining. All remaining tissues or tumors were fixed in 10% buffered formalin for histopathology and immunohistochemistry (IHC). Peripheral blood was also obtained via cardiac puncture and put into K₂EDTA coated Microtainer (purple) tubes. Red blood cells were lysed and samples washed, leaving only mononuclear cells (PBMC) for flow cytometric staining and analysis to determine tumor cell engraftment level.

[0159] To identify human MV4-11 tumor cells, BM and PBMC samples were stained with antibodies specific for mouse CD45, human CD45, human HLA, and human CD33 cell surface markers. Flow cytometric staining and analysis was then performed using this multiple marker strategy to identify and quantitate the percent of human MV4-11 leukemia cells present in randomly selected samples from **VII** treated and vehicle treated animals (Appendices 5 and 6). The percent of human MV4-11 cells was defined as the number of CD33+ HLA+ events out of total events (defined as the sum of human CD33+ HLA+ and murine CD45+ events). Every effort was made to collect and analyze all animals in the study; however, due to the abrupt nature of death in vehicle treated mice in this model fewer samples were available for analysis and the number of animals was variable among the

groups. For details of the cell preparation and flow staining and analysis procedure, see Appendices 5 and 6.

[0160] Statistical Analyses. Survival of mice was evaluated by Kaplan-Meier product limit method as a determination of the relative disease severity (n=18-20 animals/group) using the GraphPad Prism 4.0[®] software package. When possible, additional survival curve analyses including the Log Rank (Mantel-Haenszel) test (two-tailed p value) for curve comparison, hazard ratio for kinetics of survival, and the overall median survival were also calculated using Prism 4.0[®]. All surviving animals at study termination (day 83) were declared censored for statistical purposes in the Prism[®] analysis. The hazard statistic, defined as the slope of the survival curve, is a measure of how rapidly subjects are expiring; therefore, the reported hazard ratio is a comparison of the death rate between two groups. For example, if the hazard ratio is 2.0, then the rate of deaths in one group is twice that of the other group. In general, Prism 4.0[®] uses the standard calculations as detailed in D.G. Altman, Practical Statistics for Medical Research, 1991, Chapman and Hall. All results are expressed as mean $\pm$ SEM, unless otherwise indicated.

[0161] Additionally, the percent increase in life-span (%ILS) was determined using the calculated median day of death for each group compared to the vehicle control group using the following equation, where DOD is defined as day of death:

$$\%ILS = (\text{median DOD}_{\text{treated}} - \text{median DOD}_{\text{vehicle}}) / \text{median DOD}_{\text{vehicle}} * 100$$

[0162] Mice treated with 40mg/kg **VII** had an undefined median survival time, since inadequate numbers of mice (<50%) had expired by study termination; therefore, calculations of %ILS assumed the maximum median survival of 80 days for this group and denoted as greater than the calculated value.

[0163] Engraftment data was analyzed using a One-way ANOVA, unless unequal variances were detected by the Bartlett's test. In this case, an Unpaired Two-tailed student's t test was performed using a Welch correction to account for unequal variances. Normalization of the percent CD33+ HLA+ cells per total number of cells was performed. The mean percent tumor cells were determined using the following equation for each sample and then determining mean of these values. The total number of mouse cells was determined using a

single cell surface marker, murine CD45 and the total number of human events was determined using dual human surface markers CD33 and HLA.

$$\text{Percent Tumor cells} = [(\#CD33+ \text{ HLA+ dual positive events}) / (\text{total \# murine CD45+ and \#CD33+ HLA+ events})] * 100$$

[0164] Due to the abrupt nature of death in vehicle treated mice in this model, few samples were available for analysis limiting our ability to achieve statistical significance. Sufficient sample size and random sampling was achieved with **VII** treated mice; therefore, comparisons were made between 20, 40, and 80mg/kg **VII** treated mice and statistics performed as described above.

[0165] For categorical variables such as the total number of tumored animals per group, p values reflect the results of a comparison of treated versus vehicle groups using a two-tailed Fisher's Exact test with a 95% confidence interval also performed with GraphPad Prism 4.0[®] software package.

7.3.2 Results

[0166] Effect of Compound **VII** Treatment on Survival: Overall, animals treated with **VII** demonstrated prolonged survival and decreased numbers of palpable tumor masses on examination at necropsy. Vehicle treated mice inoculated with 5 million MV4-11 cells had a median survival time of 54 days compared to 79 days in 40mg/kg **VII** treated mice. Vehicle treated animals inoculated with 10 million tumor cells had a median survival time of 54 days with median day of death undefined for 40mg/kg **VII** treated mice (>83 days). Mice treated with 40mg/kg **VII** in the 10 million MV4-11 cell group had an undefined median survival time, since inadequate numbers of mice (<50%) had expired by study termination; therefore, calculations of %ILS assumed the maximum median survival of 83 days for this group and denoted as greater than the calculated value. Significant differences were seen in overall survival between **VII** treated and vehicle treated animals ((LogRank p<0.0022 and %ILS =45% for 40mg/kg **VII** with 5 million cells, LogRank p<0.0001 and % ILS >54% for 40mg/kg **VII** with 10 million cells (assuming median DOD=83 for this group)).

[0167] Table 2: Median Day Of Death (DOD) And Percent Increase In Life-Span (% ILS) Of MV4-11 Tumor Bearing NOD-SCID Mice.

MV4-11 Cell Titer	Treatment	Median DOD	% ILS*	Hazard ratio	Log Rank P value
5E6	Vehicle	54	NA	NA	NA
5E6	40mg/kg VII	79	45	3.5	0.0022
10E6	Vehicle	54	NA	NA	NA
10E6	40mg/kg VII	Undefined (>83)	>54% **	7.6	<0.0001

NA= not applicable

*as compared to vehicle control (median DOD=54 days for both)

**assumes median day of death = 83

[0168]

[0169] At day 83, there were no survivors in the vehicle treated groups. In contrast, mice from the **VII** treated group injected with 5 and 10 million MV4-11 cells had 5 and 9 surviving mice, respectively. Over 50% of the mice survived to Day 83 in the 10 million cell group treated with **VII** at 40 mg/kg, as compared to 0% survival of vehicle controls.

[0170] Effect of Compound **VII** on Disease Severity and Tumor Frequency and Distribution.

Significant phenotypic differences were apparent when comparing the disease progression of vehicle and **VII** treated animals. Upon necropsy, palpable tumors in vehicle treated animals were observed in various lymph nodes (LN), on the spinal cord in lower region near inferior vena cava (possible LN), in the chest wall and surrounding the ribcage and sternum, near the trachea, jaw and throat area (presumably salivary gland and LN related). Tumors were also found on the kidney, ovary and heart. Additionally, very large tumors were found surrounding the bone in many cases in the shoulder, arms and legs. Some mice were noted to have soft and smooth brains or obvious tumor and one mouse had a severely enlarged eye that appeared to be tumored on necropsy, similar to several mice from MOLM13 leukemia model in an initial study.

[0171] Vehicle treated animals manifested hind limb paralysis as early as 42 days post MV4-11 injection in the 5 million cell group and 48 days in the 10 million cell group. Upon necropsy, palpable tumors in vehicle treated animals were observed in similar locations as in previous studies. In total, 8 of 8 mice inoculated with 5 million tumor cells had palpable tumor masses on necropsy, with many mice having multiple anatomical sites identified as having tumors. Five mice from the vehicle treated group were found dead and 4 of these mice did not have a necropsy report due to advanced decay resulting in only 8 total necropsies in

this group. By the end of the study (day 83 post MV4-11 implantation), 12 out of 12 vehicle treated animals had been terminated with 5 of the animals being found dead, 3 of them were moribund upon examination, 2 were sacrificed >20% body weight loss, and 2 were sacrificed for HLP associated with severe wasting.

[0172] Similarly, 11 of 12 mice had visible tumor masses with many having multiple sites identified in the vehicle treated mice inoculated with 10 million cells. A total of 1 vehicle treated animal was found dead and 1 had no necropsy performed due to advanced decay resulting in a total of 11 necropsies in this group. At study termination (day 83 post MV4-11 implantation), 13 out of 13 vehicle treated animals had been terminated with 1 of the animals being found dead, 3 of them were moribund upon examination, 7 had >20% body weight loss, and 2 were sacrificed for HLP associated with severe wasting.

[0173] In marked contrast, mice treated with 40mg/kg **VII** demonstrated very few tumors with only 3 out of 11 total animals necropsied in the 5 million cell group and 7 out of 13 animals in the 10 million cell group showing some visible or palpable tumors. Most of these animals in the treated groups demonstrated much smaller tumors than vehicle treated animals. Tumors were located in similar anatomic sites and by study termination, 7 of 12 animals had been terminated in the 5 million cell 40mg/kg **VII** treated group with 2 of the animals being found dead, 1 animal was moribund upon examination, 4 had >20% body weight loss, and none were sacrificed for HLP. Similarly, 40mg/kg **VII** treated animals inoculated with 10 million MV4-11 cells demonstrated very few tumors with some mice having enlarged salivary glands or slightly enlarged LN. At study termination, only 4 out of 13 of the 40mg/kg **VII** treated mice had been terminated to date, with 3 mice having >20% body weight loss, and 1 animal found moribund upon examination. Overall, **VII** treated mice showed a significant reduction in total number of tumors, relative size of tumors observed at necropsy, and overall severity of disease in this study, with 40mg/kg **VII** treatment showing similar efficacy seen in an initial study.

[0174] Effect of Compound **VII** on Engraftment of MV4-11 Tumor Cells in Bone Marrow (BM) and Peripheral Blood (PB): To correlate with a previous study, the level of MV4-11 engraftment in the bone marrow or peripheral blood was also examined in some animals at termination. Despite our best efforts, due to the abrupt nature of death in vehicle treated mice

in this experiment some samples were unavailable for analysis. Unlike the initial study, a statistically significant decrease in mean percent MV4-11 tumor cells in the bone marrow and peripheral blood of **VII** treated animals at time of sacrifice was observed when compared to vehicle treated animals, regardless of the titer of cells inoculated (Table 3).

[0175] Vehicle treated mice inoculated with 5 million cells had 25% and 61% CD33+/HLA+ human tumor cells in BM (n=6) and PB (n=4), respectively. In contrast, **VII** treated mice inoculated with 5 million cells had 1% and 0.6% CD33+/HLA+ human tumor cells in BM (n=8) and PB (n=8), respectively (for BM p= 0.13 and p=0.02 for PB for 40mg/kg vs. vehicle control). The 10 million cell vehicle treated group had 13% and 45% CD33+/HLA+ human tumor cells in BM (n=8) and PB (n=8), respectively. In contrast, the 10 million cell **VII** treated group had 5% and 3% CD33+/HLA+ human tumor cells in BM (n=13) and PB (n=11), respectively (for BM p= 0.05 and p=0.007 for PB for 40mg/kg vs. vehicle control).

[0176] Table 3: Mean % MV4-11 Human Tumor Cells In Bone Marrow And Peripheral Blood of NOD-SCID Mice at Sacrifice.

A.	Bone Marrow: Mean % Tumor Cells				
	Naïve* No cells)	5 million MV4-11 Cells		10 million MV4-11 Cells	
		Vehicle	40mg/kg VII	Vehicle	40mg/kg VII
Number of values (n=)	6	6	8	9	13
Mean % Tumor Cells*	0.85	24.51	1.03	12.60	5.21
Std. Deviation	0.65	31.64	1.52	8.44	7.70
Std. Error	0.27	12.92	0.54	2.81	2.14
Statistical Significance (versus Vehicle control)***	--	--	p=0.13	--	p=0.05

B.	Peripheral Blood: Mean % Tumor Cells				
	Naïve** no cells injected	5 million MV4-11 Cells		10 million MV4-11 Cells	
		Vehicle	40mg/kg VII	Vehicle	40mg/kg VII
Number of values (n=)	6	4	8	9 (8)	12 (11)
Mean % Tumor Cells*	0.05	61.12	0.59	(44.68) 50.57	(2.80) 10.32
Std. Deviation	0.08	26.78	0.76	(30.99) 33.95	(4.76) 26.46

Std. Error	0.03	13.39	0.27	(10.96) 11.32	(1.44) 7.64
Statistical Significance (versus Vehicle control)***	--	--	p=0.02	--	(p=0.007) p=0.01

[0177] Numbers in () reflect values adjusted to exclude two suspected contaminated PB samples (n=1 in each group). These samples were noted to have milky white exudates while attempting blood collection by cardiocentesis and later noted to have tumors near heart or thymus on necropsy. The two suspected values showed 98 and 93% tumor cells in vehicle and 40 mg/kg **VII** groups (10 million cells), respectively.

[0178] *Mean % Tumor cells are defined as # of CD33+ HLA+ events / total events; where total events = # of murine CD45+ cells + # of CD33+ HLA+ events.

[0179] **Naïve samples were stained on 3 separate days among 3 separate studies. Values are reflective of the detection limit of assay, since mice were not injected with MV4-11 tumor cells.

[0180] ***Statistical significance was determined using an unpaired two-tailed Student's t test with Welch's correction to account for unequal variances in the data, even in the event that the sample size was too small for the Bartlett's test for equal variance.

[0181] These data demonstrate 96% and 59% reductions (5 and 10 million cell injected groups, respectively) in tumor burden in BM with **VII** treatment when compared to vehicle group and 99% and 94% reductions in tumor cell engraftment with **VII** treatment in the PB (5 and 10 million cell, respectively) when compared to vehicle group (Table 4).

[0182] Table 4: Engraftment Data Summary

Group ID	% Reduction from Vehicle Control	
	Bone Marrow	Peripheral blood
5 million cells, VII 40 mg/kg PO, bid daily	96	99
10 million cells, VII 40 mg/kg PO, bid daily	59	80 (94)

[0183] Numbers in () reflect values adjusted to exclude suspected contaminated samples (n=1 in each 10million cell PB group). These samples were noted to have milky white exudates while attempting blood collection by cardiocentesis and subsequently noted to have tumors near heart or thymus on necropsy. The two suspected values showed 98 and 93% tumor cells in vehicle and 40 mg/kg **VII** groups (10 million cells), respectively.

[0184] Pharmacokinetics of Compound **VII** in NOD-SCID Mice: Initial PK data in NOD-SCID mice was generated in an intial study prior to dosing in this study. Results indicate that dose-proportional increases in plasma levels of **VI** with 20, 40 or 80 mg/kg doses and exposures were limited to 2-4 hours post dose with plasma levels of **VI** virtually undetectable by 6 hours post-dose in all female mice. Males showed higher exposures with equivalent doses and longer duration of **VI** levels in the plasma when compared to females.

[0185] Due to precipitates observed in on-study formulations prepared in the initial study and other studies, additional PK data was obtained using remaining dosing solutions after study termination. NOD-SCID female mice (n=4) were dosed with 40 mg/kg dose solution of **VII** and plasma obtained at 1 hour post dose. As shown in Table 5, PK data using remaining formulations was moderately variable between the four animals with percent Coefficient of Variation (% CV) value of 25%. Plasma **VI** concentrations obtained for 1 hour exposures were similar to the values obtained in a previous PK study, indicating that these formulations were comparable.

[0186] Table 5A and 5B: PO administration of **VII** Calcium Salt: Mean Concentration of Compound **VI** in Plasma (ng/mL).

Table 5A					
Dose (mg/kg)	20	40	80	20	40
Group	1	2	3	4	5
Time (hrs)	Female	Female	Female	Male	Male
0.25	3717	5140	3847	1872	3587
0.5	2240	3597	7433	3377	2860
1	1737	5497	7267	3280	5250
2	1438	2823	8193	2130	6370
4	55.0	201	2529	1209	1730
6	13.0	105	465	454	1909

Table 5B

Dose (mg/kg)	20	40	80	20	40
Group	1	2	3	4	5
Parameter	Female	Female	Female	Male	Male
Dose, mg/kg	20	40	80	20	40
C _{-max}	3717	5497	8193	3377	6370
T _{-max}	0.25	1.00	2.00	0.50	2.00
AUC _{last} , ng*hr/mL	5351	11498	27013	10261	20831
AUC _{inf} , ng*hr/mL	5362	11625	27662	11434	27170
T _{1/2} , hrs	0.59	0.84	0.97	1.79	2.30

[0187] Table 6: PK Data in NOD-SCID Mice Using Remaining Dosing Solutions.

N=3 animals per group	
Dose =>	40 mg/kg VII
Average Conc. (ng/mL VI)	4210
Standard Deviation	1035
Coefficient of Variation (%)	25

7.3.3 Conclusions

[0188] Intravenous administration of MV4-11 tumor cells at 10 million cells per mouse resulted in greater dissemination of tumors to multiple anatomical sites in comparison to 5 million cells. Moreover, mice inoculated with 10 million cells more consistently had high levels of engraftment in the bone marrow and peripheral blood. Administration of 40 mg/kg **VII** to mice bearing disseminated MV4-11 tumors demonstrated marked efficacy when compared to vehicle treated mice with respect to overall survival and disease-related sequelae regardless of cell titer. Additionally, **VII** was well tolerated by NOD-SCID mice for greater than 60 days in this study and formulations used appeared adequate to achieve similar systemic exposures as in previous PK study.

[0189] In summary, **VII** treated mice reproducibly demonstrated a drug-related reduction in the number of total tumors identified, the total number of tumored animals, and in disease severity as measured by body weight loss, condition at necropsy, and other in-life clinical observations. Data from this study was similar to a previous efficacy study, showing

repeated prolongation of survival of MV4-11 bearing mice with **VII** treatment. Additionally, mice treated with **VII** demonstrated fewer detectable MV4-11 tumor cells in the bone marrow and peripheral blood when compared to vehicle controls. These data suggest that Syk/Flt-3 kinase inhibitors may be a valuable therapeutic for the treatment of leukemia patients bearing **FLT-3-ITD** or other leukemias associated with Flt-3 mutations.

7.4 Example 4: Inhibition of Constitutive Flt-3 Activity in Lysates from MV4-11 Tumor Grafts

[0190] Initial studies had shown that treatment of MV4-11 human AML cells resulted in the inhibition of constitutive phosphorylation of Flt-3. Given these data, the phosphorylation of Flt-3 versus total Flt-3 was assessed *ex vivo* in tumor cell lysates from randomly selected mice from this study using immunoprecipitation (IP) of human Flt-3 and subsequent Western blot analysis of phosphorylated Flt-3 was carried out as follows.

7.4.1 Experimental Protocol

[0191] Preparing Tumor Lysates.

1. Harvest the tumor as soon as possible.
2. Pulverize frozen tumor in liquid nitrogen.
3. Weigh and normalize in 250mg/1ml ice-cold RIPA buffer (Santa Cruz Biotechnology, sc-24948) containing protease inhibitor and phosphatase inhibitor cocktail(Sigma, P5726, 1ml/100ml lysis buffer).
4. Homogenize on ice.

Microcentrifuge and collect the clear supernatant which is the tumor lysate.

[0192] BCA Assay to measure the protein concentration.

BCA assay kit was obtained from Pierce Prod#23227)

1. Preparation of BSA standard.:
2000ug/ml→1500ug/ml→1000ug/ml→750ug/ml→500ug/ml→250ug/ml→125
ug/ml→25ug/ml
2. Pre-dilute test sample with cold PBS (tumor lysate 1:20 dilution)
3. 200ul BCA working solution + 25ul standard/sample, incubate at 37°C for 30 min.
4. Cool plate and read OD 570nm.

[0193] Immunoprecipitation.

1. Take 1mg tumor lysate and add primary antibody 2ug (Anti-Flt-3 S-18, Santa Cruz, #sc-480) adjust volume to 500ul with lysis buffer. Incubate with gentle rocking for 1h at 4°C.
2. Add protein A/G plus-agarose beads (Santa Cruz, SC-2003) 40ul. Incubate with gentle rocking for 1h at RT.
3. Microcentrifuge for 30 second at 4°C. Wash pellet three times with 500ul of 1X lysis buffer. Keep on ice during washes.
4. Resuspend the pellet with 30ul 2X Tris-Glycine SDS loading buffer + 5ul reducing agent 10X (Invitrogen).
5. Heat the sample to 95-100°C for 5 minutes.
6. Store the sample at -80°C

[0194] Western Blot Procedure.

1. Load 20ul of the sample on 8% Tris-Glycine gel.
2. Run in 1X Tris-glycine SDS running buffer.
3. Transfer to PVDF membrane in 1X Tris-Glycine transfer buffer .
4. Block in 1% BSA/TBST (TBS + 0.1% Tween-20) at RT for 1h.
5. Add primary antibody 1:5000 overnight at 4°C.
Anti-Phosphotyrosine clone 4G10 (mouse monoclonal, Upstate, #05-321).
Anti-Phospho-Flt-3 (Tyr591) (mouse mAb, Cell Signaling, #3466S)
6. Wash 2h in TBST.
7. 2°Ab anti-mouse IgG-HRP (Amersham, NA931V) 1:5000 in 5% milk/ TBST (Blotting grade Blocker, non-fat dry milk, Bio-Rad, #170-6404) for 1h.
ECL-plus (Amersham, RPN2132)short exposure.

[0195] Stripping and reprobing membranes.

1. Submerge the membrane in stripping buffer (Pierce, #21062) and incubate 37°C for 1h.
2. Wash the membrane in TBST for 15 mins, twice.
3. Block the membrane with 1% BSA/ TBST for 1h.
4. Add anti-Flt-3 1:5000 (Santa Cruz, #sc-480) in 5% milk/TBST, incubate overnight at 4°C.

5. Wash 2h in TBST.
6. 2°Ab anti-rabbit IgG-HRP (Amersham, NA934V) 1:5000 in 5% milk/ TBST at RT for 1h .
7. ECL-plus short exposure.

[0196] In addition, the blot was reprobed for total Flt-3 levels. Constitutive phosphorylation of Flt-3 was reduced in tumors from 40mg/kg **VII** treated animals in a dose-dependent manner when compared to vehicle treated controls..

7.4.2 Phosphorylated Histone H3 Analysis of MV4-11 Tumor Xenografts.

[0197] Proliferation was assessed ex vivo in formalin fixed tumor sections from three randomly selected mice from this study using immunohistochemical staining of human-specific phosphorylated histone H3 (phH3) as a marker for tumor cell proliferation. Human phH3 expression was reduced in tumor sections in a dose-dependent manner following treatment with **VII**. When compared to MV4-11 tumor xenografts from vehicle treated mice, treatment with 20 and 40mg/kg **VII** resulted in a 53% and 71% inhibition of phH3 staining, respectively. These data indicate that **VII** inhibition reduced the proliferative capacity of MV4-11 tumors in vivo, correlating with the reduced tumor volumes observed during the in-life portion of the study. The reduced proliferation is likely to be due to reduced constitutive Flt-3 phosphorylation, as this activity has been shown to be required for survival of MV4-11 cells in vitro.

7.4.3 Inhibition of Downstream Signaling Events

[0198] Additional immunohistochemical staining of tumor sections from mice treated for 26 days with 40mg/kg **VII** demonstrated reduced STAT5 and ERK1/2 phosphorylation, which are downstream molecules in the Flt-3:ITD signaling pathway in MV4-11 cells (FIG. 11).

7.5 Example 5: Examination of Compound **VI** on Acute Myelogenous Leukemic Cells and Comparison to Kinase Inhibitor AG1296

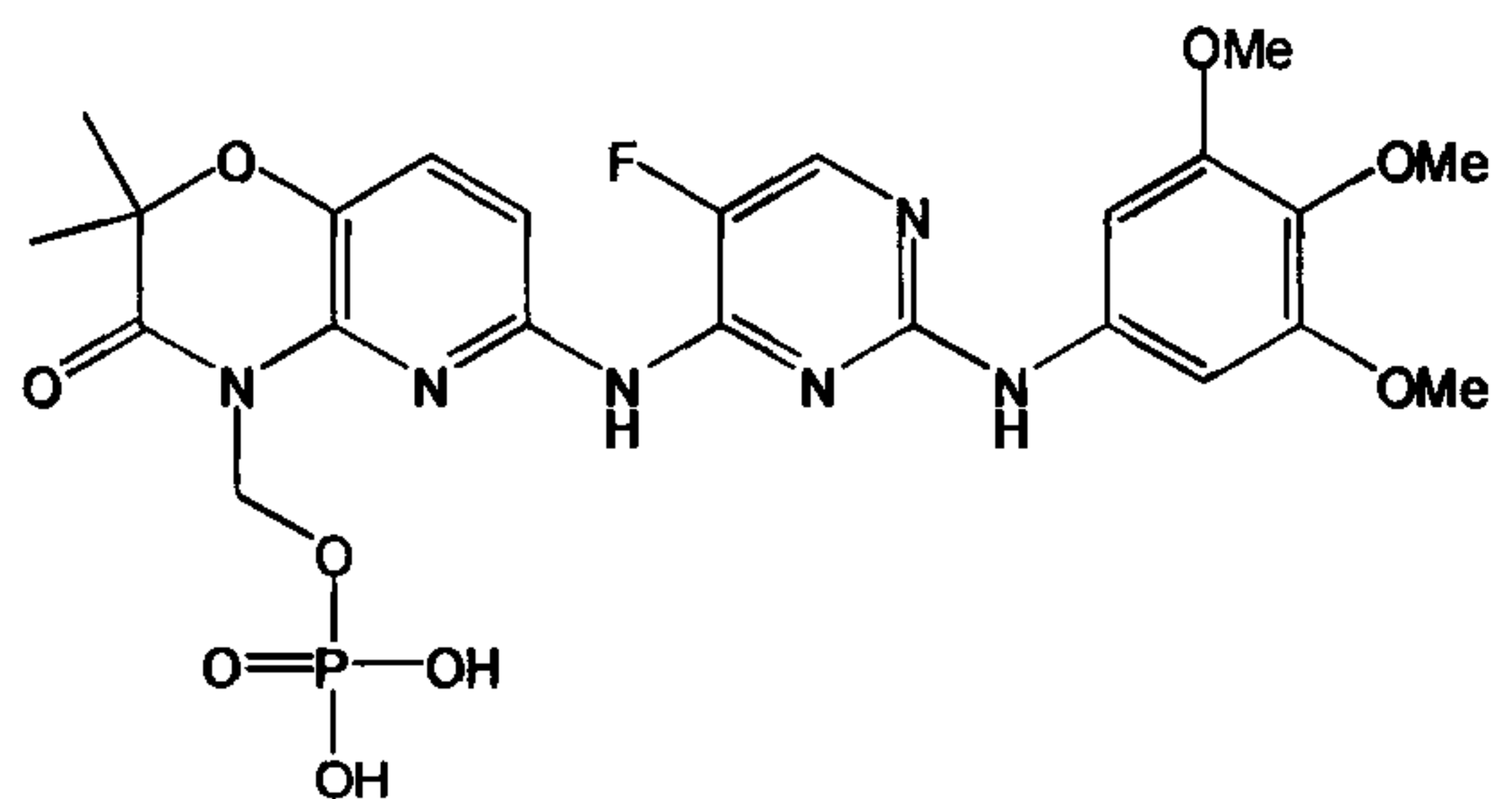
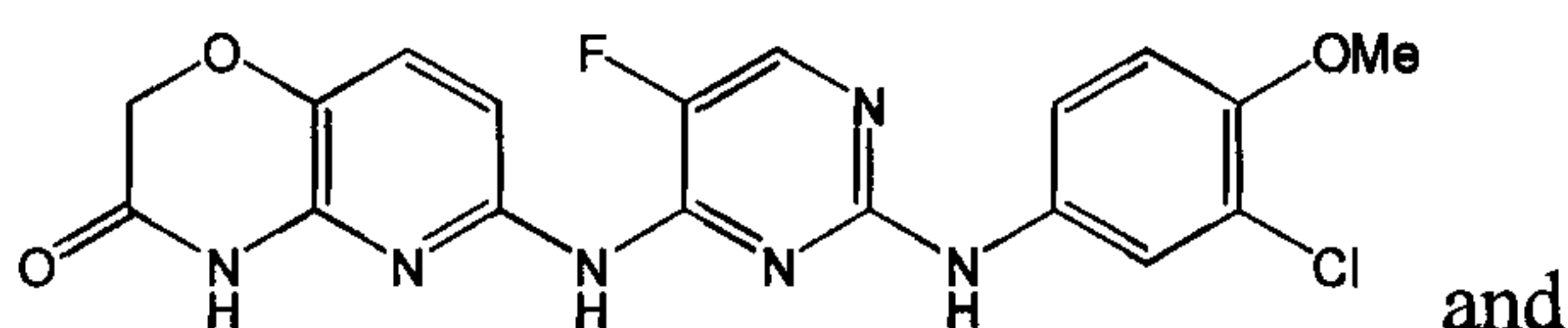
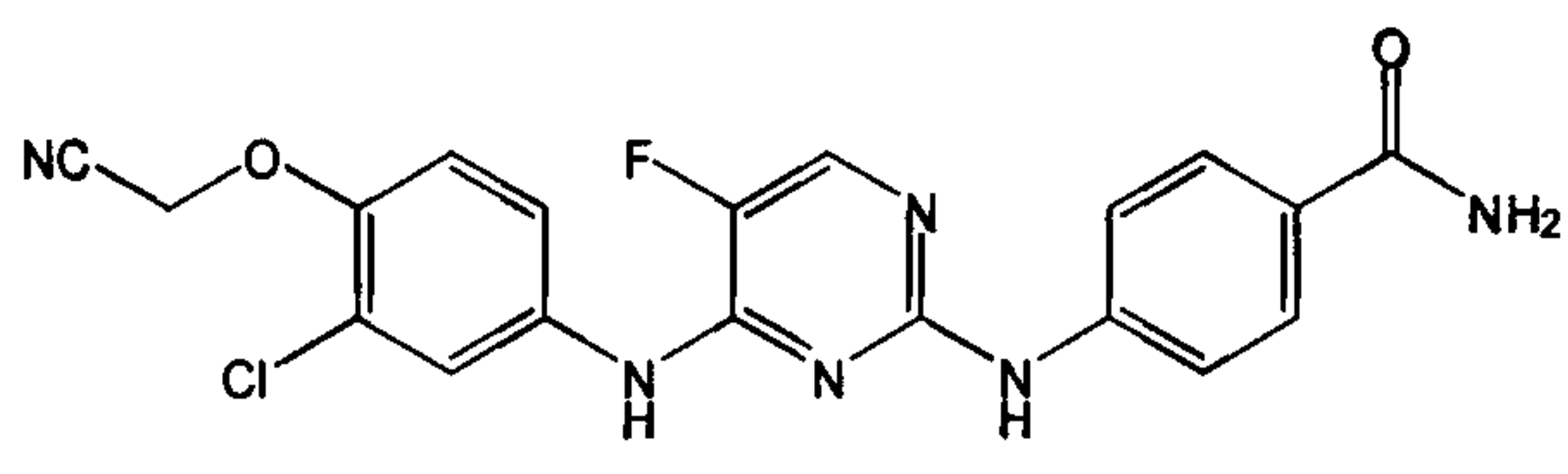
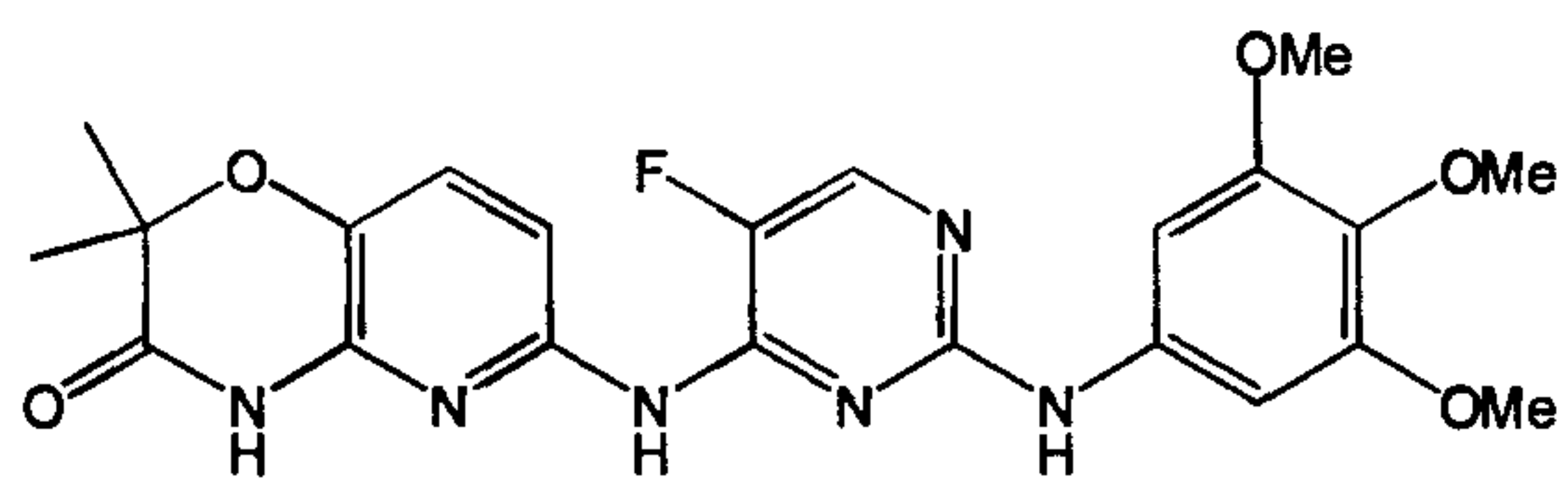
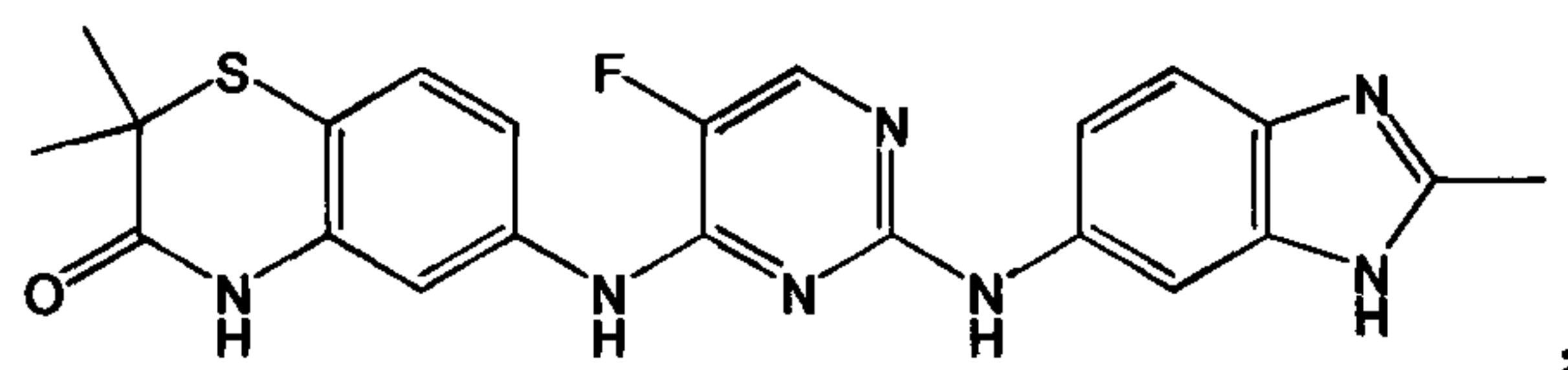
[0199] AML cells or 32D transfectants were treated for 48 h (AML) or 24 h (32D) with 1 uM **VI**, and the increase in % of apoptotic cells was determined using Annexin V and PI staining. The data show that AML cells harboring Flt-3 ITD are uniquely sensitive to **VI**-induced

apoptosis. Similarly, 32D transfected with ITD or point mutated Flt-3 TDK undergo more apoptosis at 24 h than 32D cells transfected with wt Flt-3.

[0200] The foregoing descriptions of specific embodiments of the present invention have been presented for purposes of illustration and description. They are not intended to be exhaustive or to limit the invention to the precise forms disclosed, and obviously many modifications and variations are possible in light of the above teaching. The embodiments were chosen and described in order to best explain the principles of the invention and its practical application, to thereby enable others skilled in the art to best utilize the invention and various embodiments with various modifications as are suited to the particular use contemplated. It is intended that the scope of the invention be defined by the Claims appended hereto and their equivalents.

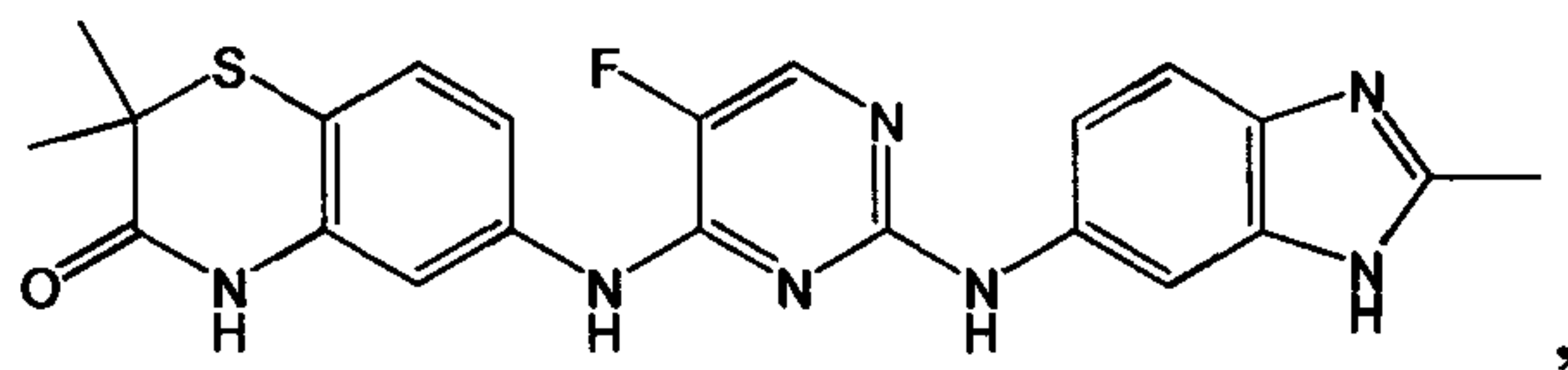
THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. Use of a therapeutically effective amount of a compound selected from the group consisting of



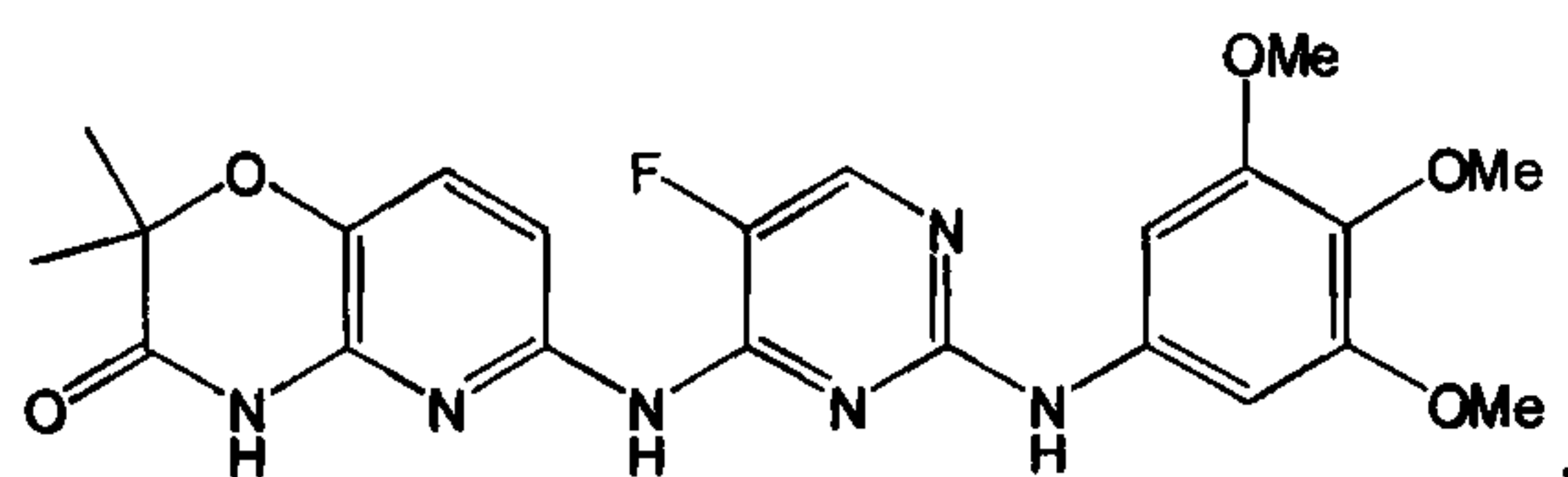
and salts, N-oxides, hydrates and solvates thereof, in treatment of a cell proliferative disorder.

2. The use according to claim 1 in which the compound is



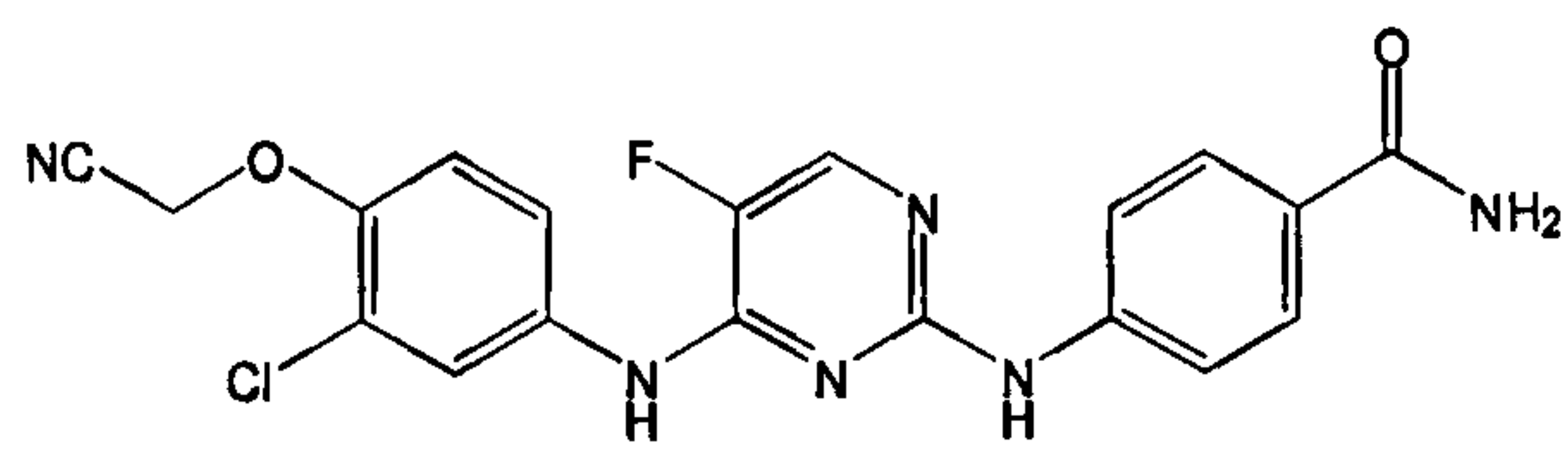
or a salt, hydrate, solvate or N-oxide thereof.

3. The use according to claim 1 in which the compound is



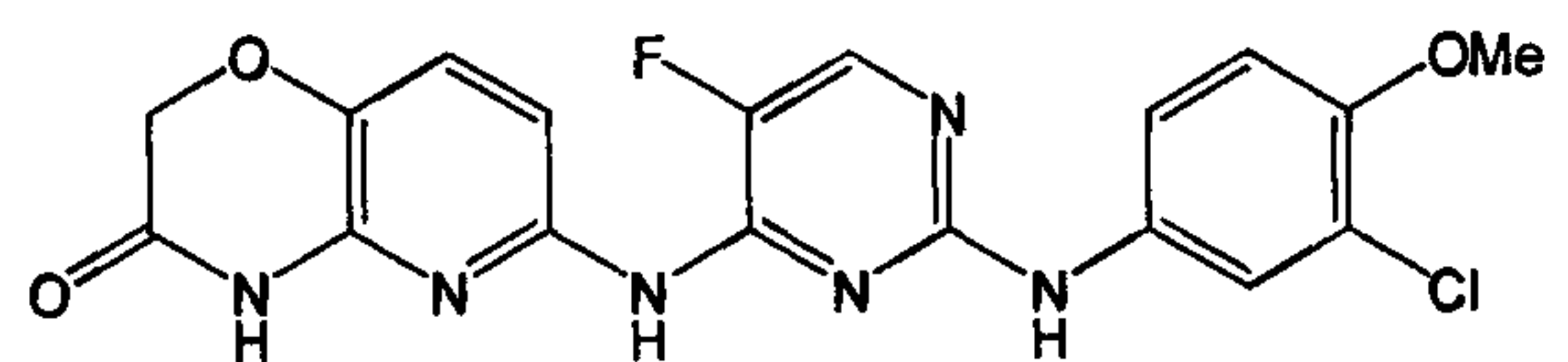
or a salt, hydrate, solvate or N-oxide thereof.

4. The use according to claim 1 in which the compound is



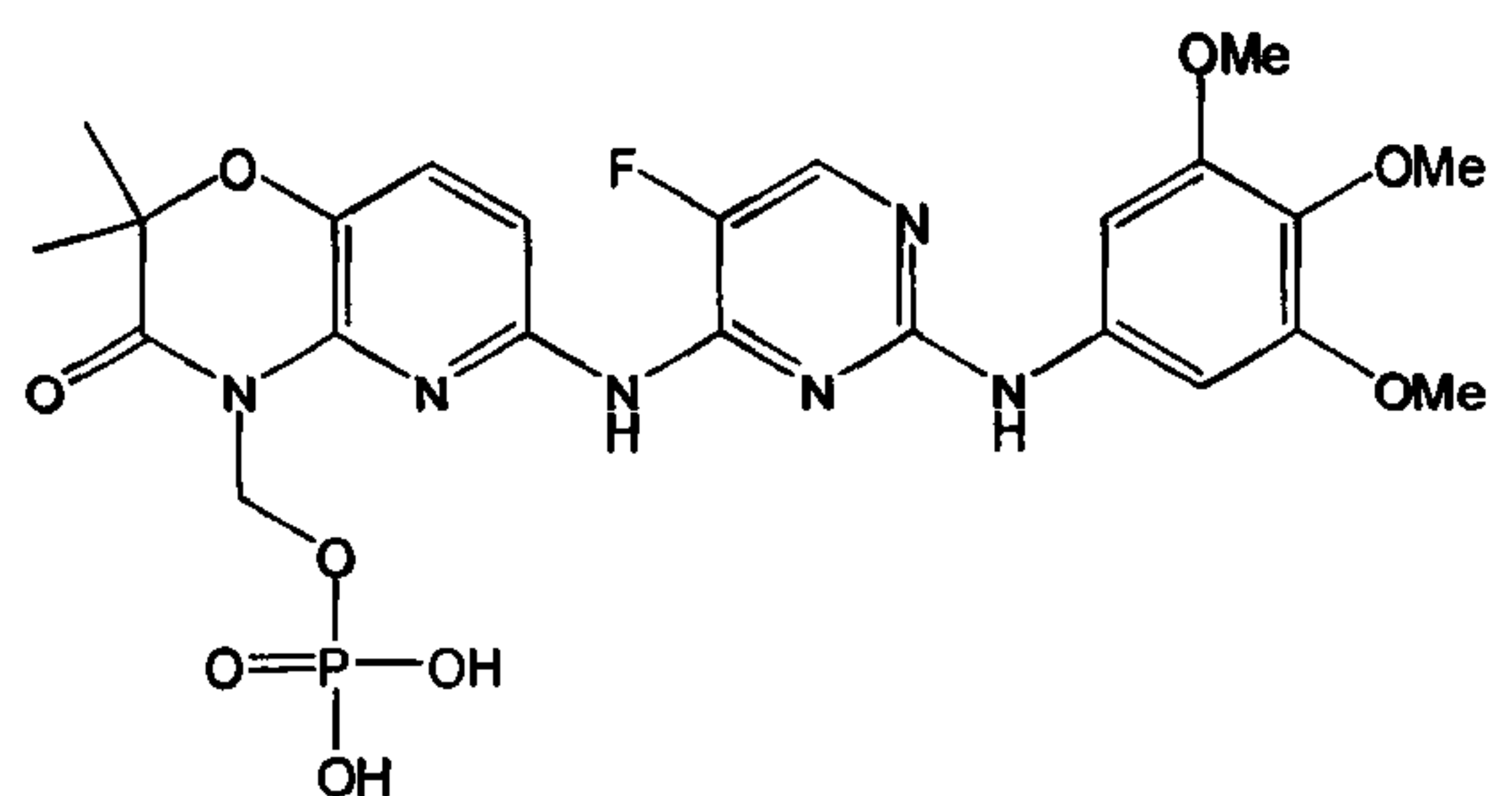
or a salt, hydrate, solvate or N-oxide thereof.

5. The use according to claim 1 in which the compound is



or a salt, hydrate, solvate or N-oxide thereof.

6. The use according to claim 1 in which the compound is



or a salt, hydrate, solvate or N-oxide thereof.

7. The use according to any of claims 1 to 6 in which the cell proliferative disorder is a hematopoietic neoplasm.

8. The use according to claim 7 in which the hematopoietic neoplasm is a lymphoid

neoplasm.

9. The use according to claim 8 in which the lymphoid neoplasm is a T cell neoplasm.

10. The use according to claim 9 in which the T cell neoplasm is T lymphoblastic leukemia.

11. The use according to claim 8 in which the lymphoid neoplasm is a B cell neoplasm.

12. The use according to claim 11 in which the B cell neoplasm is B-lymphoblastic leukemia.

13. The use according to claim 11 in which the B cell neoplasm is Burkitt's lymphoma.

14. The use according to any one of claims 1 to 6 in which the cell proliferative disorder is a myeloid neoplasm.

15. The use according to claim 14 in which the myeloid neoplasm is a myeloproliferative disease.

16. The use according to claim 15 in which the myeloproliferative disease is chronic myelogenous leukemia (CML).

17. The use according to claim 14 in which the myeloid neoplasm is myelodysplastic disease.

18. The use according to claim 17 in which the myelodysplastic disease is chronic myelomonocytic leukemia.

19. The use according to claim 14 in which the myeloid neoplasm is myelodysplastic syndrome.

20. The use according to claim 14 in which the myeloid neoplasm is acute myeloid leukemia.
21. The use according to claim 20 in which the acute myeloid leukemia is associated with activity of a TEL/Syk fusion protein.
22. The use according to claim 7 in which the hematopoietic neoplasm is selected from acute myelogenous leukemia, B-precursor cell acute lymphoblastic leukemia, T-cell acute lymphoblastic leukemia, myelodysplastic syndrome, and chronic myelogenous leukemia.
23. The use according to any one of claims 1 to 6 in which the proliferative disorder is a virally-mediated tumor arising from modulation of Syk kinase activity.
24. The use according to claim 23 in which the virally mediated tumor is associated with a virus encoding an ITAM motif.
25. The use according to claim 23 in which the virally mediated tumor is associated with Kaposi's sarcoma associated herpes virus.
26. The use according to claim 25 in which the virally mediated tumor is associated with the activity of K1 protein of Kaposi's sarcoma associated herpes virus.
27. The use according to claim 23 in which the virally mediated tumor is associated with Epstein Barr Virus.
28. The use according to claim 27 in which the virally mediated tumor is associated with the activity of LMP2A protein of the Epstein Barr Virus.
29. The use according to claim 23 in which the virally mediated tumor is associated with

HTLV-1 virus.

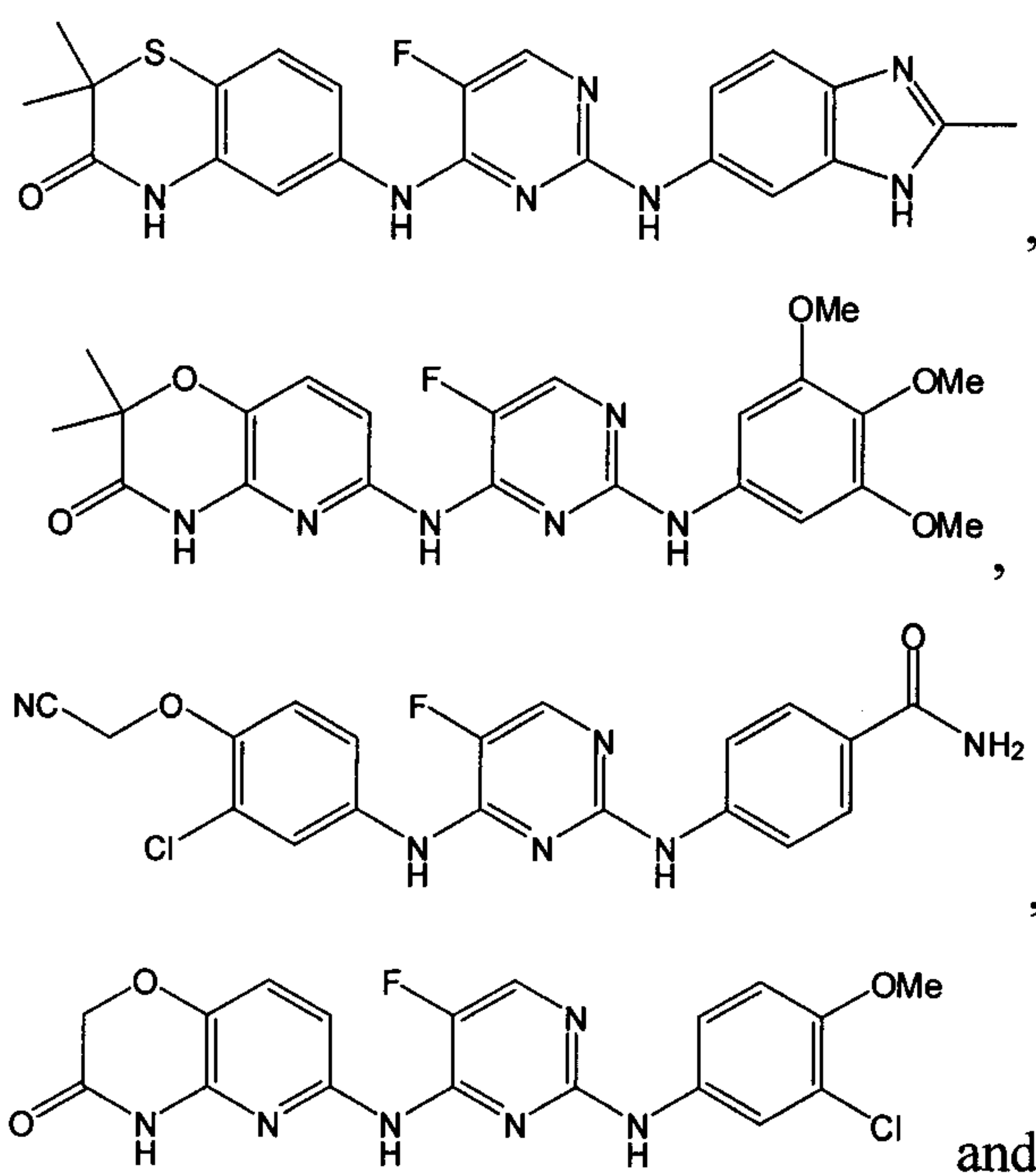
30. The use according to any one of claims 1 to 6 , further comprising use of an Abl kinase inhibitor.

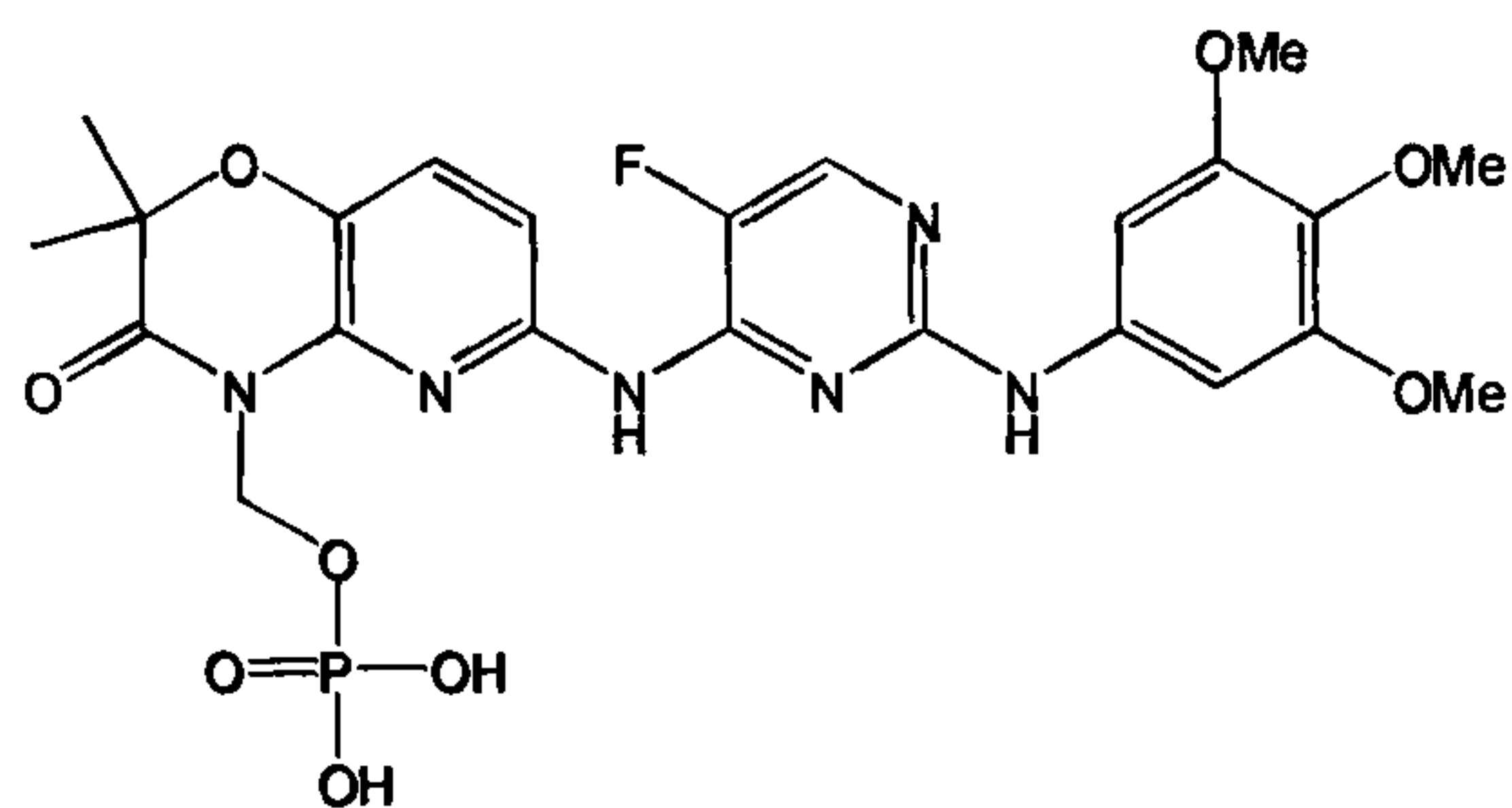
31. The use according to claim 30 in which the Abl kinase inhibitor is 2-phenylaminopyrimidine.

32. The use according to claim 30 in which said use of said compound and said use of said Abl kinase inhibitor is sequential.

33. The use according to claim 30 in which said use of said compound and said use of said Abl kinase inhibitor is simultaneous.

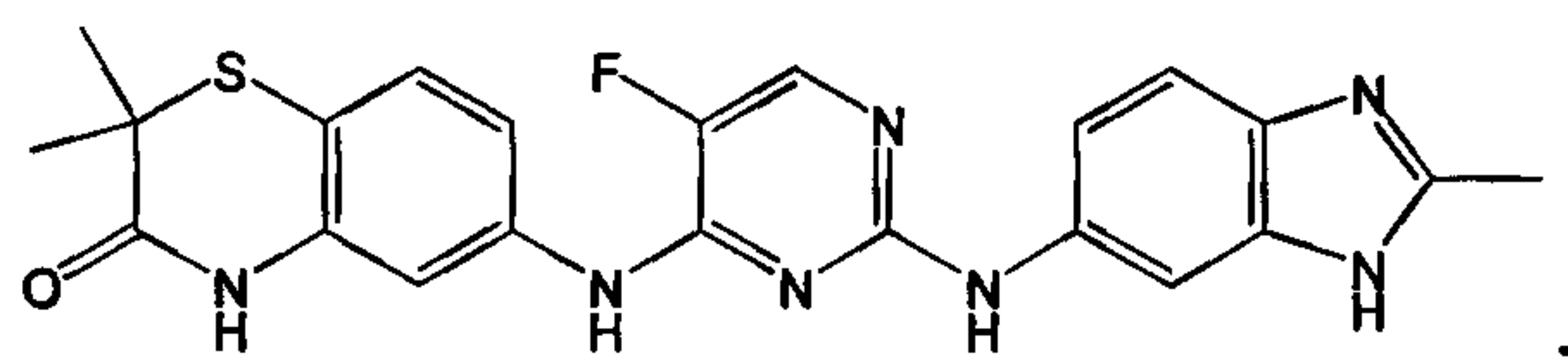
34. Use of a therapeutically effective amount of a compound selected from the group consisting of





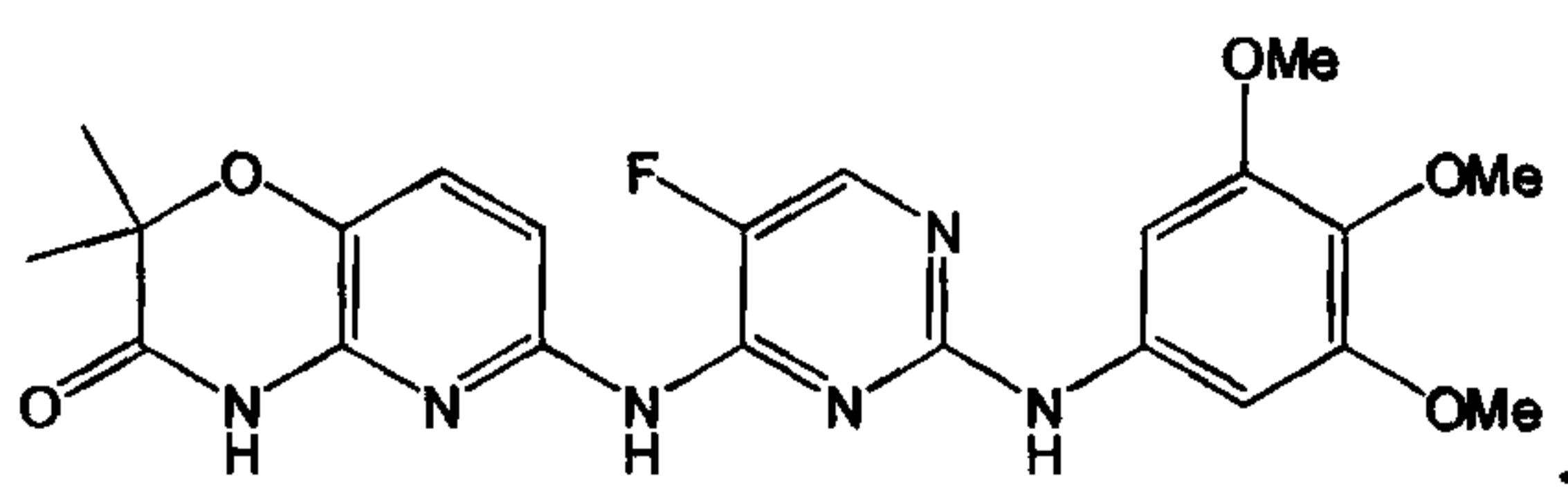
and salts, N-oxides, hydrates and solvates thereof in inhibition of tumor metastasis.

35. The use according to claim 34 in which compound is



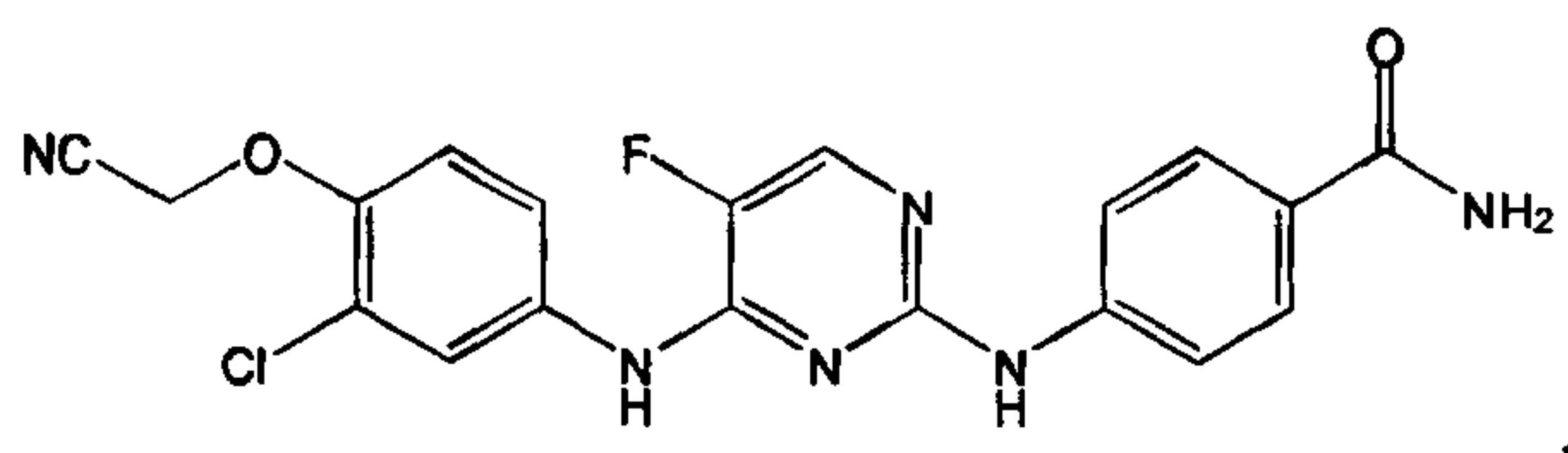
or a salt, hydrate, solvate or N-oxide thereof.

36. The use according to claim 34 in which the compound is



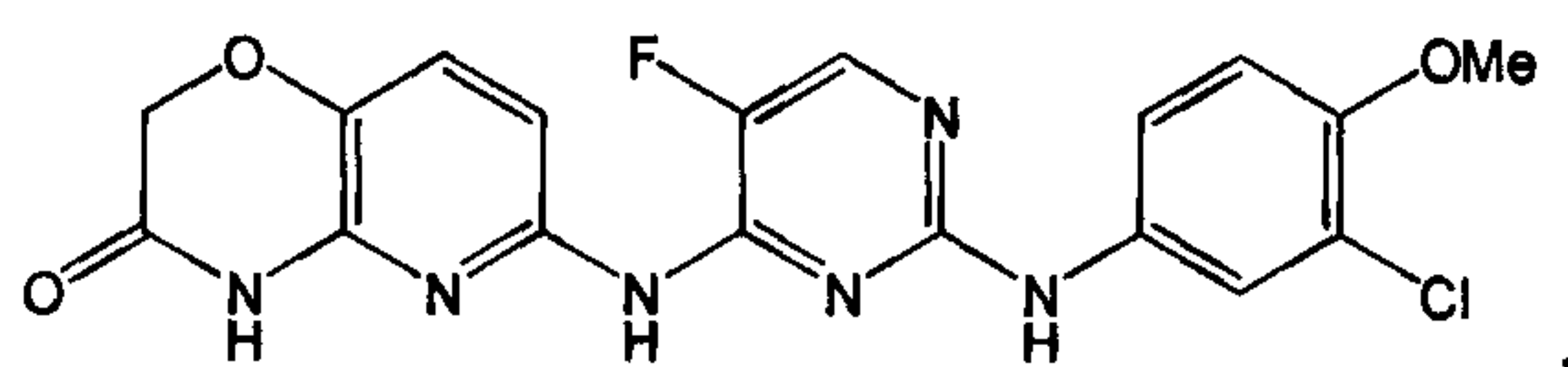
or a salt, hydrate, solvate, or N-oxide thereof.

37. The use according to claim 34 in which the compound is



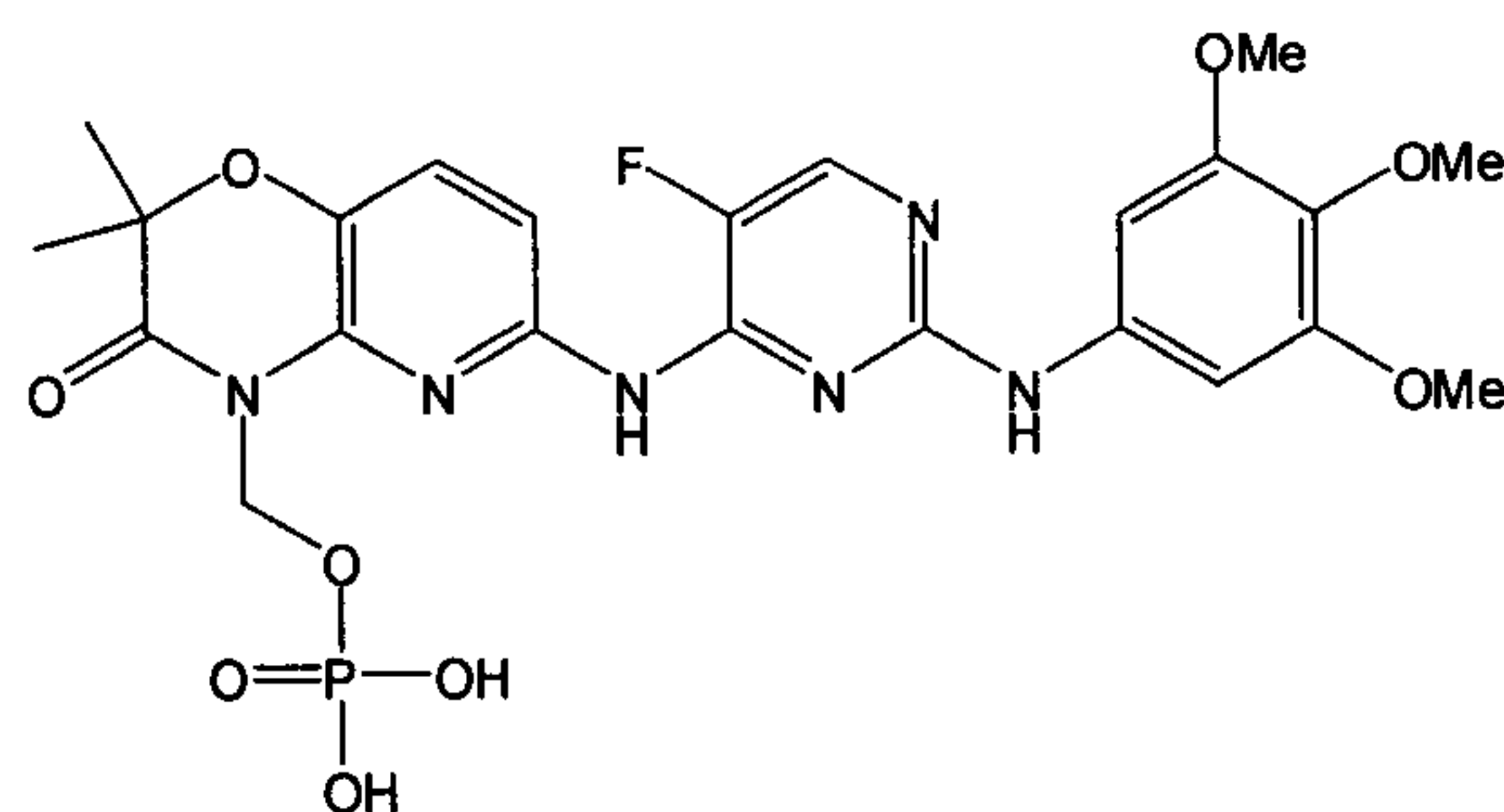
or a salt, hydrate, solvate, or N-oxide thereof.

38. The use according to claim 34 in which the compound is



or a salt, hydrate, solvate or N-oxide thereof.

39. The use according to claim 34 in which the compound is



or a salt, hydrate, solvate or N-oxide thereof.

40. The use according to any one of claims 34 to 39 in which the tumor is selected from breast cancer, ovarian cancer, renal cancer, gastrointestinal cancer, kidney cancer, bladder cancer, pancreatic cancer, lung squamous carcinoma, and adenocarcinoma.

41. The use according to claim 40 in which said use is prior to diagnosis of tumor metastasis.

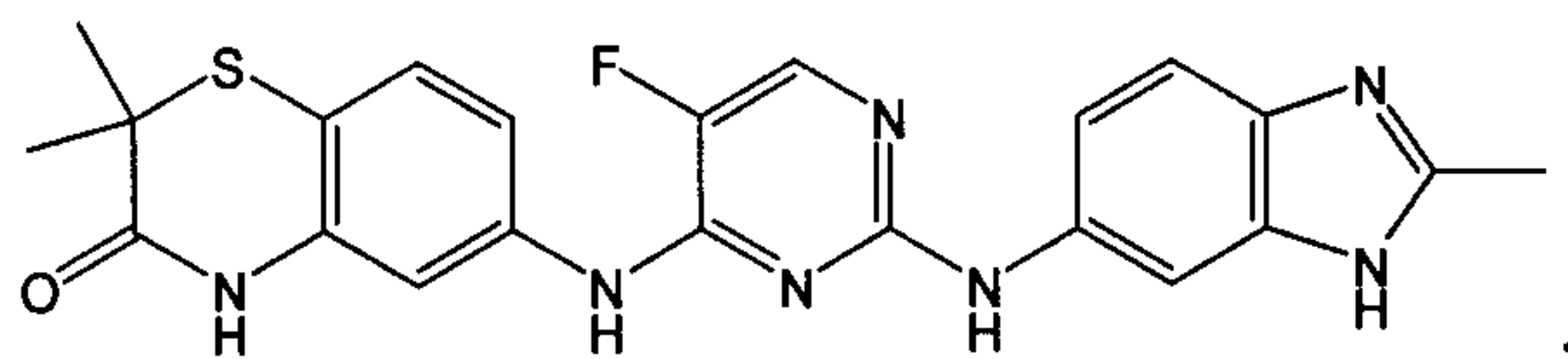
42. The use according to claim 40 in which the metastasis is caused by integrin signalling through Syk.

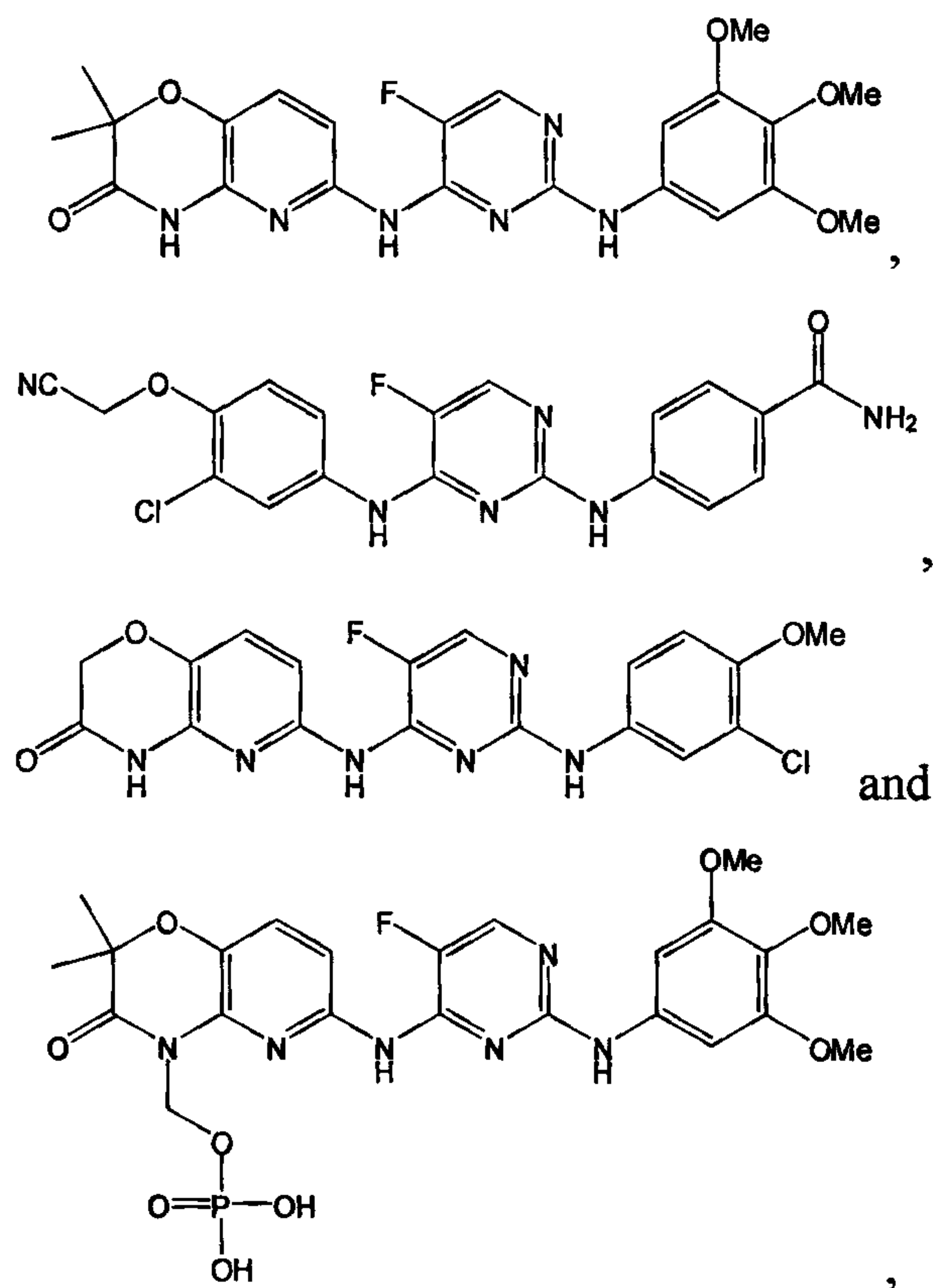
43. The use according to claim 42 in which the metastasis is caused by integrin signalling by $\beta 1$ integrins.

44. The use according to claim 44 in which the metastasis is caused by integrin signalling by $\beta 2$ integrins.

45. The use according to claim 44 in which the metastasis is caused by integrin signalling by $\beta 3$ integrins.

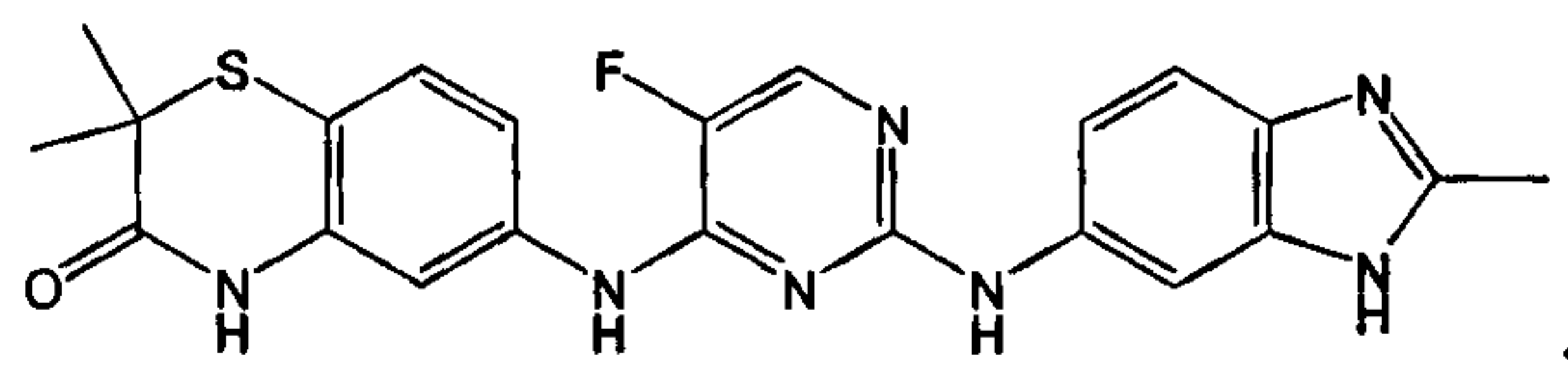
46. Use of a compound selected from the group consisting of





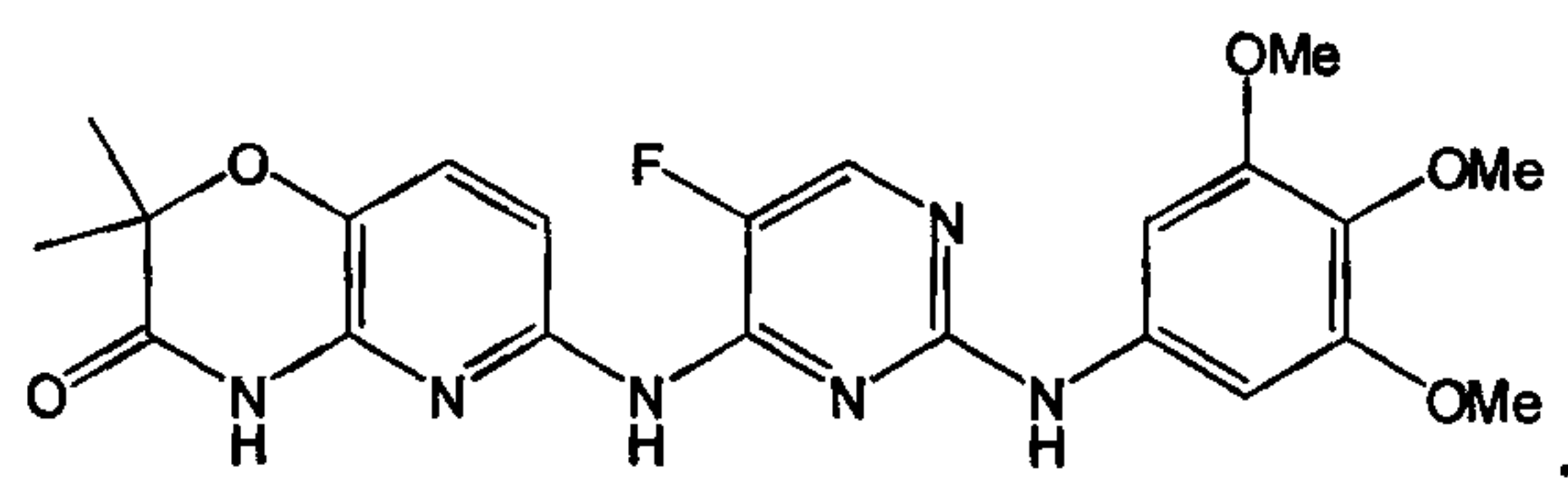
and salts, N-oxides, hydrates and solvates thereof, in the preparation of a medicament for treatment of a cell proliferative disorder.

47. The use according to claim 46 in which the compound is



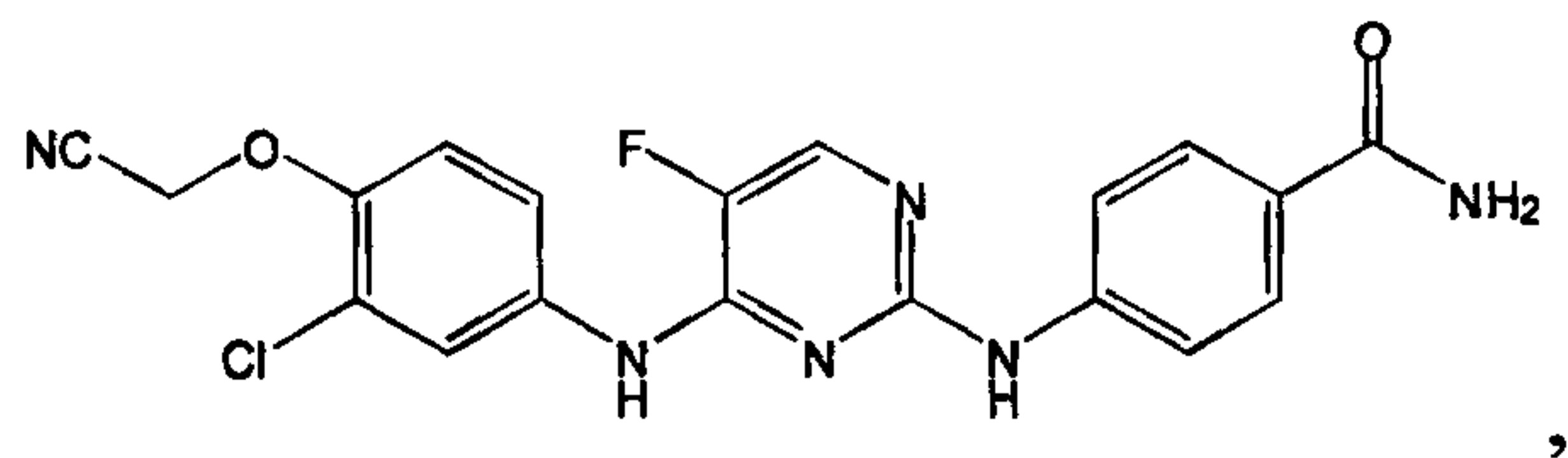
or a salt, hydrate, solvate or N-oxide thereof.

48. The use according to claim 46 in which the compound is



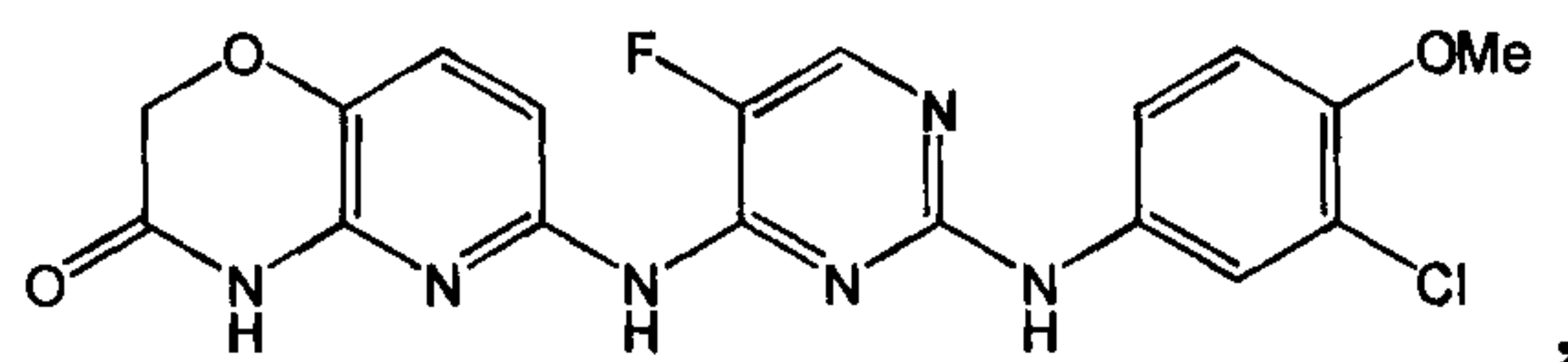
or a salt, hydrate, solvate or N-oxide thereof.

49. The use according to claim 46 in which the compound is



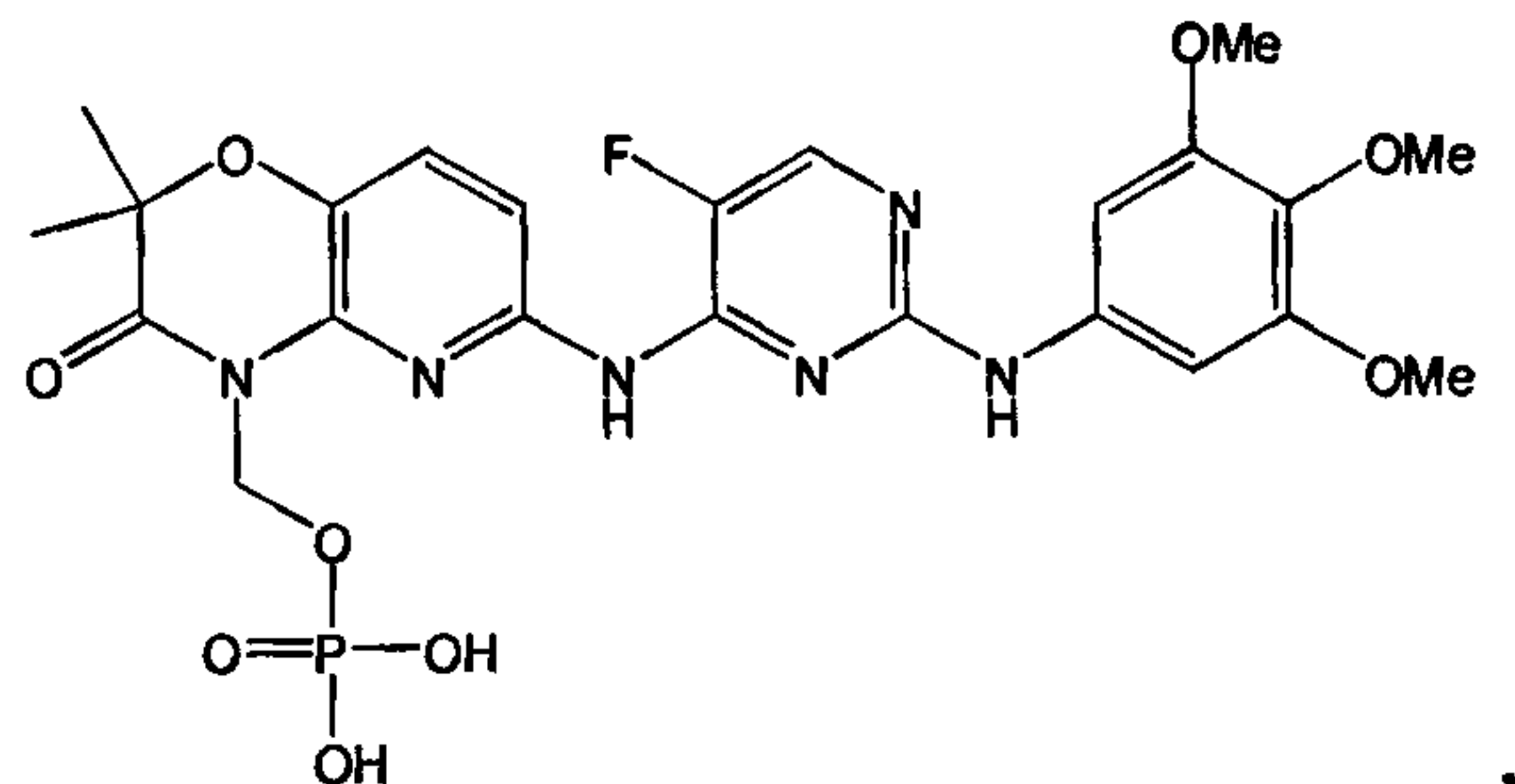
or a salt, hydrate, solvate or N-oxide thereof.

50. The use according to claim 46 in which the compound is



or a salt, hydrate, solvate or N-oxide thereof.

51. The use according to claim 46 in which the compound is



or a salt, hydrate, solvate or N-oxide thereof.

52. The use according to any of claims 46 to 51 in which the cell proliferative disorder is a hematopoietic neoplasm.

53. The use according to claim 52 in which the hematopoietic neoplasm is a lymphoid neoplasm.

54. The use according to claim 53 in which the lymphoid neoplasm is a T cell neoplasm.

55. The use according to claim 54 in which the T cell neoplasm is T lymphoblastic leukemia.

56. The use according to claim 53 in which the lymphoid neoplasm is a B cell neoplasm.

57. The use according to claim 56 in which the B cell neoplasm is B-lymphoblastic leukemia.
58. The use according to claim 56 in which the B cell neoplasm is Burkitt's lymphoma.
59. The use according to any one of claims 46 to 51 in which the cell proliferative disorder is a myeloid neoplasm.
60. The use according to claim 59 in which the myeloid neoplasm is a myeloproliferative disease.
61. The use according to claim 59 in which the myeloproliferative disease is chronic myelogenous leukemia (CML).
62. The use according to claim 59 in which the myeloid neoplasm is myelodysplastic disease.
63. The use according to claim 62 in which the myelodysplastic disease is chronic myelomonocytic leukemia.
64. The use according to claim 59 in which the myeloid neoplasm is myelodysplastic syndrome.
65. The use according to claim 59 in which the myeloid neoplasm is acute myeloid leukemia.
66. The use according to claim 65 in which the acute myeloid leukemia is associated with activity of a TEL/Syk fusion protein.
67. The use according to claim 52 in which the hematopoietic neoplasm is selected from

acute myelogenous leukemia, B-precursor cell acute lymphoblastic leukemia, T-cell acute lymphoblastic leukemia, myelodysplastic syndrome, and chronic myelogenous leukemia.

68. The use according to any one of claims 46 to 51 in which the proliferative disorder is a virally-mediated tumor arising from modulation of Syk kinase activity.

69. The use according to claim 68 in which the virally mediated tumor is associated with a virus encoding an ITAM motif.

70. The use according to claim 68 in which the virally mediated tumor is associated with Kaposi's sarcoma associated herpes virus.

71. The use according to claim 70 in which the virally mediated tumor is associated with the activity of K1 protein of Kaposi's sarcoma associated herpes virus.

72. The use according to claim 68 in which the virally mediated tumor is associated with Epstein Barr Virus.

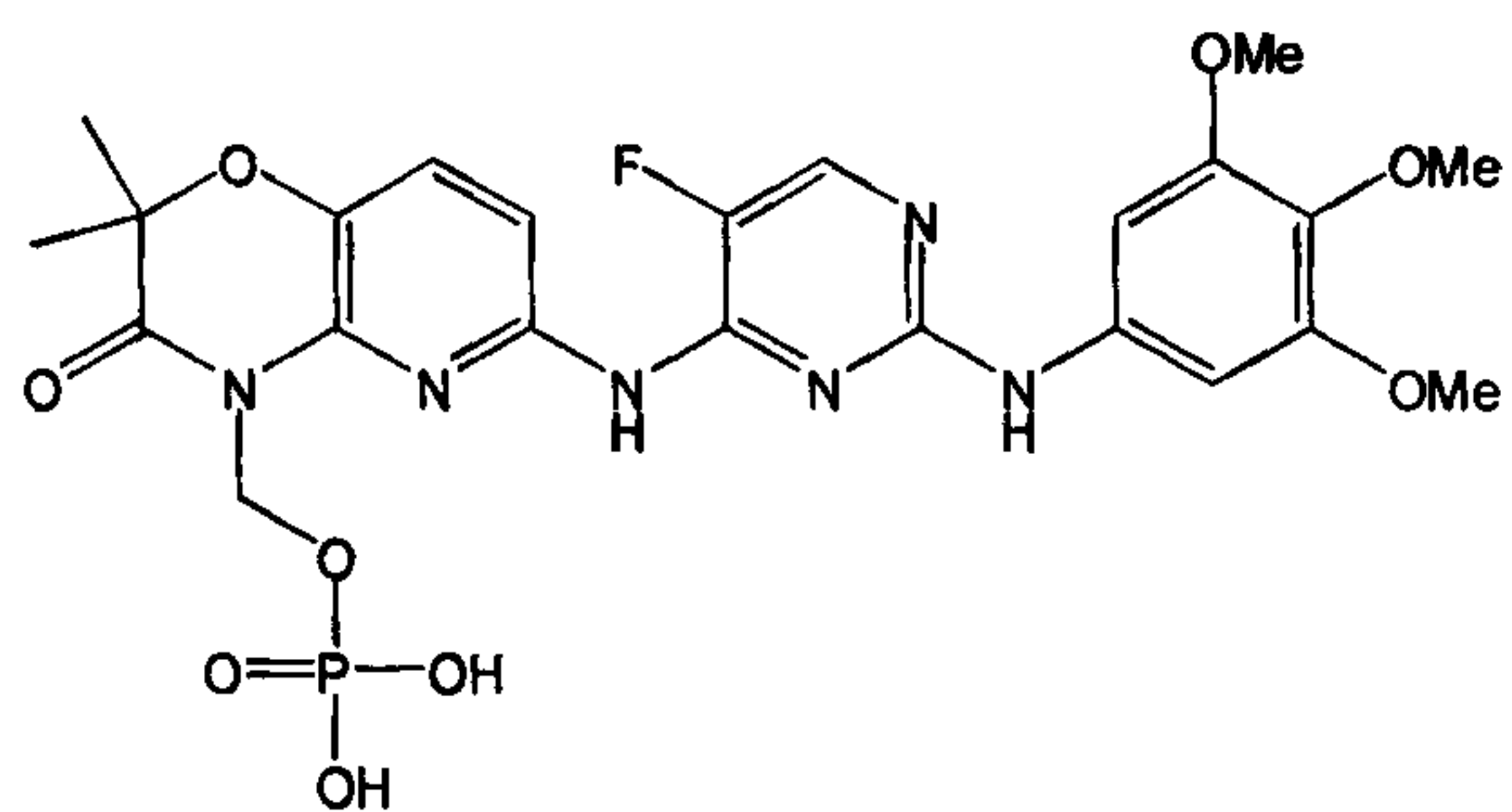
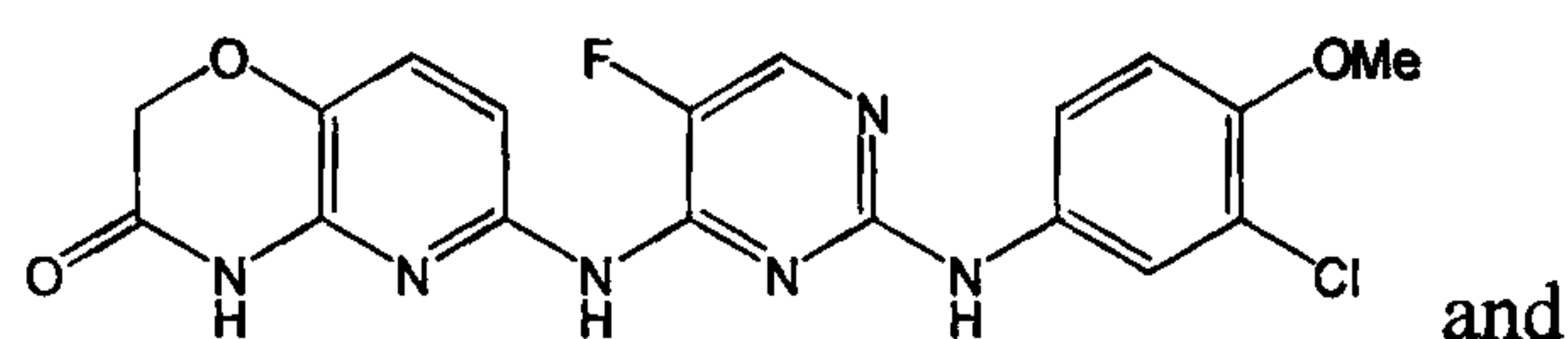
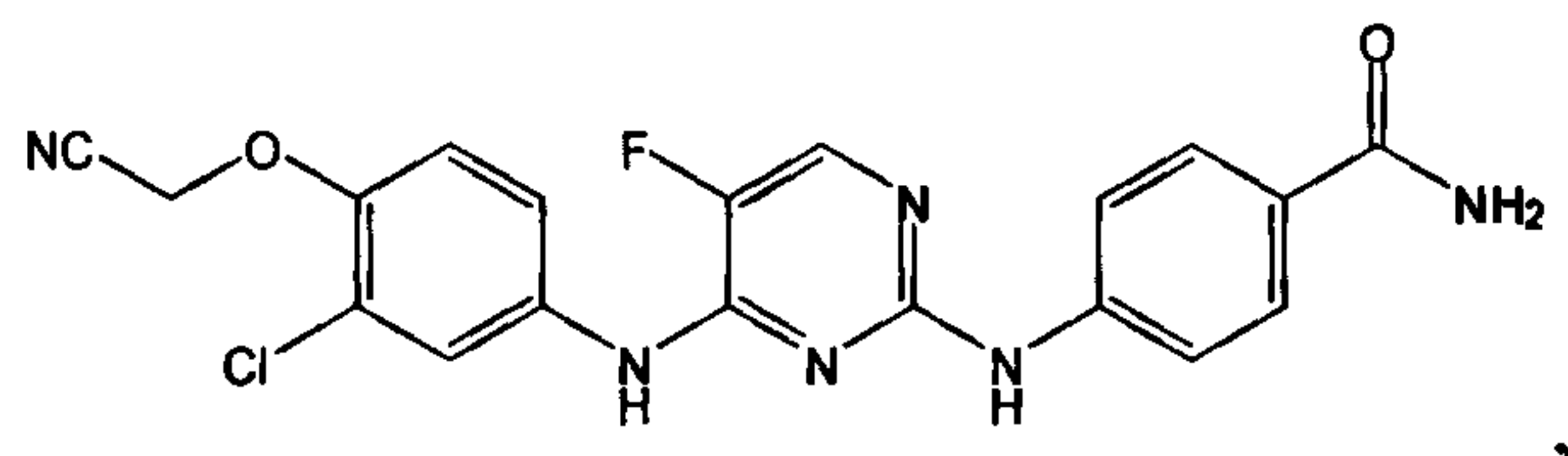
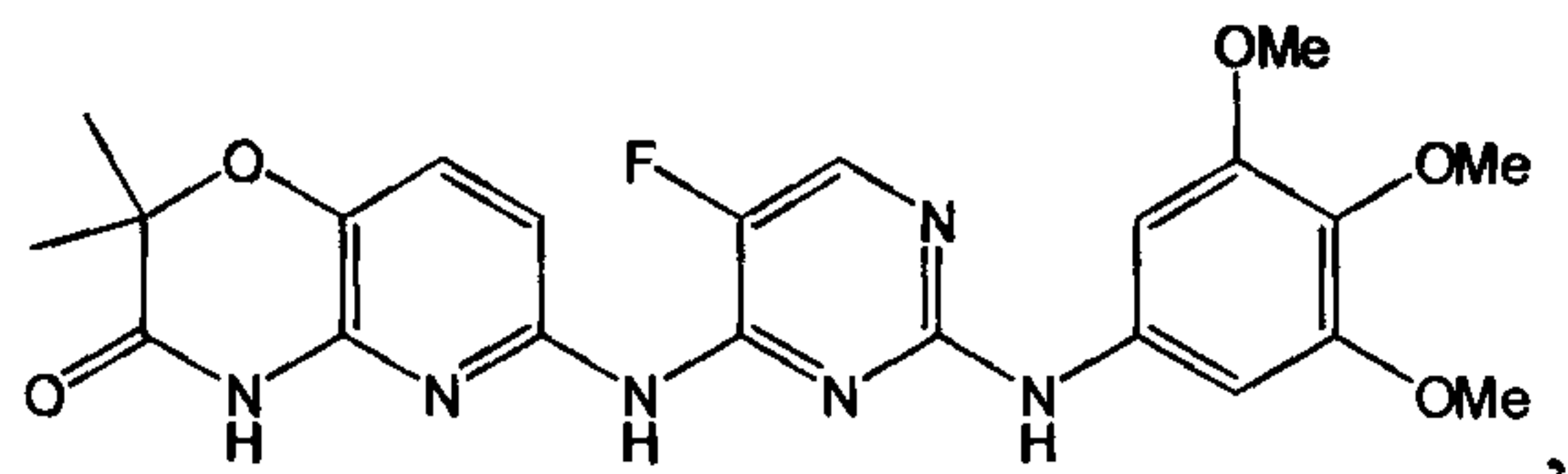
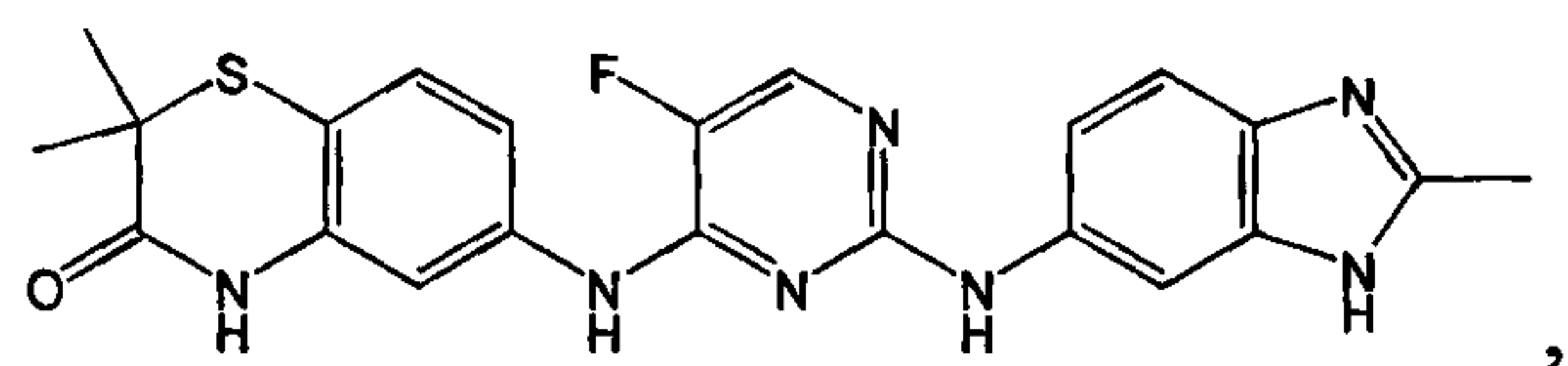
73. The use according to claim 72 in which the virally mediated tumor is associated with the activity of LMP2A protein of the Epstein Barr Virus.

74. The use according to claim 68 in which the virally mediated tumor is associated with HTLV-1 virus.

75. The use according to any one of claims 46 to 51, further comprising use of an Abl kinase inhibitor.

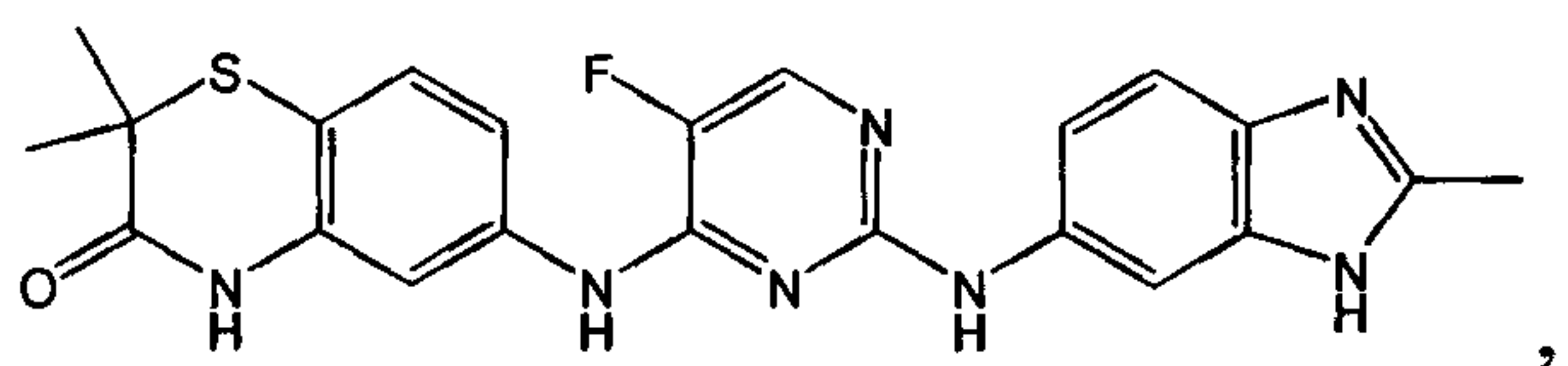
76. The use according to claim 75 in which the Abl kinase inhibitor is 2-phenylaminopyrimidine.

77. Use of a compound selected from the group consisting of



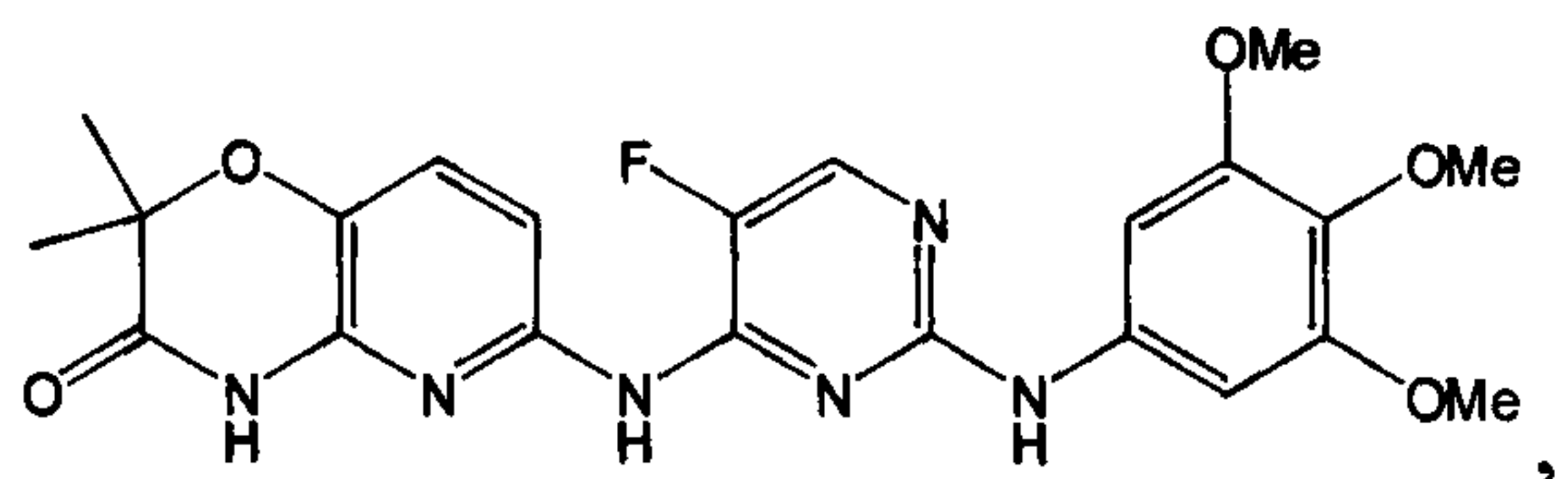
and salts, N-oxides, hydrates and solvates thereof in the manufacture of a medicament for inhibition of tumor metastasis.

78. The use according to claim 77 in which compound is



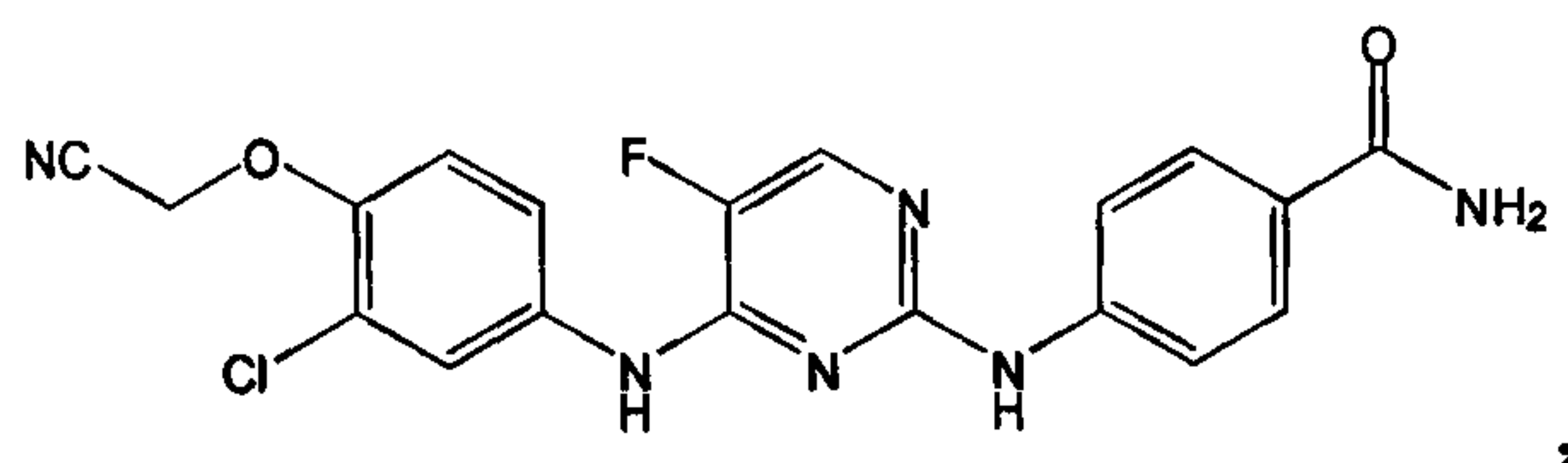
or a salt, hydrate, solvate or N-oxide thereof.

79. The use according to claim 77 in which the compound is



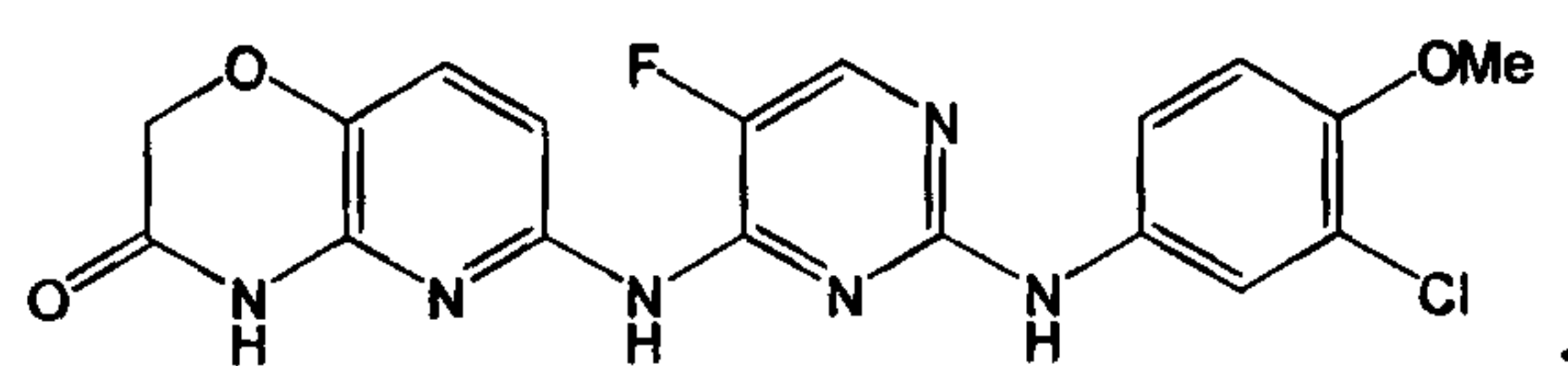
or a salt, hydrate, solvate, or N-oxide thereof.

80. The use according to claim 77 in which the compound is



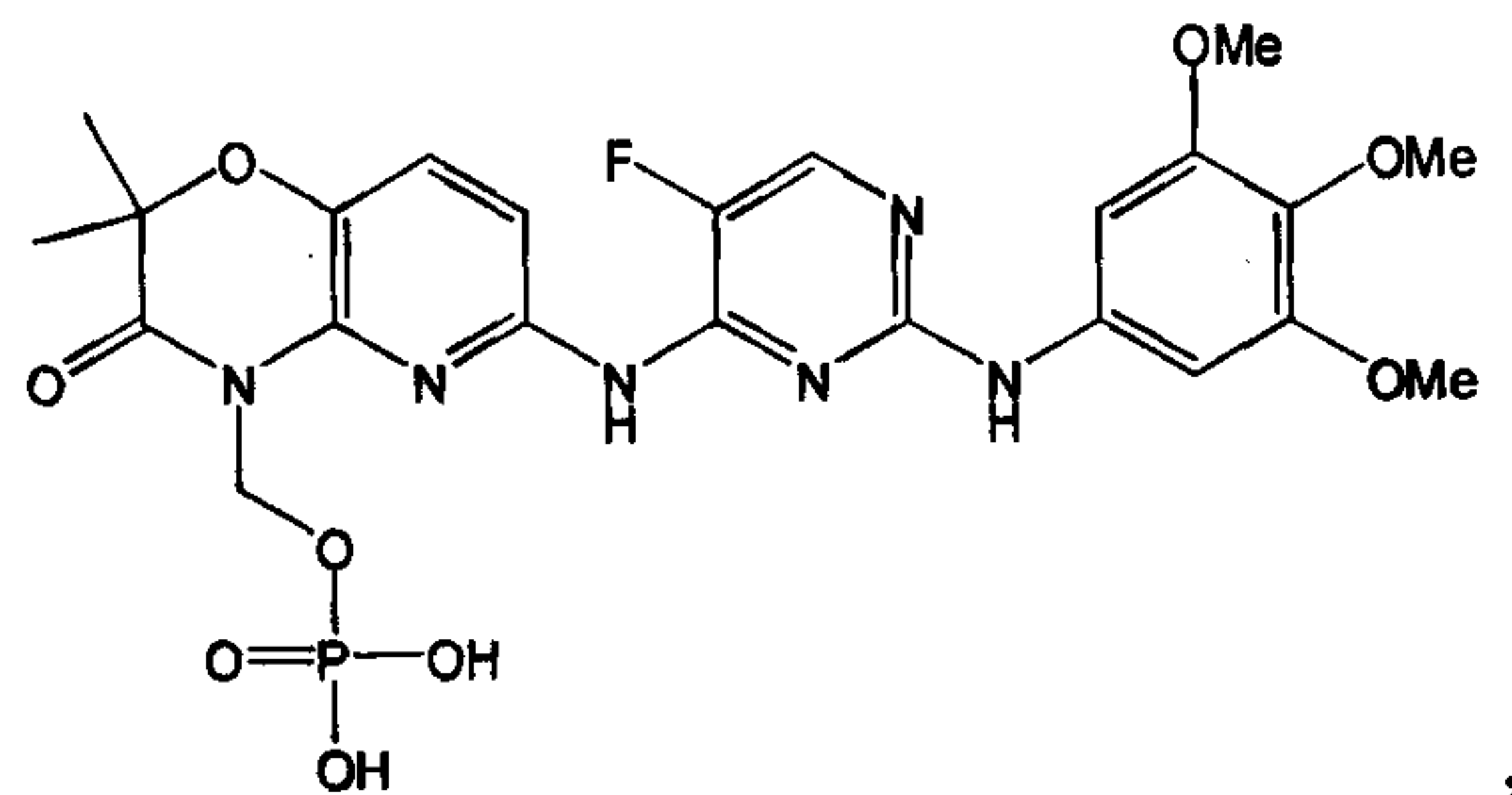
or a salt, hydrate, solvate, or N-oxide thereof.

81. The use according to claim 77 in which the compound is



or a salt, hydrate, solvate or N-oxide thereof.

82. The use according to claim 77 in which the compound is



or a salt, hydrate, solvate or N-oxide thereof.

83. The use according to any one of claims 77 to 82 in which the tumor is selected from breast cancer, ovarian cancer, renal cancer, gastrointestinal cancer, kidney cancer, bladder cancer, pancreatic cancer, lung squamous carcinoma, and adenocarcinoma.

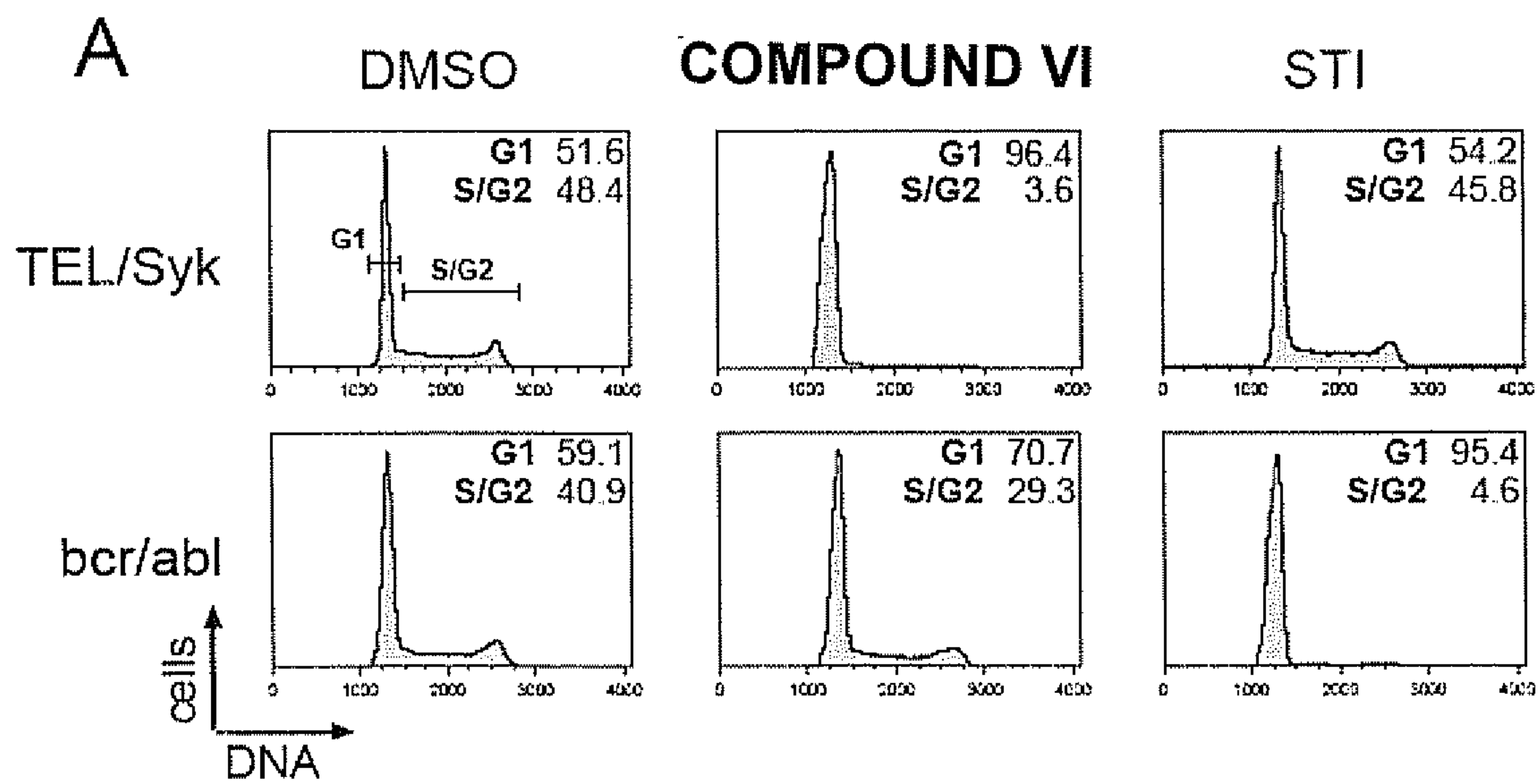
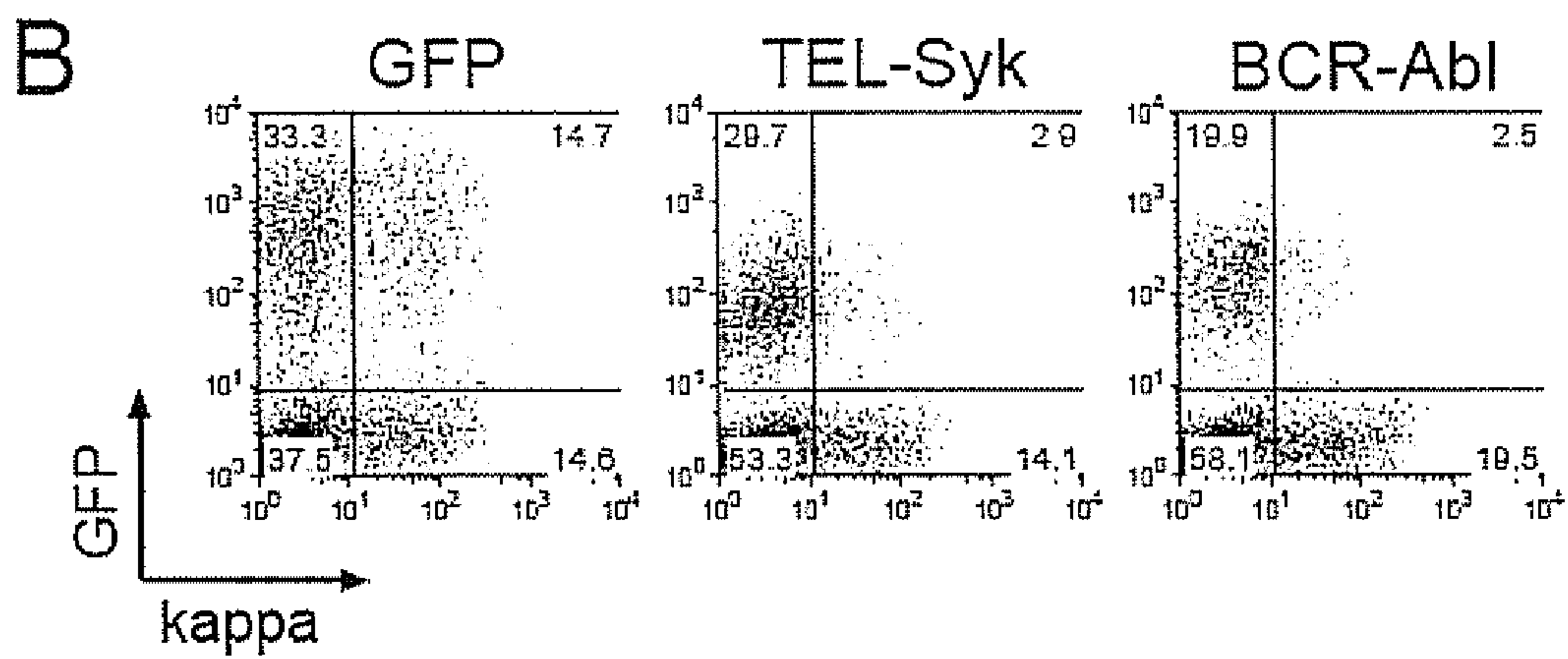
84. The use according to claim 77 in which said medicament is for administration prior to diagnosis of tumor metastasis.

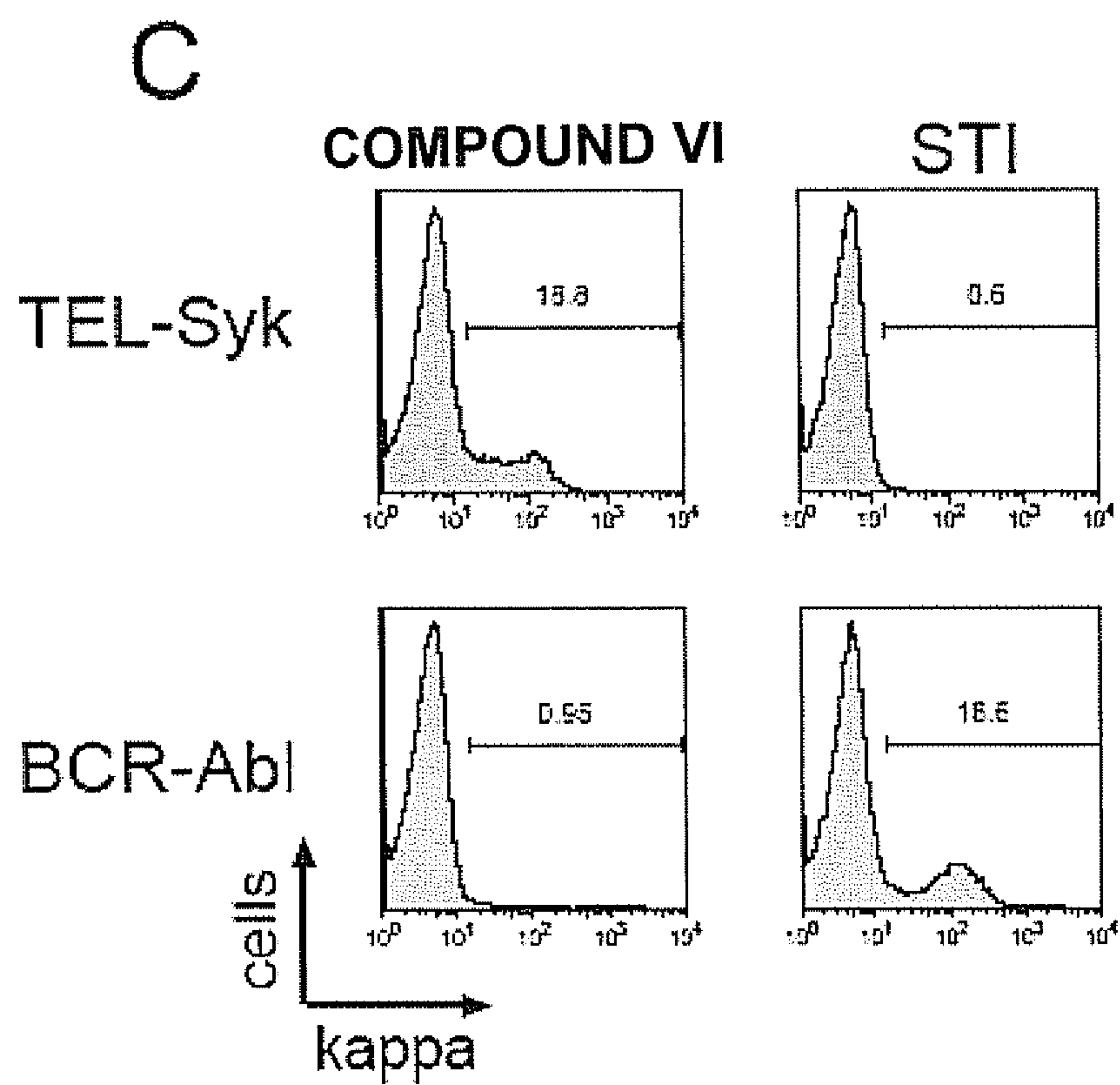
85. The use according to claim 77 in which the metastasis is caused by integrin signalling through Syk.

86. The use according to claim 77 in which the metastasis is caused by integrin signalling by $\beta 1$ integrins.

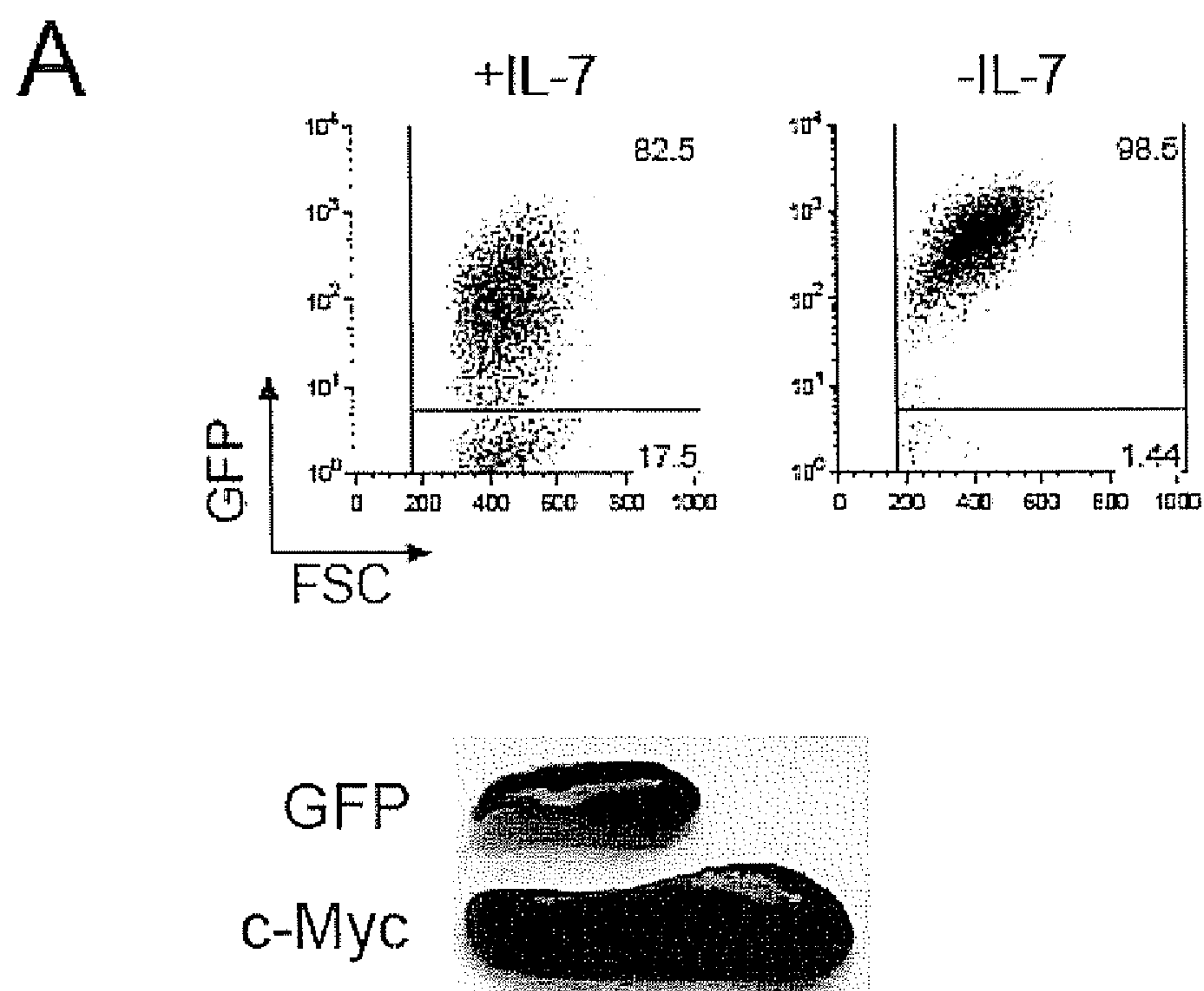
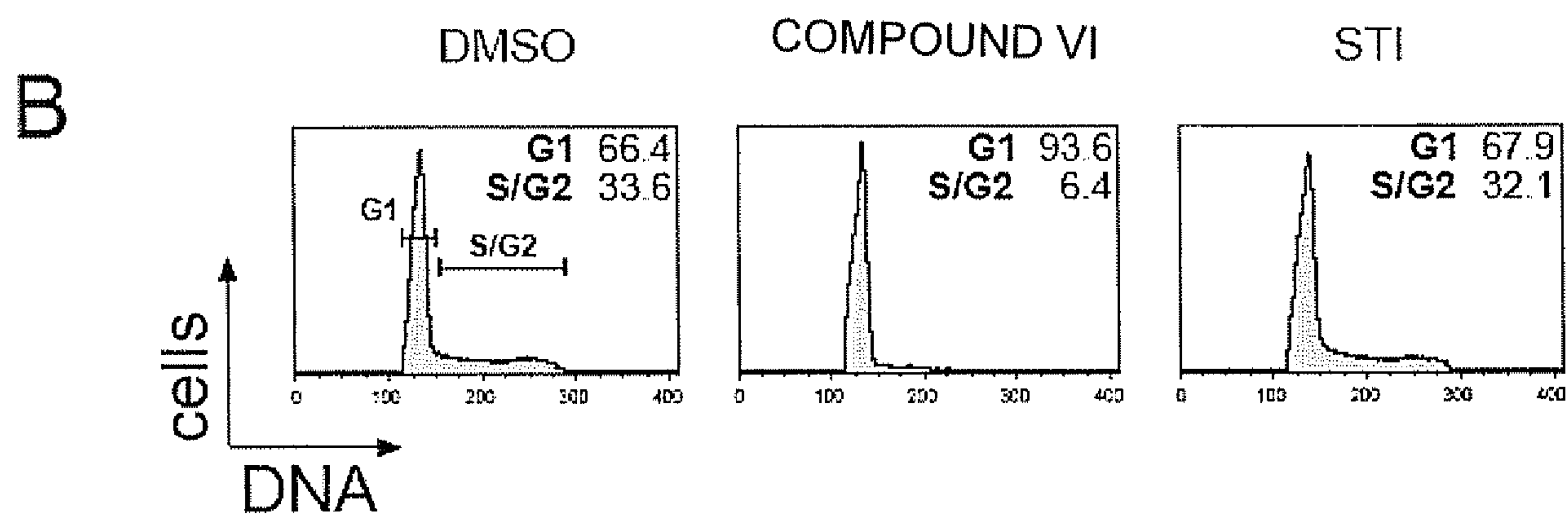
87. The use according to claim 77 in which the metastasis is caused by integrin signalling by $\beta 2$ integrins.

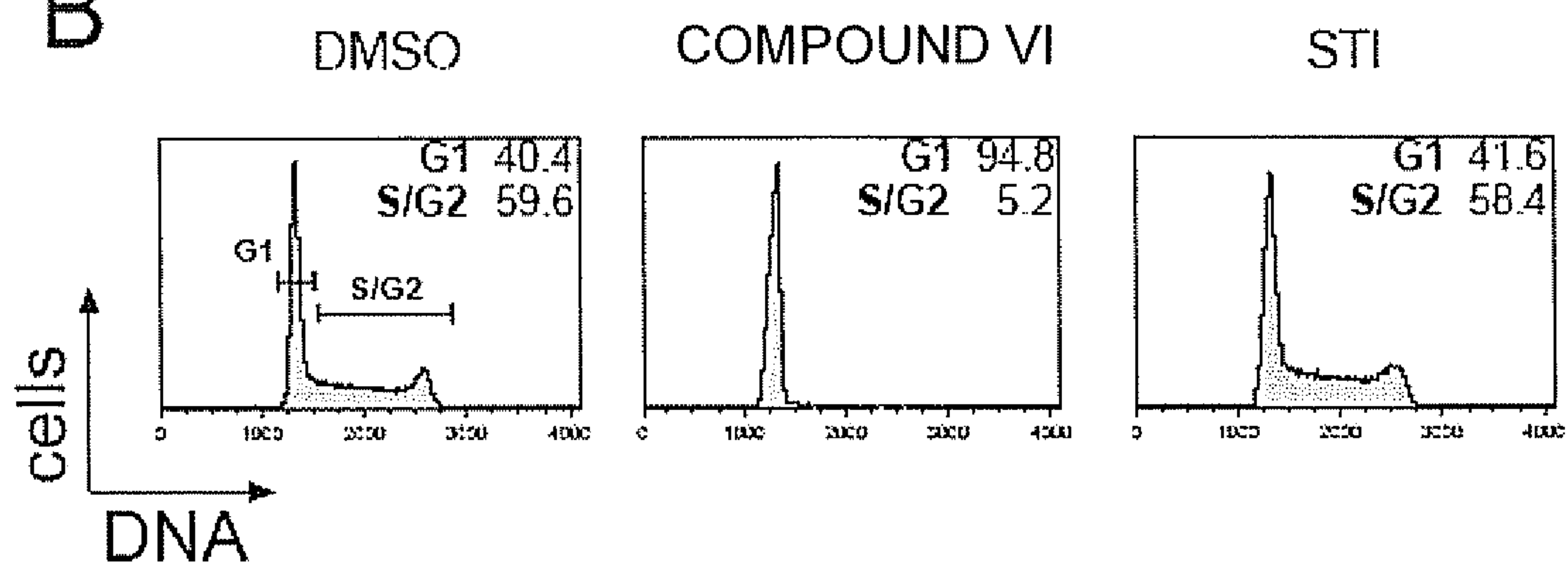
88. The use according to claim 77 in which the metastasis is caused by integrin signalling by $\beta 3$ integrins.

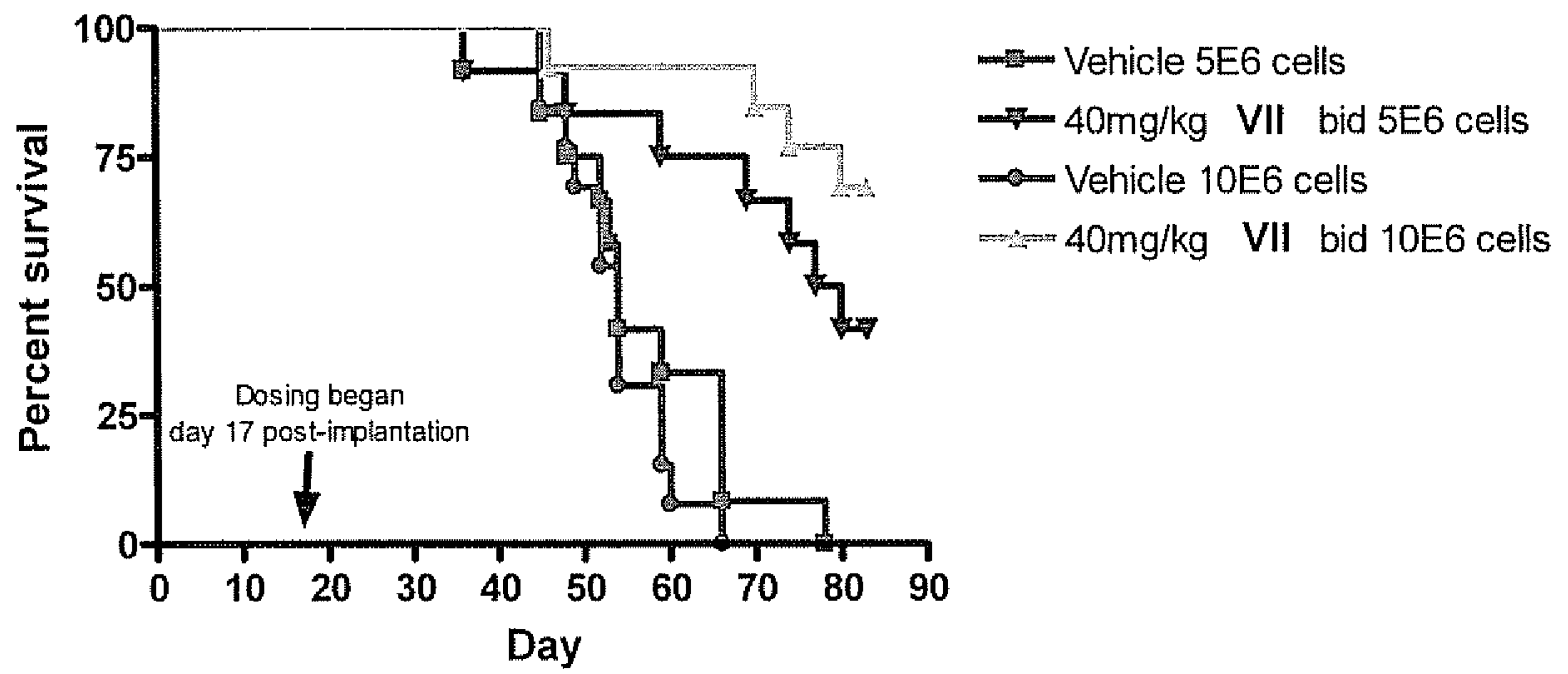
**FIG. 1A****FIG. 1B**

**FIG. 1C**

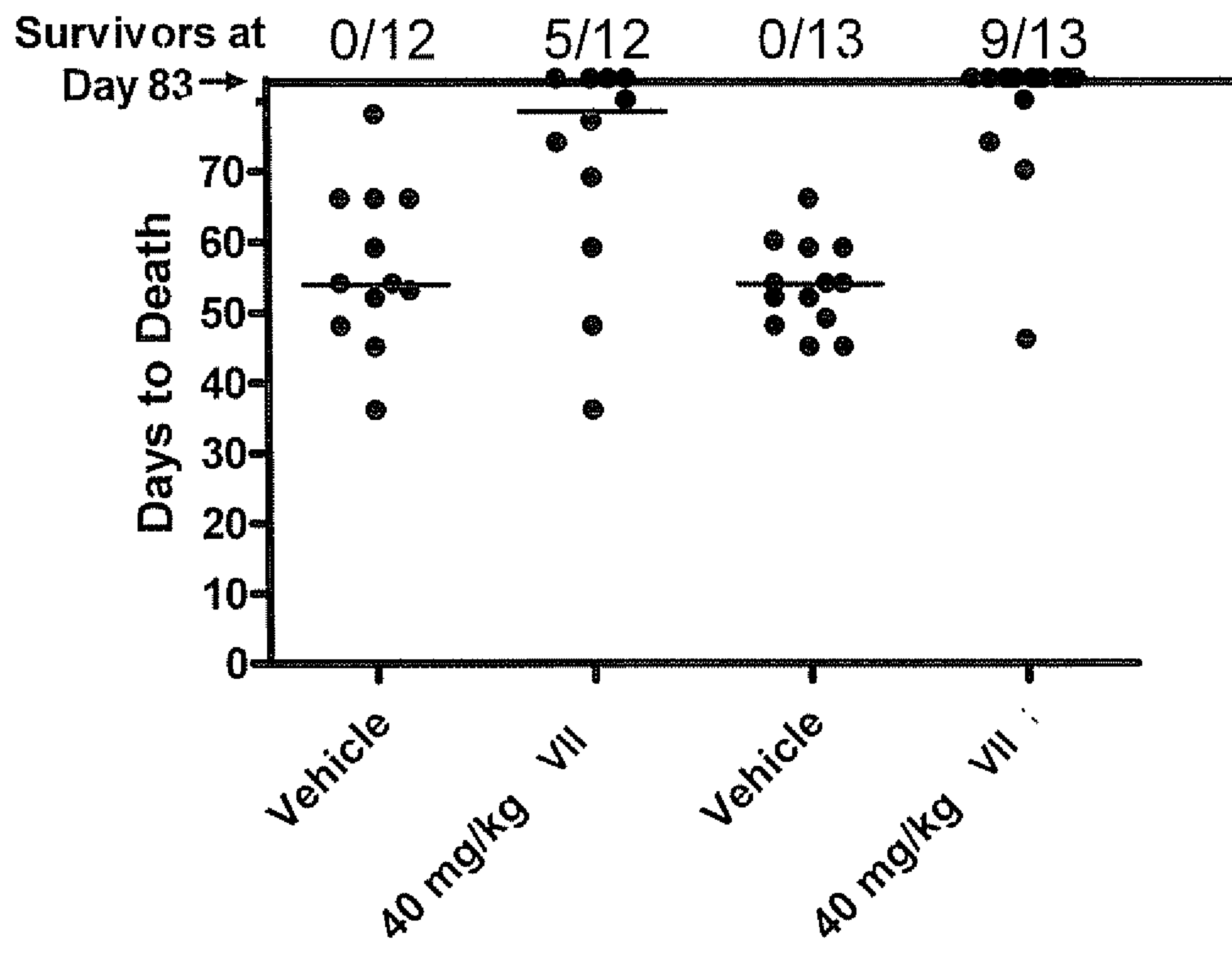
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**FIG. 2A****FIG. 2B**

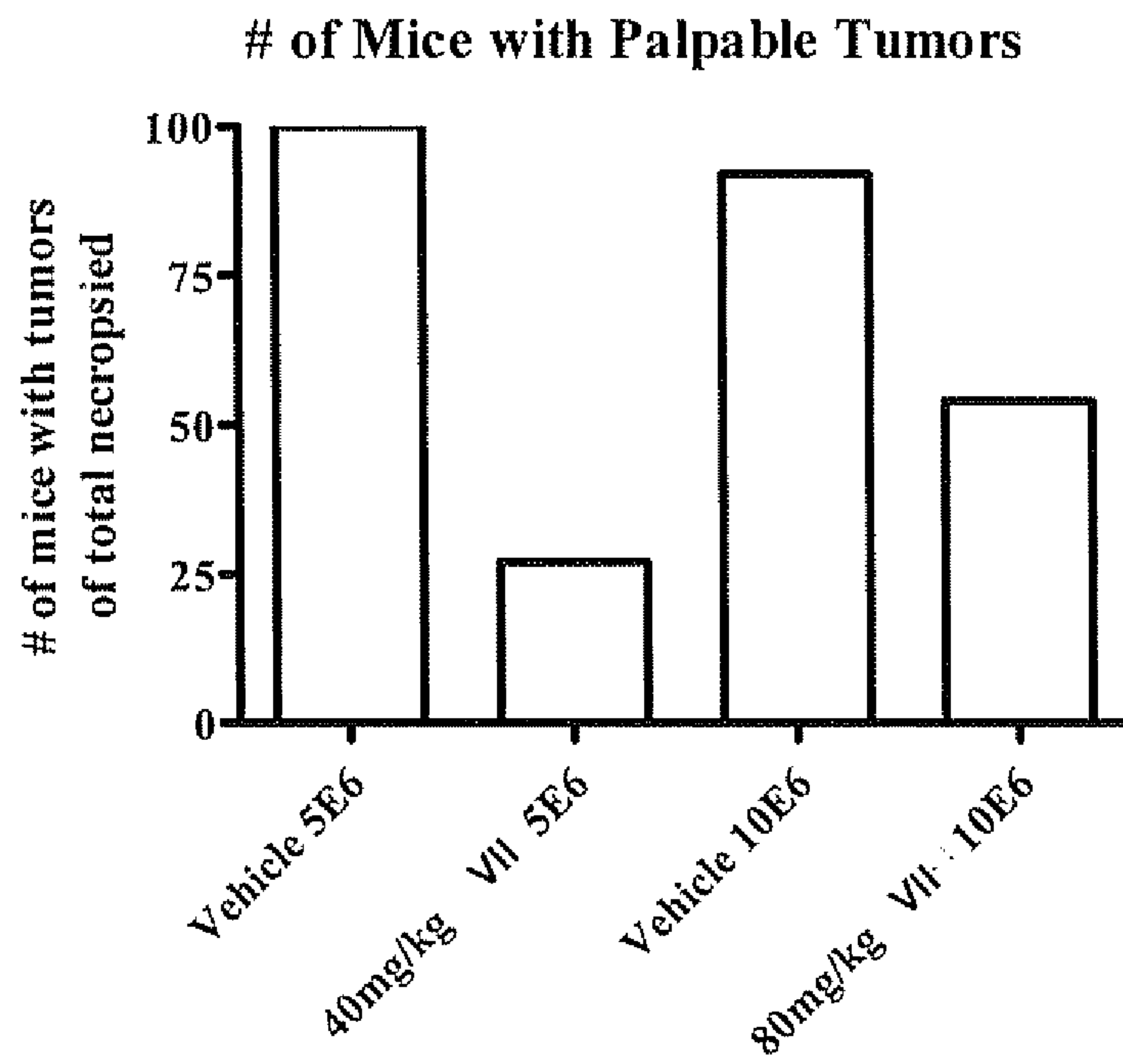
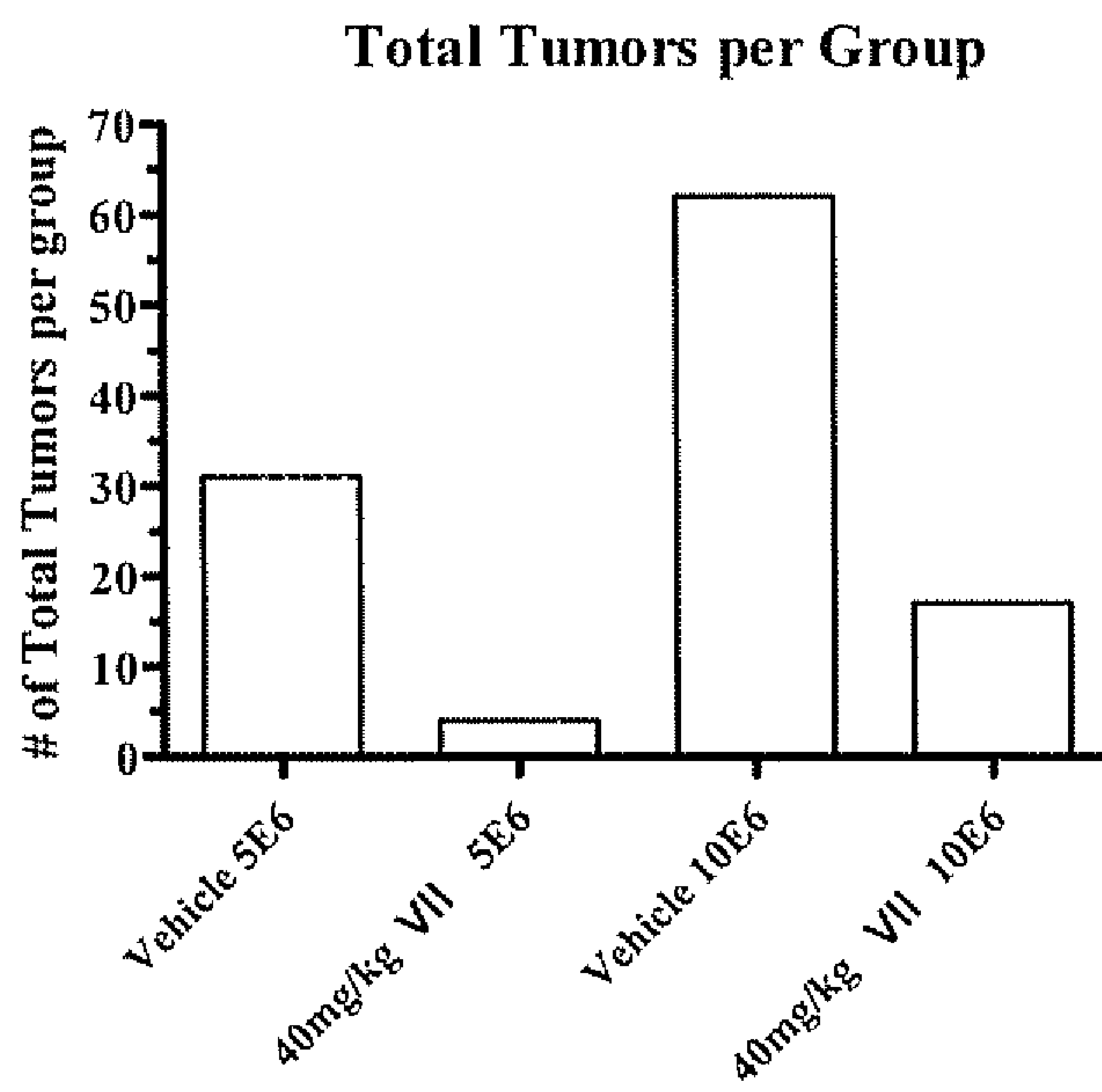
A**FIG. 3A****B****FIG. 3B**

**FIG. 4**

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**FIG. 5**

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**FIG. 6A****FIG. 6B**

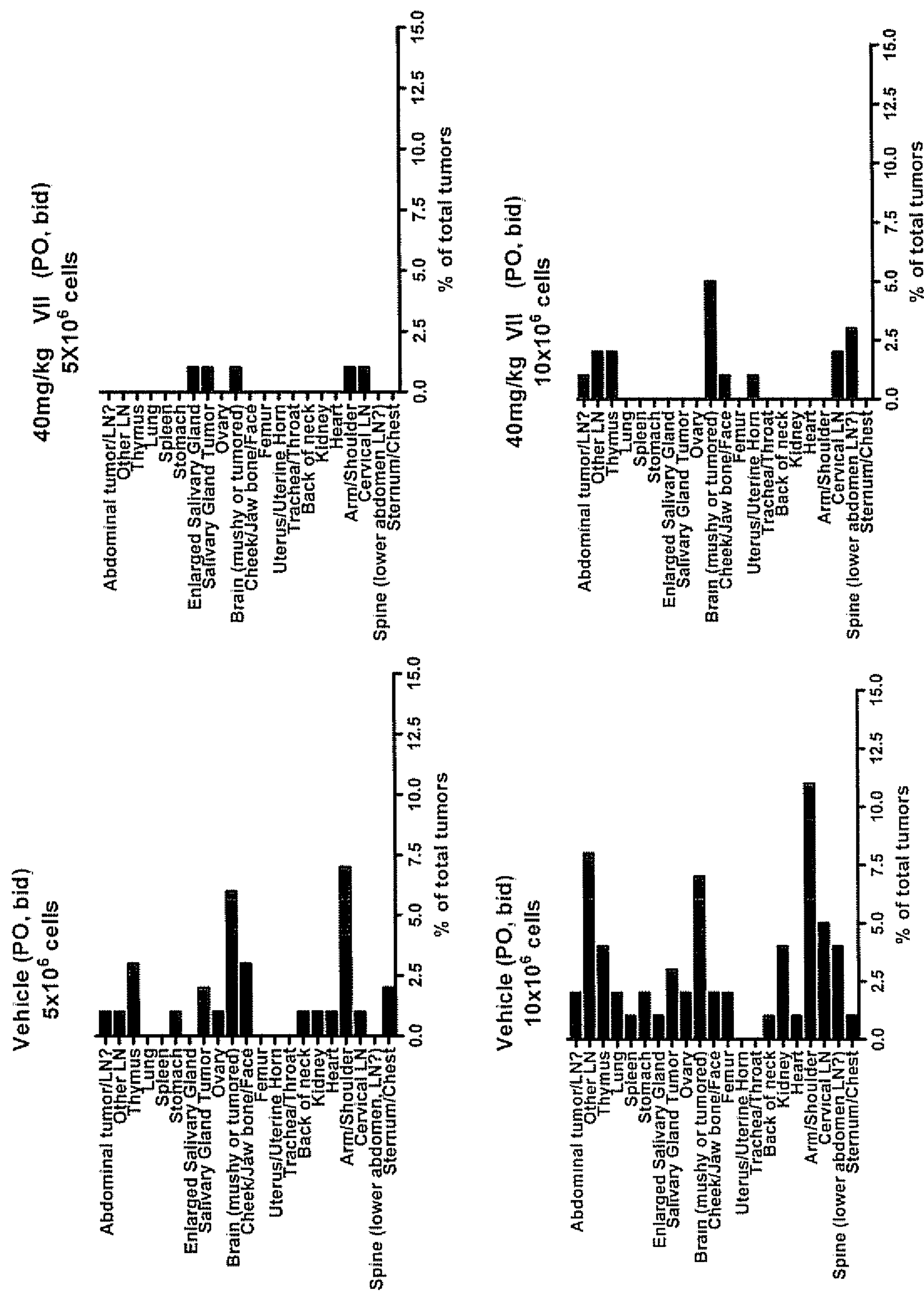


FIG. 7

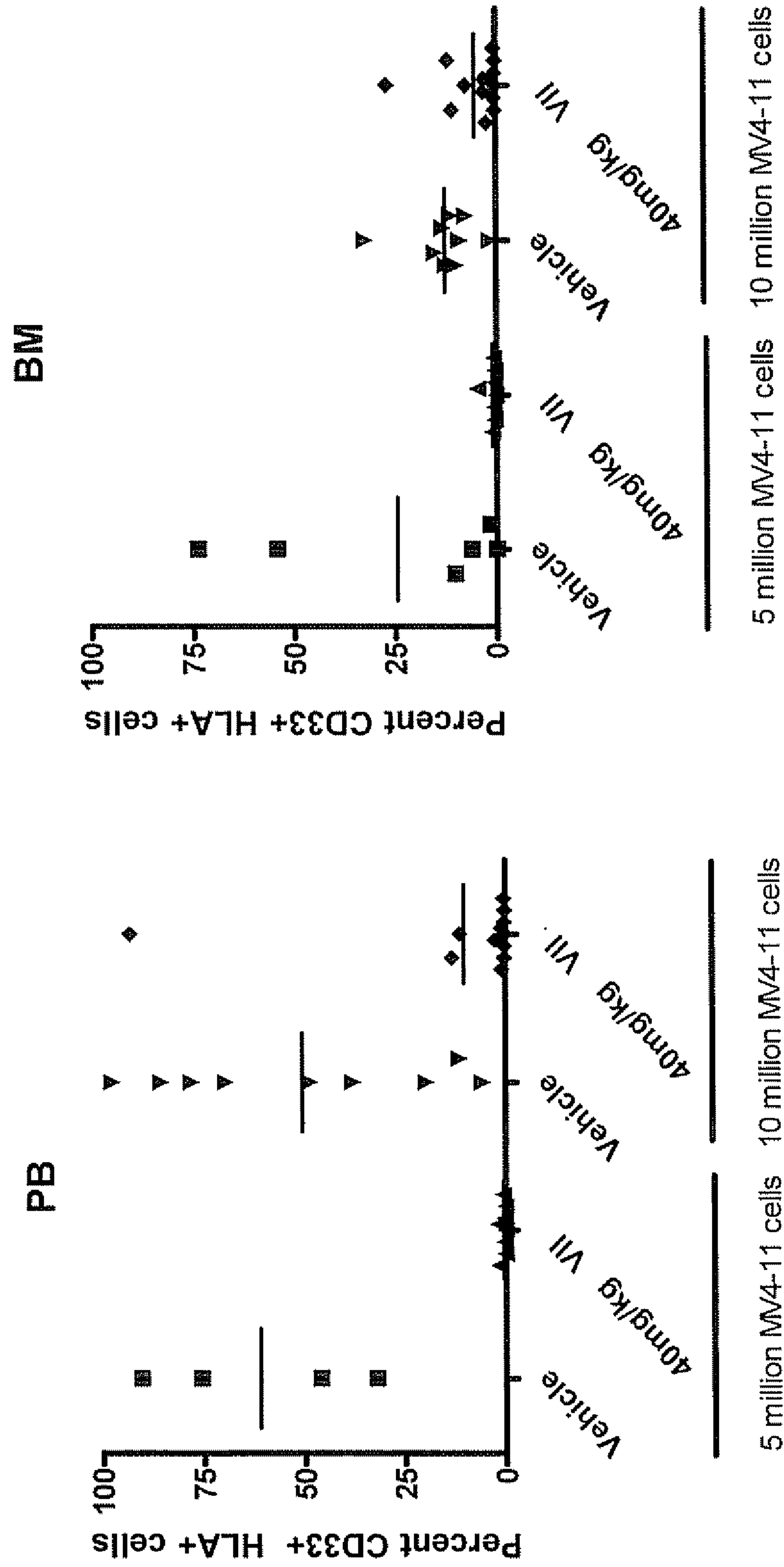


FIG. 8

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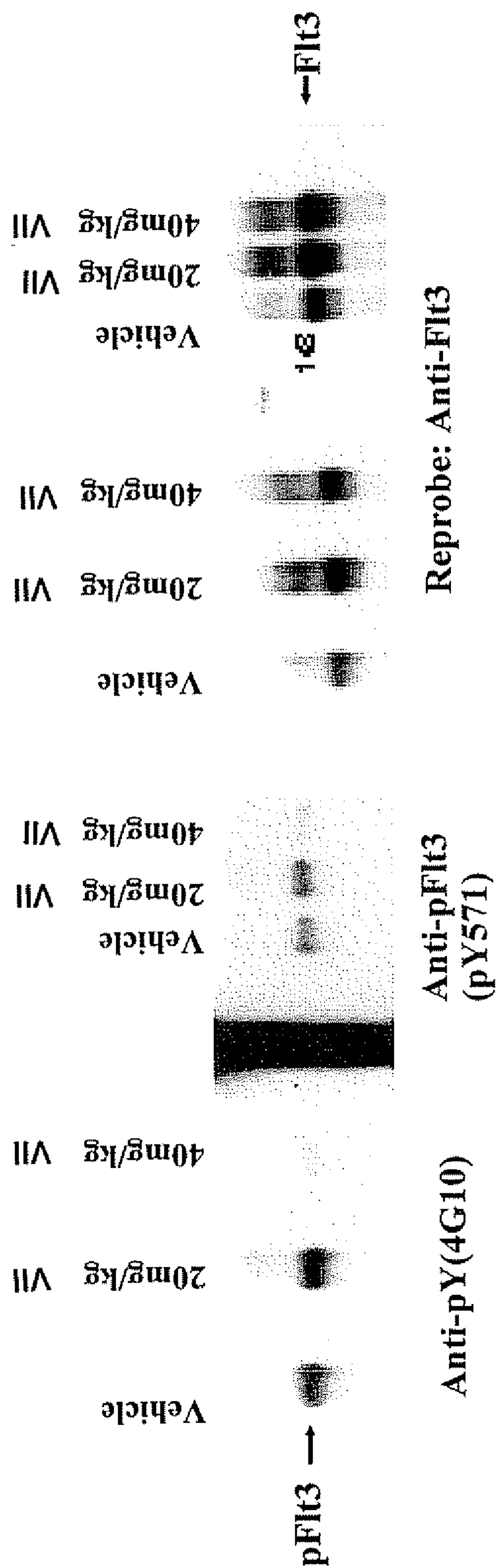
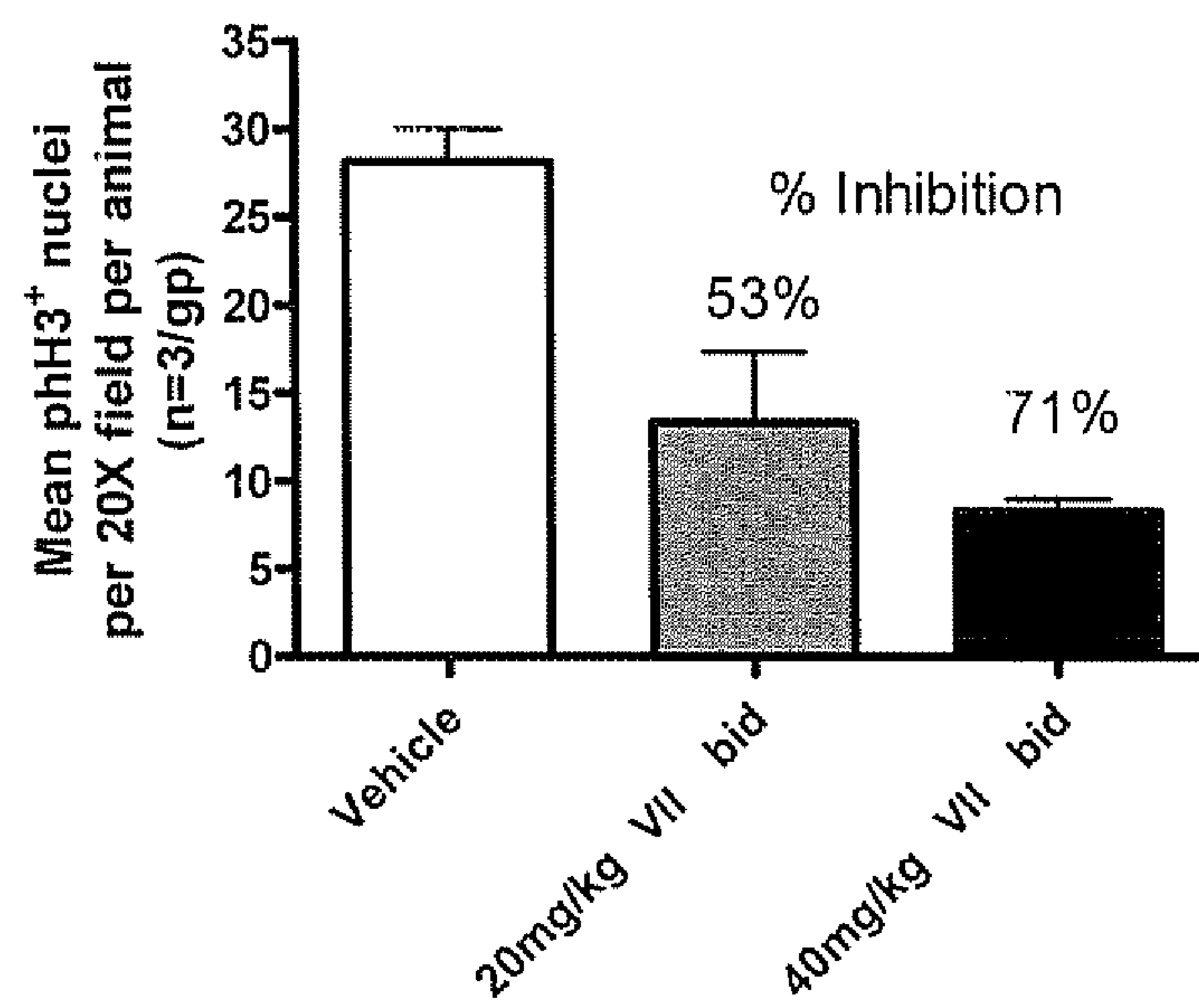
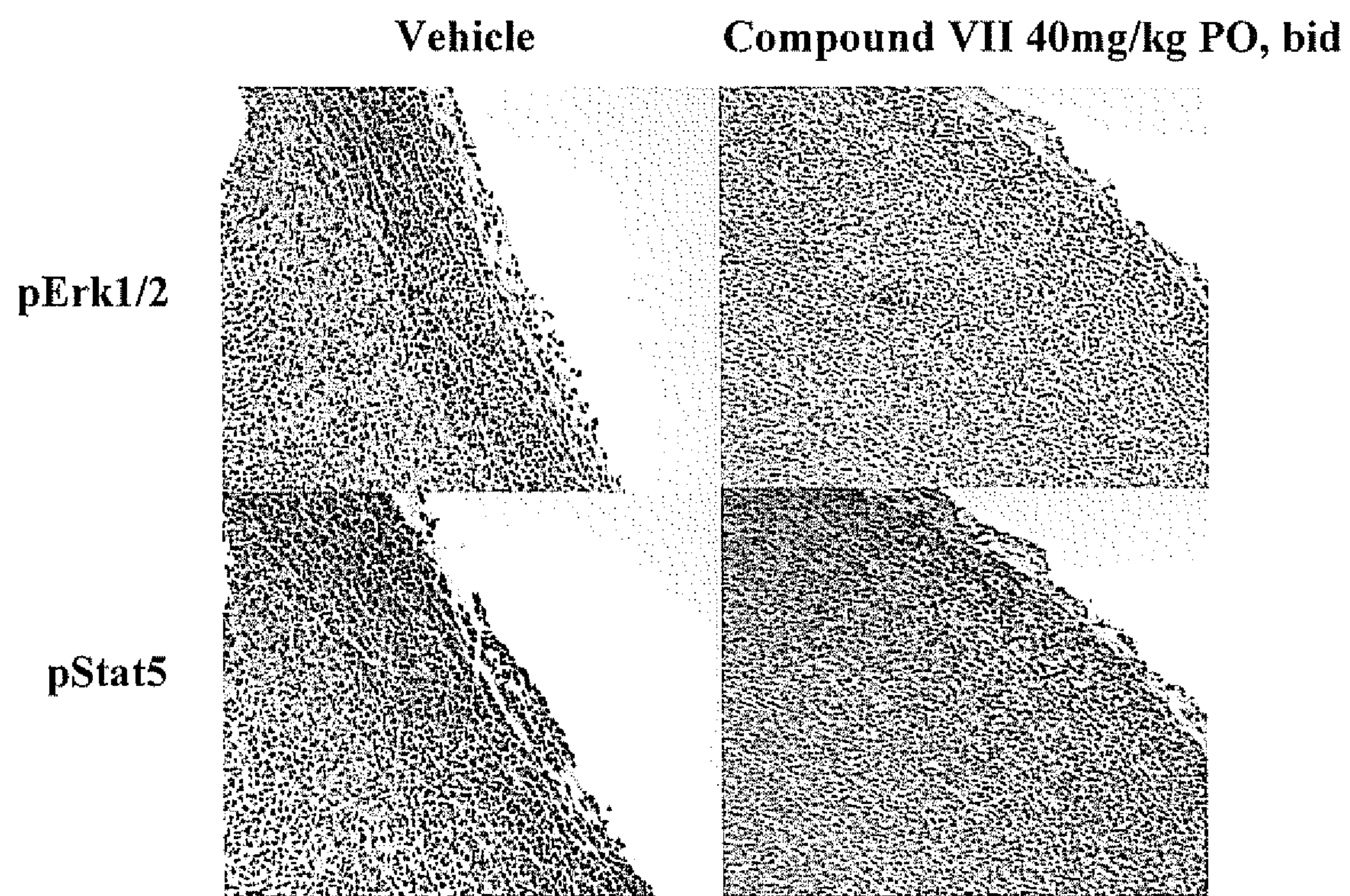


FIG. 9

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**FIG. 10**

**FIG. 11**

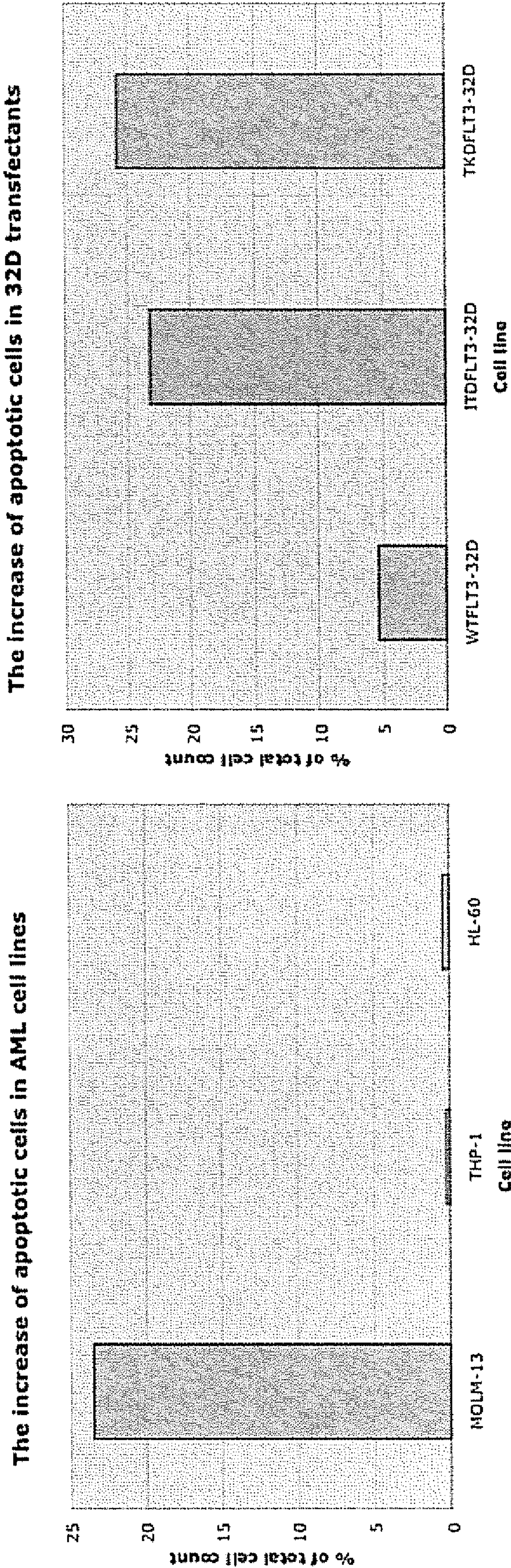


FIG. 12