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(54) **PHARMACEUTICAL COMPOSITION
USEFUL FOR TREATING CHRONIC
MYELOID LEUKEMIA**

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(57) **ABSTRACT**

The present invention relates to a pharmaceutical composition useful for treating chronic myeloid leukemia where Bcr-Abl kinase is constitutively expressed in animals and humans, said composition comprising an effective amount of analogs of chlorogenic acid such as neochlorogenic acid (5-O-caffeoyl quinic acid), cryptochlorogenic acid (4-O-Caffeoyl quinic acid), 3-O-(3'-methylcaffeoyl) quinic acid and 5-O-(Caffeoyl-4'-methyl) quinic acid and/or its salts such as sodium, potassium and ammonium together with pharmaceutically acceptable additives.

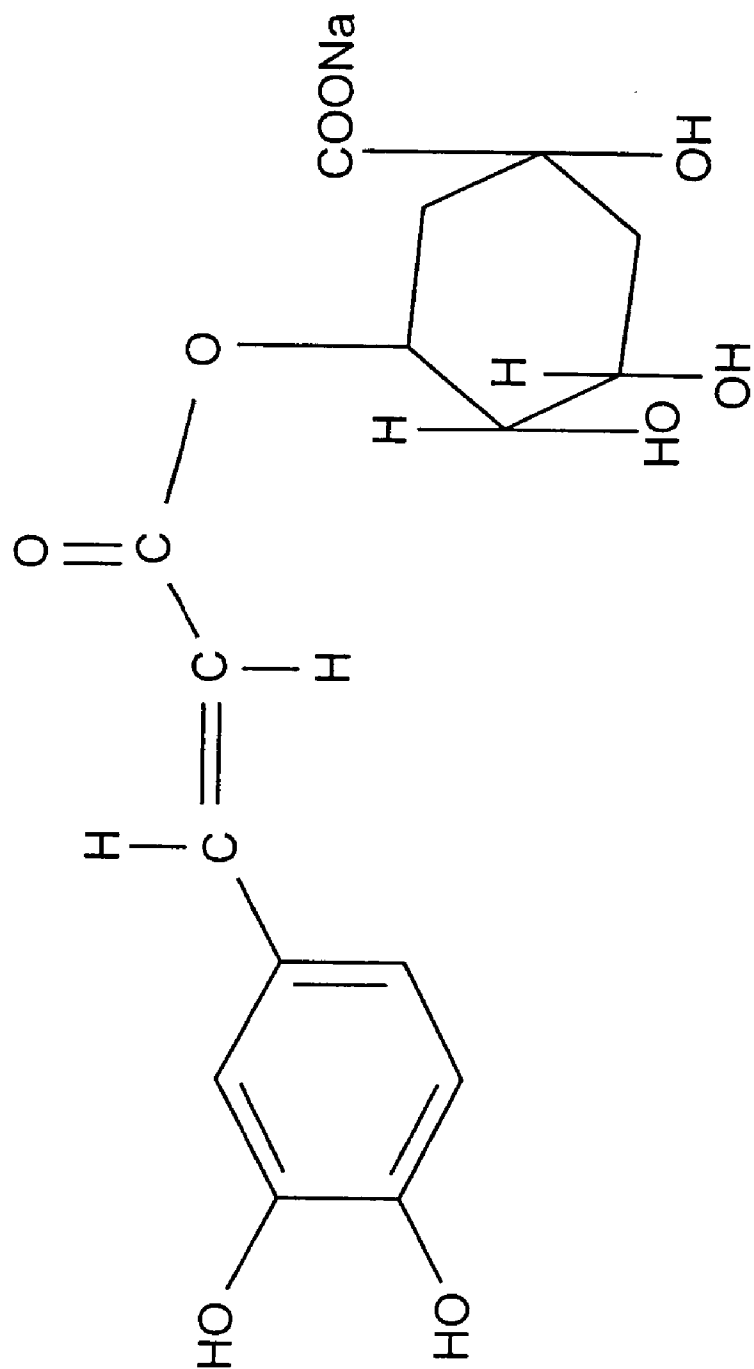
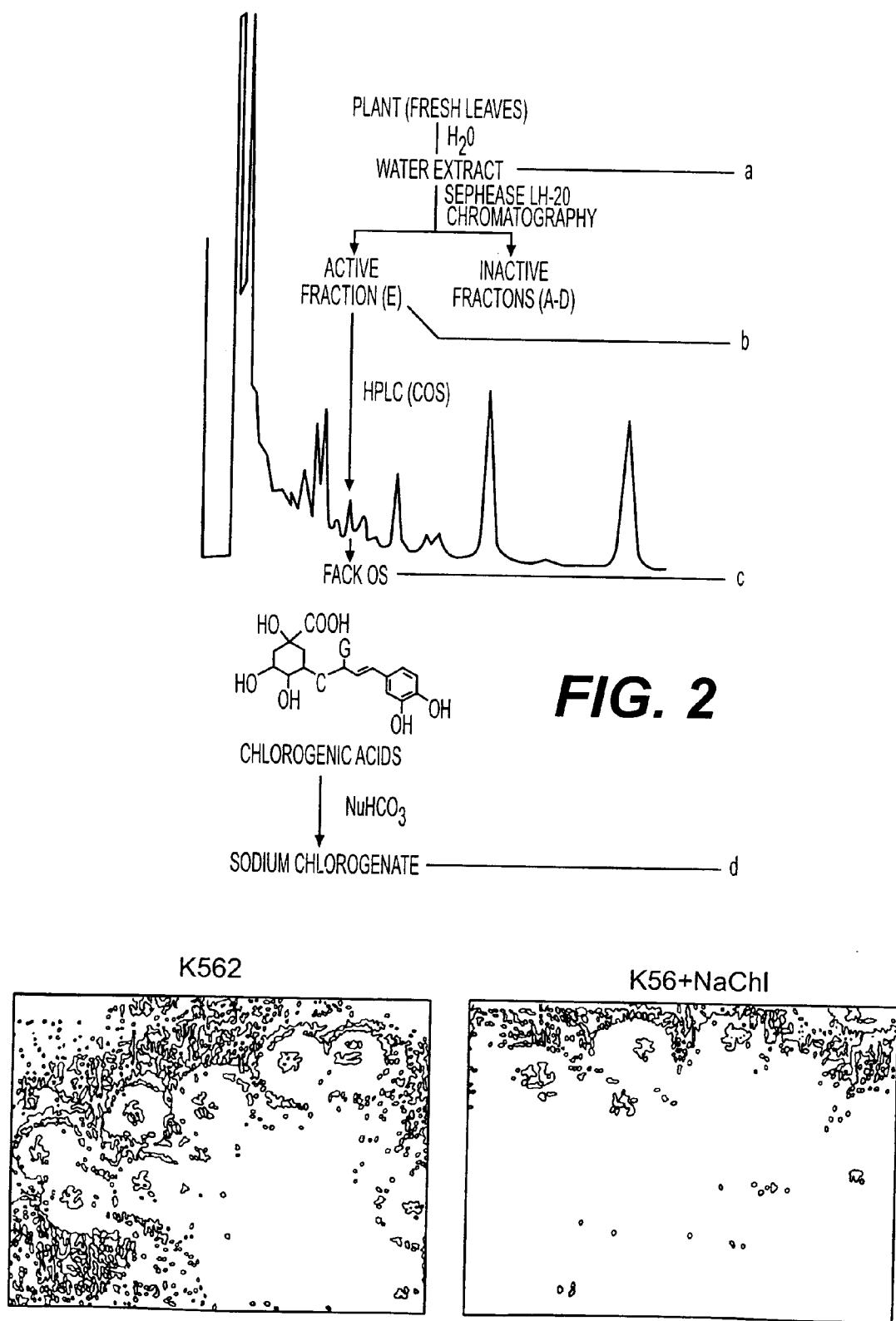


FIG. 1



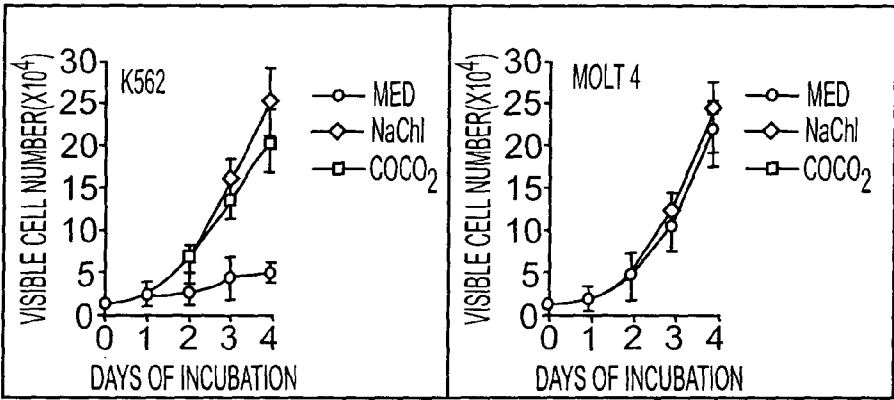


FIG. 2a

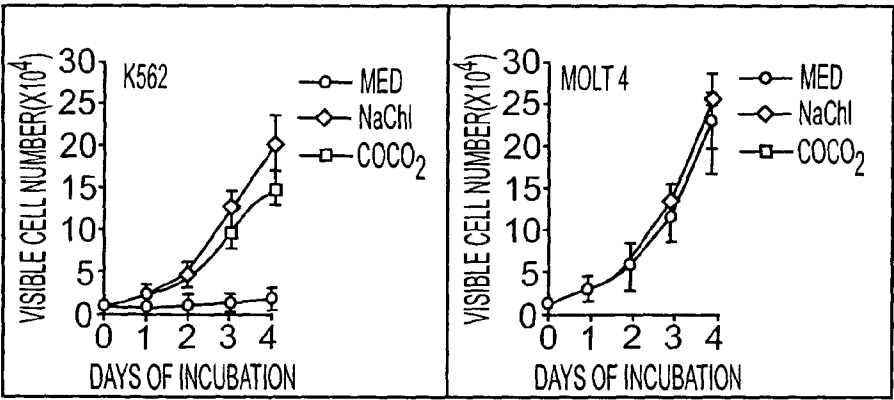


FIG. 2b

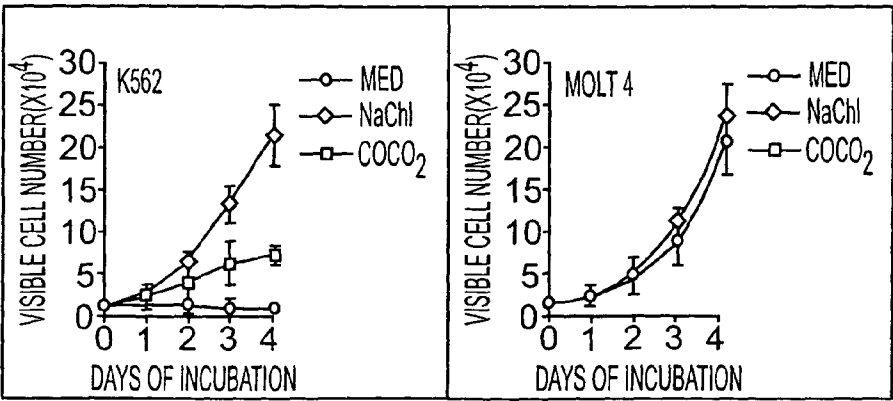


FIG. 2c

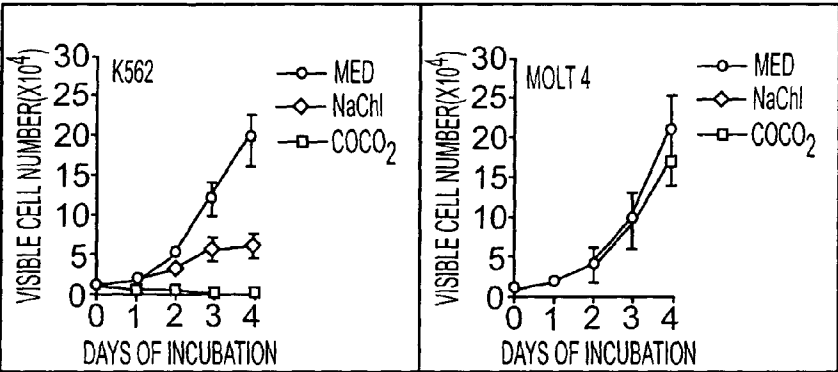


FIG. 2d

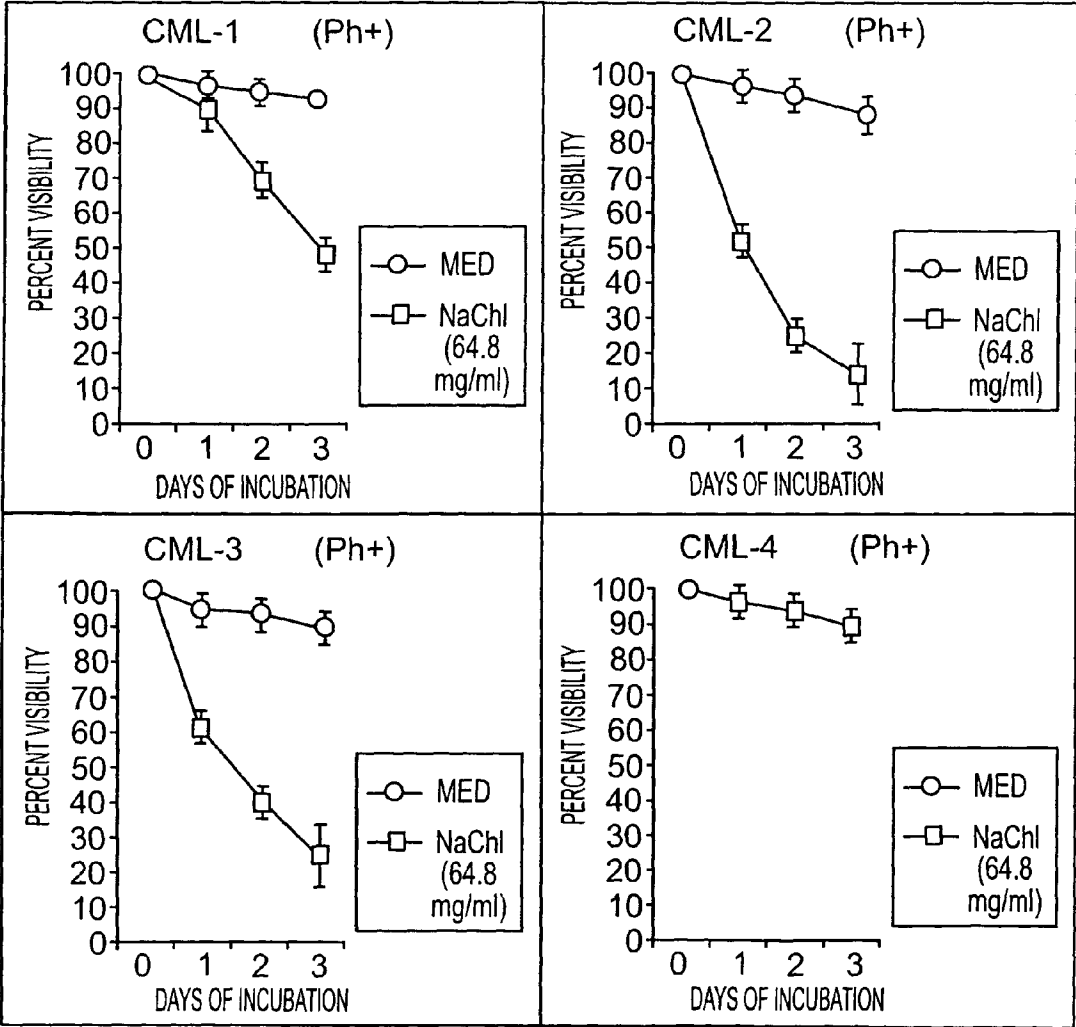


FIG. 2e

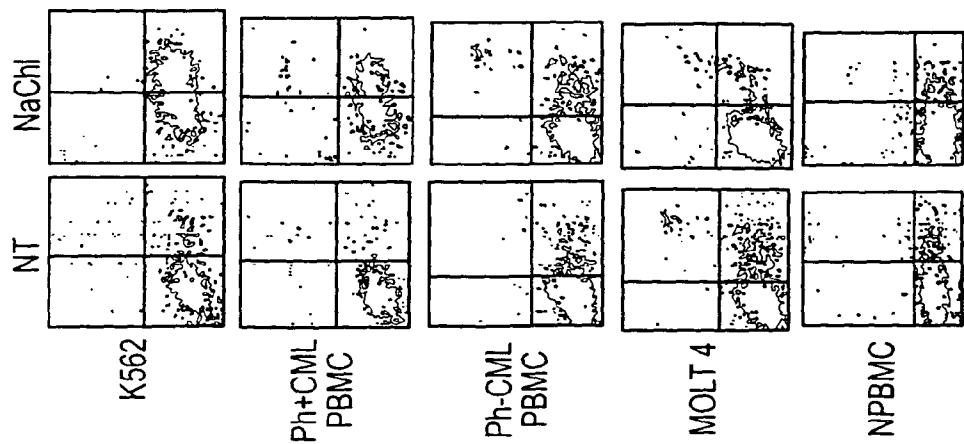


FIG. 3a

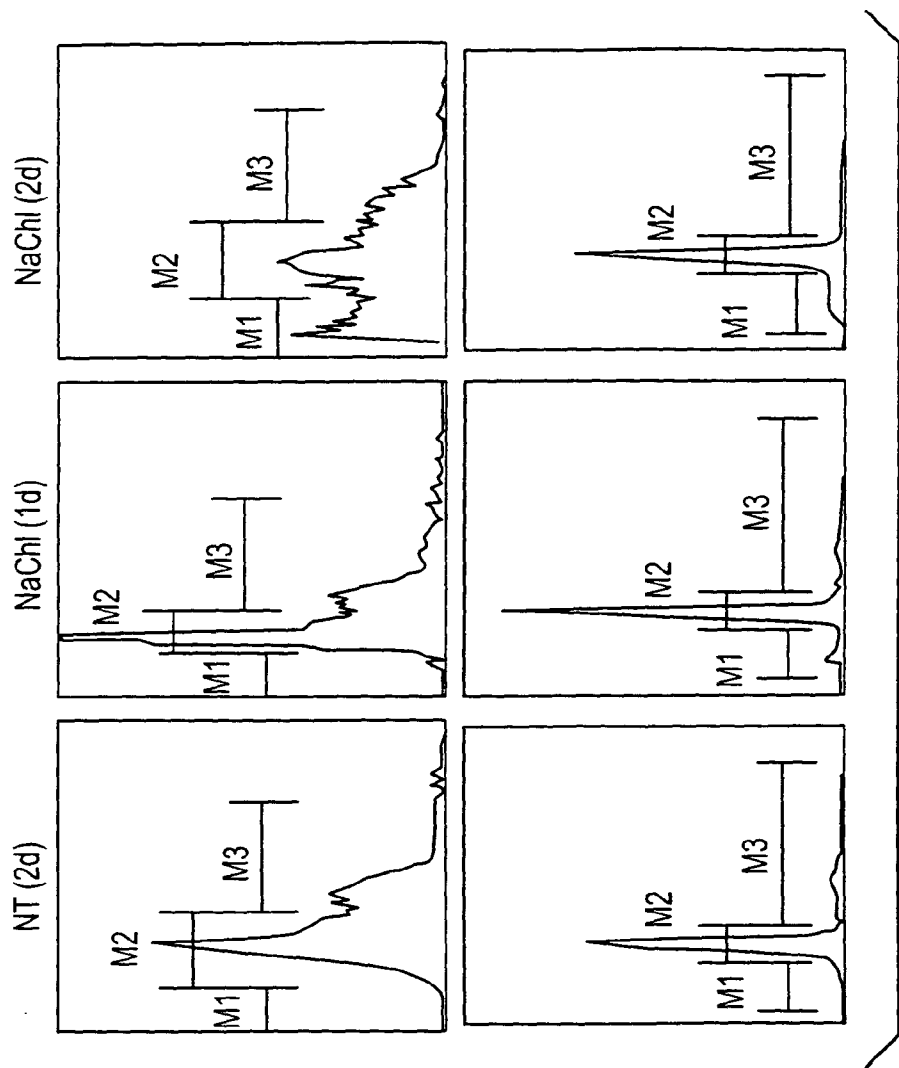


FIG. 3b

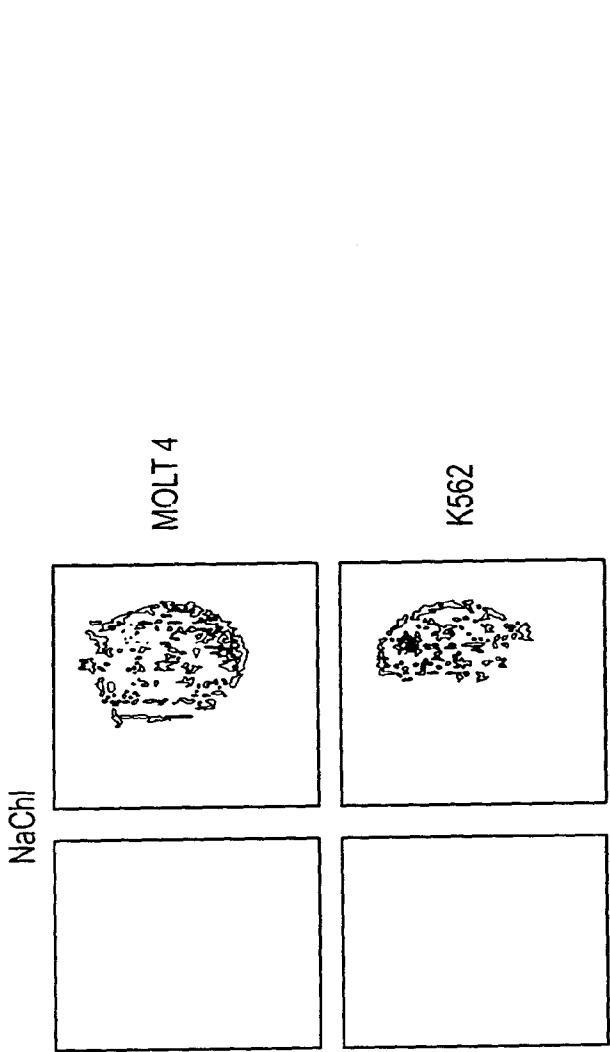


FIG. 3c

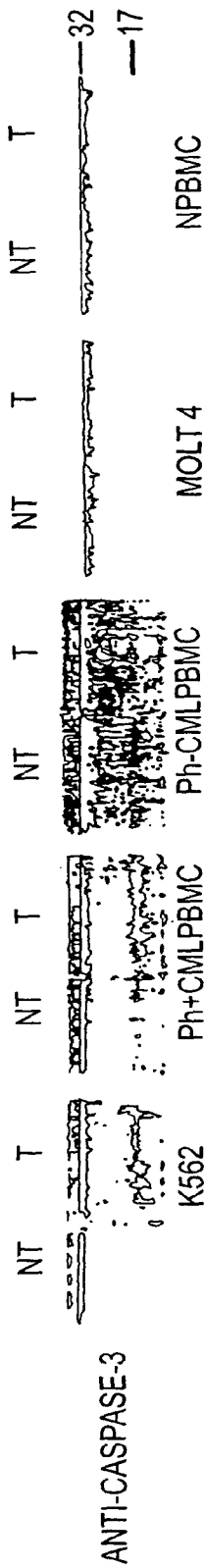
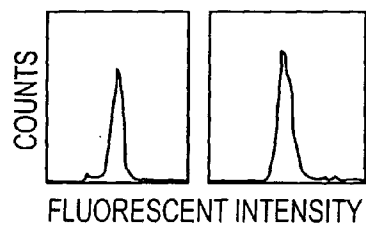
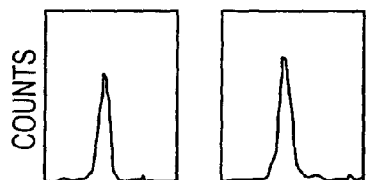


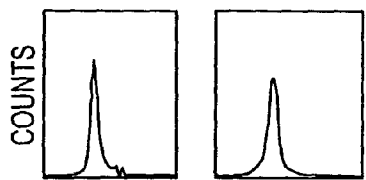
FIG. 3d



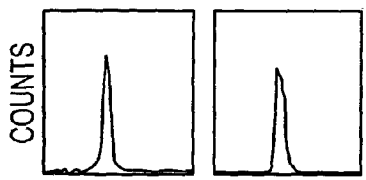
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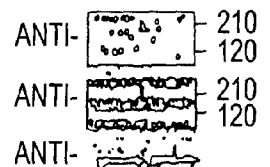
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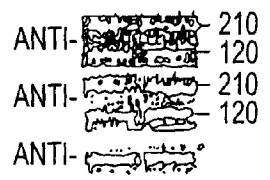
FIG. 4a



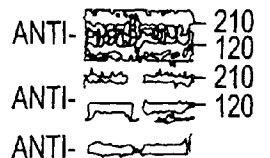
Ph+CML PBMC



Ph-CML PBMC



MOLT 4



NORMAL INDIVIDUAL'S
PBMC

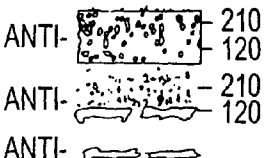
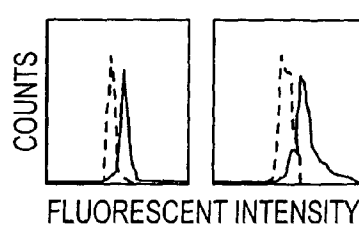
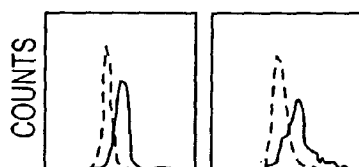


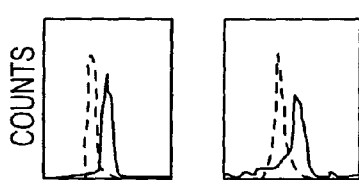
FIG. 4b



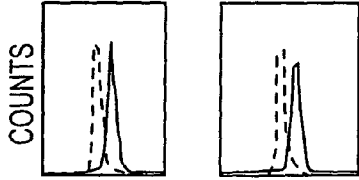
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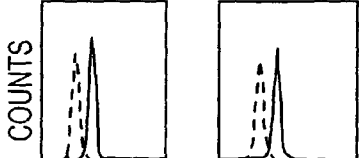
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FIG. 4c

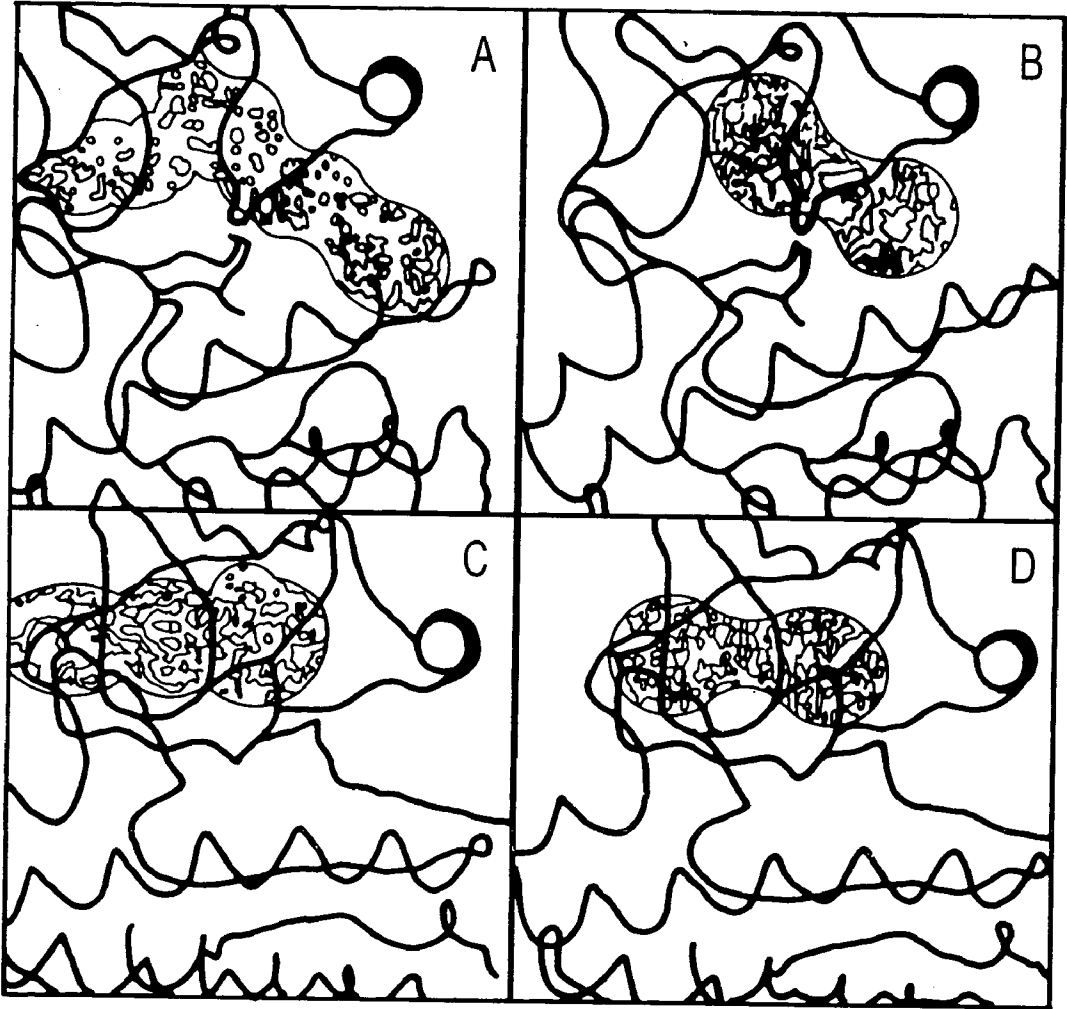


FIG. 4d

PHARMACEUTICAL COMPOSITION USEFUL FOR TREATING CHRONIC MYELOID LEUKEMIA

[0001] This application claims benefit of U.S. Provisional application No. 60/393,750 filed on Jul. 8, 2002 and is a Continuation-In-Part of an application entitled "An Herbal Molecule As Potential Anti-leukemic Drug" filed Jan. 9, 2003.

FIELD OF THE INVENTION

[0002] This invention relates to treatment of chronic myeloid leukemia (CML) by a composition comprising analogs of chlorogenic acid and/or its salts such as sodium chlorogenate (Na-Chl) or potassium or ammonium salts, which were prepared from Chlorogenic acid or its analogs.

[0003] Chronic myeloid leukemia is lethal, there is no drug directing towards the destruction of the leukemic cells, and these cells poorly respond to chemotherapy which is always non-specific thus adversely affecting normal cells. Unique property of the therapy with NaChl is the killing of myeloid cancer cells leaving other normal cells unaffected.

BACKGROUND AND PRIOR ART REFERENCES

[0004] Myeloid leukemia is usually subdivided into two groups: Acute Myeloid Leukemia (AML) and Chronic Myeloid Leukemia (CML). AML is characterized by an increase in the number of myeloid cells in the bone marrow and an arrest in their maturation. In the United States, the annual incidence of AML is approximately 2.4 per 100,000 and it increases progressively with age, to a peak of 12.6 per 100,000 adults 65 years of age or older. The CML is a malignant clonal disorder of hematopoietic stem cells. The median age at presentation is 53 years, but it occurs at all age groups, including children. The natural history of CML is progression from a benign chronic phase to a rapidly fatal blast crisis within three to five years or even earlier. The prognosis of CML is also poor in spite of vast advancement of clinical medicine (1). CD33 represents a specific and useful marker in the process of myeloid cell differentiation (2). Recent reports suggest that engagement of CD33 by monoclonal antibody induced apoptosis leading to growth inhibition of proliferation of AML and CML cells in vitro (2,3). Exploiting the myeloid specific expression of CD33, humanized anti-CD33 monoclonal antibody conjugated with anti-cancer drug has been tried in AML patients with significant success (4). Similarly, lymphoid leukemia is also subdivided in two groups: acute lymphocytic leukemia (ALL) and chronic lymphocytic leukemia (CLL). Lymphoid leukemia may affect both T and B cell lineages and are prevalent in children. With the extracts from *Piper betel* leaves anti-myeloid activity was claimed earlier (Patent filed no. PCT/INOO/00118 dated Dec. 12, 2000). Two compounds isolated from *Piper betel* extract also showed anti myeloid leukemic activity. One compound was 3-O-Coumaryl quinic acid (U.S. patent application Ser. No. 60/384, 163 dated May 31, 2002). The other compound was Chlorogenic acid (U.S. patent application Ser. No. 60/393750 dated Aug. 7, 2002).

[0005] Hence, applicant's earlier findings are in direct consonance with the present patent filing on sodium chlorogenate prepared from the fractions of the betel leaf extracts for treating chronic myeloid leukemia. Chlorogenic

acid (Chl) is known to have anti-allergic activity (5). Chl also inhibits hepatic and renal glucose-6-phosphatase systems (6). Chl is an inhibitor of epidermal lipoxygenase activity and TPA-induced ear inflammation (7). Chl also renders inhibitory effects on TPA-induced tumor promotion in mouse skin (7). Anti-HIV activity of Chl has also been reported (8). The inadvertent fusion of Bcr with the Abl gene results in a constitutively active tyrosine kinase (Bcr-Abl) that transforms cells to chronic myelogenous leukemia (CML)⁹. Highly potent small molecules are known to inhibit Bcr-Abl dependent cell growth^{10,11}. Recent reports on the development of resistance to one such compound emphasizes the need for further therapeutic search to control CML¹². Here we claim that a chemical compound isolated from a herb, *Piper betel* inhibits Abl protein tyrosine kinase triggering apoptosis of Bcr-Abl expressing CML cell line K562. Elucidation of structure identifies this molecule as chlorogenic acid. Its sodium, potassium, ammonium and other salts also exhibit killing activity against CML cells although the sodium salt, sodium chlorogenate (NaChl) exhibits little more potency than other salts of chlorogenic acid. Sodium chlorogenate shows 2.0 fold greater efficiency in killing K562 cells compared to chlorogenic acid. Interestingly, NaChl also destroys Bcr-Abl expressing peripheral blood cells of CML patients without any effects on peripheral blood cells of Bcr-Abl negative CML patients. Analysis of molecular models indicates that Na-Chl occupies the ATP-binding site of the kinase domain of Abl. NaChl therefore stands as an additional therapeutic agent for CML. In the present patent application, anti myeloid leukemic activity of Na-Chl is claimed for the first time.

[0006] References

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OBJECTS OF THE INVENTION

[0019] The main object of the invention is to provide a pharmaceutical composition comprising analogs of chlorogenic acid and/or its salts.

[0020] Another object of the present invention is to provide a pharmaceutical composition comprising analogs of chlorogenic acid and/or its salts for treating chronic myeloid leukemia.

[0021] Another object of the invention is to provide pharmaceutical composition comprising salts of chlorogenic acid and/or its salts such as sodium chlorogenate (Na-Chl) or potassium or ammonium salts, which were prepared from Chlorogenic acid or its analogs for the treatment of chronic myeloid leukemia.

[0022] Another object of the invention is to provide a new use of the compound sodium chlorogenate (NaChl) prepared from Chlorogenic acid (Chl) isolated from the *Piper betel* leaf extract or from any other sources for the treatment of chronic myeloid leukemia.

[0023] Another objective of the invention is to provide a new pharmaceutical composition comprising a carrier along with the compound sodium chlorogenate for the treatment of chronic myeloid leukemia.

[0024] Yet another objective of the invention is to provide a process for the preparation of sodium chlorogenate from Chlorogenic acid to treat CML.

[0025] Yet another objective of the invention is to provide a simplified method of preparation of NaChl from Chlorogenic acid which was isolated from all plant parts of *Piper betel* possessing biological activities relevant to the treatment of CML.

[0026] Yet another objective of the invention is to provide sodium salt of Chlorogenic acid a herbal product from leaves or any other plant parts of *Piper betel* for the treatment of CML.

SUMMARY OF THE INVENTION

[0027] Accordingly, the present provides a pharmaceutical composition for the treatment of chronic myeloid leukemia (CML) by a composition comprising analogs of chlorogenic acid and/or its salts such as sodium chlorogenate (Na-Chl) or potassium or ammonium salts, which were prepared from Chlorogenic acid or its analogs.

[0028] In particular, the present invention provides a pharmaceutical composition, which kills myeloid cancer cells leaving other normal cells unaffected. Chronic myeloid leukemia is lethal, there is no drug directing towards the destruction of the leukemic cells, and these cells poorly respond to chemotherapy which is always non-specific thus adversely affecting normal cells.

[0029] Unique property of the therapy with analogs and/or salts of chlorogenic acid is the killing of myeloid cancer cells leaving other normal cells unaffected.

DETAILED DESCRIPTION OF THE INVENTION

[0030] Accordingly, the present invention provides a pharmaceutical composition useful for treating chronic myeloid leukemia where Bcr-Abl kinase is constitutively expressed in animals and humans, said composition comprising an effective amount of analogs of chlorogenic acid and/or its salts along with pharmaceutically acceptable additives.

[0031] In an embodiment of the invention, the said composition is also useful in treating diseases caused by over expression of Abl type of kinase

[0032] In another embodiment, the analogs of chlorogenic is selected from a group consisting of neochlorogenic acid (5-O-caffeoyl quinic acid), cryptochlorogenic acid (4-O-Caffeoyl quinic acid), 3-O-(3'-methylcaffeoyl) quinic acid and 5-O-(Caffeoyl-4'-methyl) quinic acid.

[0033] Still another embodiment, the analogs of chlorogenic acid are obtained either from natural sources or synthetically prepared.

[0034] Still another embodiment, the salt of chlorogenic acid analog is selected from sodium, potassium and ammonium.

[0035] Yet another embodiment, the additive is selected from a group consisting of nutrients such as proteins, carbohydrates, sugars, talc, magnesium stearate, cellulose, calcium carbonate, starch-gelatin paste and/or pharmaceutically acceptable carriers, excipients, diluents or solvents.

[0036] Another embodiment of the invention related to method of administering the composition to a subject. The composition may administered through oral, intravenous, intramuscular or subcutaneous routes.

[0037] Yet another embodiment, the said composition is administered at a dose level ranging between about 1 and 100 mg per kg body weight/day, preferably in the range of 1 to 25 mg/kg body weight.

[0038] Yet another embodiment, the said composition may be administered for period ranging between at least four weeks and up to twelve weeks, and in case of relapse it can again can be administered to the subject without any toxicity.

[0039] Yet another embodiment, the said composition inhibits the growth of leukemic cell types K562. and Molt-4.

[0040] Yet another embodiment, the said composition inhibits the growth of leukemic cell types Molt-4.

[0041] Yet another embodiment, the said composition, IC_{50} value for in vitro activity against K562 cells of concentration of 10^4 /well is up to 27.0 nanomole/ml.

[0042] One more embodiment of the invention provides a method of treating a subject suffering from chronic myeloid leukemia where Bcr-Abl kinase is constitutively expressed in animals and humans or any diseases caused by the over expression of Abl type of kinase.

[0043] In another embodiment, the IC_{50} values for chlorogenic acid and sodium chlorogenate on K562 cells is 13.5 and 27.0 $\mu\text{M}/10^4$ cells respectively (FIGS. 2C & 2D). Another embodiment, the acute toxicity of sodium chlorogenate in mouse model, wherein the compound is non-toxic up to a dose level of 2 gm/kg body weight in oral route.

[0044] The invention is described in details with reference to the examples given below which are provided to illustrate the invention and therefore, should not be construed to limit the scope of the invention.

BRIEF DESCRIPTION OF THE ACCOMPANYING DRAWINGS

[0045] FIG. 1. Structure of sodium chlorogenate, one of the salts of chlorogenic acid.

[0046] FIG. 2. Purification of chlorogenic acid from *Piper betel* leaves and its effects on cell lines and Ph⁺ CML patients' PBMC in vitro. The flow diagram of purification is shown. The structure of peak 09 isolated from fraction E by HPLC was deduced as chlorogenic acid by spectroscopic methods (IR, NMR, ¹³CNMR, FABMS). Cell count assays were performed by plating cells in the presence of regular growth medium with or without indicated amount of extract (a), fraction (b), purified compound (c) and its sodium salt (d). Each day, viable cells were counted as assessed by exclusion of trypan blue. (e), Viability of PBMC obtained from three Ph⁺ CML patients and one Ph⁻ CML patient after treatment with Na-Chl (67.5 nmole/10⁵ cells). (f), Morphological changes of K562 cells after treatment with NaChl (67.5 nmole/10⁵ cells) for 6 h (phase contrast micrographs, ×400).

[0047] FIG. 3. Sodium chlorogenate induces apoptosis in Ph⁺ CML cell line and CML patient's PBMC.

[0048] a). Cells were left untreated (NT) or incubated with NaChl (67.5 nmole/10⁵ cells) for 6 h and processed for flow cytometry after staining with annexin V-FITC and PI. Viable cells are in the lower left quadrant. Apoptotic cells stained by annexin V are in the lower right quadrant. Late stage apoptotic cells stained by both annexin V and PI are in the upper right quadrant.

[0049] b). Treatment with NaChl results in early cell cycle arrest followed by apoptosis in Ph⁺ cells. Cells were cultured in the presence or absence of NaChl. After 1 or 2 days in culture, cells were collected, permeabilized, and stained with PI for DNA content analysis. Gates were set to assess the percentages of dead (<2n DNA, M1), G0/G1 (2n DNA, M2), and S+G2+M (>2n DNA, M3) cells.

[0050] c). Fluorescence images of K562 and Molt-4 cells after treatment with NaChl followed by staining with annexin V-AlexaTM staining with annexin V are shown in left panels and bright filed phase contrast views are shown in right panels.

[0051] d). Treatment with NaChl leads to activation of procaspase-3 in Ph⁺ cells. Cells were left untreated or treated with NaChl (T) for 24 h. Cells were harvested, lysed, equivalent amount of lysates were separated by SDS-PAGE and electro-transferred. The filters were probed with anti-caspase-3 that recognize both procaspase-3 (32 Kda, upper band) and caspase-3 cleavage product (17 Kda, lower band).

[0052] FIG. 4. Sodium chlorogenate inhibits Bcr-Abl autophosphorylation in Ph⁺ cells.

[0053] a) Flow cytometric determination of Abl phosphorylation status. Cells were left untreated (NT) or incubated with NaChl (T) for 3 h, permeabilized and stained with rabbit anti-phospho-c-Abl (Tyr245) antibody. Dotted line, staining with normal rabbit IgG; solid line, staining with immune IgG.

[0054] b) Immuno-blot-based determination of Abl phosphorylation status. Cells were harvested, lysed, equivalent amount lysates were separated by SDS-PAGE and electrotransferred. The filters were probed with anti-phospho-c-Abl (Tyr 245) (upper panels) or anti-c-Abl antibody (middle panels). To demonstrate equal protein levels in the samples analysed, anti-actin antibody was also used as loading controls (bottom panes).

[0055] c) Flow cytometric determination of Abl expression status. Cells were permeabilized and stained with rabbit anti-c-Abl antibody. Dotted line, staining with normal rabbit IgG; solid line, staining with immune IgG.

[0056] d) Structures of the complexes of the inactive (Panel-A & B) form of Abl tyrosine kinase with gleevec (A) and Chl (B) and active (Panel-C & D) form of the kinase with PD173955 (C) and Chl (D). Structures of complexes of the inactive form with gleevec (A) and active form with PD173955 (C) were obtained by x-ray crystallography. Structures of complexes of the inactive form with Chl (B) and active form with Chl (D) were modeled using those x-ray structures. Ribbons show the binding pocket of the kinase; yellow lines are the parts of activation loop, which differs in inactive (A&B) and active (C&D) conformations. Envelopes around the inhibitor molecules are their Connolly surfaces.

EXAMPLES

Example 1

[0057] Preparation of Sodium Chlorogenate:

[0058] 4.7 kg of *Piper betel* leaves freshly collected, washed with distilled water and then cut into small pieces. Small pieces of leaves were gathered together and mixed with 1.0 litre of distilled water and thoroughly homogenized in a mixture blender. The homogenate was passed through a fine cheesecloth to filter out the large particles and the filtrate was collected. The process was repeated 2-3 times to have maximum yield. The combined filtrate was then centrifuged, the aliquot, a clear solution, was collected and lyophilised to a semi-solid mass, which was about 110 gm. Collected material was examined for biological activity i.e. destruction of CML cells. On observing its positive activity, purification was initiated. 10 gm of above-mentioned material was loaded on Sephadex LH-20 column and chromatographed with water, water-methanol (1:1) and methanol as eluent. Three different fractions thus obtained from three different solvent systems were separately checked for biological activity. The activity was located only on Methanol-water (1:1) and termed as fraction E. Fraction E (0.23 g) was then subjected to preparative HPLC using M-Bonda pak column (19×300 mm) with a solvent system methanol:water:acetic acid (23:76:1), having flow rate of 12 ml/min and detection at 280 nm. A purified compound, chlorogenic acid (4 mg) was isolated from the peak (peak no. 09) having retention time 9.16 min.

[0059] Sodium Chlorogenate (13 mg) was prepared by sterring 10.5 mg of Chlorogenic acid with sodium hydrogen carbonate (3.6 mg) in 2 ml of water and lyophilised the resulting solution. It was tested for biological activity. The structure was thus determined as sodium chlorogenate (**FIG. 1**) IR, NMR, ¹³CNMR and FABMS m/z.

KBr

IR $\gamma_{\max}$ cm⁻¹: 3398(OH), 1685(CO), 1593, 1527
1449, 1443, 1394, 1279, 1159, 1118
1081, 1038, 975, 916 and 810

-continued

KBr	
¹ H-NMR (D ₂ O):	7.45(1H), 6.99(1H), 6.93(1H), 6.77(1H) 6.18(1H), 5.16(1H), 4.11(1H), 3.75(1H) and 2.09–1.85(4H)
¹³ C NMR (D ₂ O):	181.28, 169.49, 147.69, 146.45, 144.67, 127.14, 123.10, 116.51, 115.40, 114.68, 77.28, 73.33, 71.56, 71.17, 38.88 and 37.72
FABMS m/z:	355 (M ⁺ + H) and 377 (M ⁺ + Na)

Example 2

[0060] The Chlorogenic acid is available in the market in the pure form. The Chlorogenic acid (1 gm) was hand shaken with sodium hydrogen carbonate (024 g in 5 ml of water) solution. The solution was lyophilised to pure sodium chlogogenate (1.12 gm) and was tested for biological activity. Sodium chlorogenate prepared from chlorogenic acid which was either isolated from *Piper betel* or obtain commercially have similar structure and activity.

Example 3

[0061] Culture of Bcr-Abl positive CML cell line (K562), peripheral blood cells of CML patients, Bcr-Abl-negative ALL cell line (Molt-4) and peripheral blood cells of CML patients. Cell count assays were performed by plating cells in the presence of regular growth medium with or without indicated amount of extract, fraction, purified compound and its sodium salt. Each day, viable cells were counted as assessed by exclusion of trypan blue.

Example 4

[0062] Morphology analysis of Bcr-Abl positive CML cell line K562 by phase contrast microscopy. Cells were left untreated (NT) or treated with NaChl (Nachl; 67.5 nmole/10⁵ cells) and viewed under phase contrast microscope (magnification ×400).

Example 5

[0063] Measurement of apoptosis by flow cytometry. Cells were left untreated or treated with NaChl (67.5 nmole/10⁵ cells) for 6 h. After washing, cells were stained with fluorescein isothiocyanate (FITC) conjugated Annexin V and propidium iodide (PI) and analysed in a flow cytometer (FACS Calibur, Beckton Dickinson, USA).

Example 6

[0064] Confocal microscopy. K562 and Molt-4 cells were treated with NaChl followed by staining with Annexin-V-Alexa™ as described in example 5, and allowed to adhere onto poly-L-lysine-coated coverslips for 10 min. Representative fields of cells were analysed with a Leica TCS SP2 confocal laser scanning microscope (Heidelberg, Germany).

Example 7

[0065] DNA cell cycle analysis. Cells were cultured with NaChl as described in example 5. After 1 or 2 days culture, cells were collected, permeabilized and stained with PI for DNA cell cycle analysis.

Example 8

[0066] Immunoblot assay. Cells were harvested, lysed, equivalent amount of lysates were separated by SDS-PAGE and electro-transferred. The filters were probed with anti-caspase-3 antibody (B.D. Pharmingen), anti-c-Abl antibody or anti-phospho-c-Abl antibody (Cell Signaling Technology).

Example 9

[0067] Flow cytometric determination of Abl phosphorylation or Abl expression status. Cells were permeabilysed, stained with rabbit anti-phospho-c-Abl antibody, anti-c-Abl antibody or control rabbit antibody and analysed in a flow cytometer.

Example 10

[0068] Structures of the complexes of Chl with the inactive and active forms of the kinase were modeled using the InsightII 98.0 (Accelrys). Models of the complexes were built using two recently determined structures of two complexes of the enzyme with two important drug molecules, which have some structural and functional similarities with Chl. The structure of Chl was built and optimized by repeated minimization and dynamic simulations. The initial structure of a complex was built by superposing a functional group of Chl with a similar group of the experimental structure of the drug molecule. It was optimized by energy minimization (100 steps each of steepest descent and conjugate gradient methods) using cff91 force field. Then dynamics was run for 1000 steps of one fempto second each after 100 steps of equilibration with a conformational sampling of 1 in 10 steps at 300° K. At the end of the simulation the conformation with lowest potential energy was picked for the next cycle of simulation. This combination of minimization and dynamics were repeated until a satisfactory conformation was obtained. A series of optimizations were done with varying initial conditions in the cavity of the binding pocket. Position constraints were applied to the atoms which were more than 10 Å away during energy minimization and molecular dynamics.

[0069] Results of Examples 3 & 4:

[0070] Water extract of *Piper betel* leaves killed K562 cells in a dose dependent manner (FIG. 2a). The extract had no appreciable effect on AAL cell line Molt-4. Fraction E induced killing activity of K562 cells was restricted to peak 09 (FIG. 2b) which was subsequently identified as chlorogenic acid (FIG. 2c) and showed higher killing activity of K562 cells compare to crude extract or fraction E. Interestingly, sodium chlorogenate (NaChl) is two-fold more potent than chlorogenic acid in killing K562 cells (FIG. 2d). NaChl is also active in killing Philadelphia chromosome (Bcr-Abl) positive CML patients peripheral blood mononuclear cells (PBMC) (FIG. 2e). NaChl has no effect on Bcr-Abl negative CML patients PBMC (FIG. 2e). Phase contrast microscopy indicates that NaChl induces morphological changes (nuclear condensation) in K562 cells, sign of apoptosis (FIG. 2f).

[0071] The IC₅₀ values for chlorogenic acid and sodium chlorogenate on K562 cells is 13.5 and 27.0 μm/10⁴ cells respectively FIGS. 2C & 2D. With respect to acute toxicity of sodium chlorogenate in mouse model, the compound is non-toxic upto the dose level of 2 gm/kg body weight in oral route.

[0072] Results of Examples 5 to 9:

[0073] Treatment with NaChl did not induce apoptosis in Molt-4 cells, normal PBMC or PBMC of Bcr-Abl negative CML patients. In contrast, the same treatment cause an increase in apoptosis in Bcr-Abl positive K562 cells and PBMC of Bcr-Abl positive CML patients (FIG. 3a). DNA cell cycle analysis also indicates that NaChl induces cell cycle arrest followed by breakdown of DNA in Bcr-Abl positive CML cell line K562 cells and PBMC of Bcr-Abl positive CML patients (FIG. 3b). Confocal microscopy indicating positive Annexin V staining in K562 cells but not in Molt-4 cells after treatment with NaChl is shown in FIG. 3c. Apoptosis in K562 cells, PBMC of Bcr-Abl (Ph) positive CML patients but not in Molt-4 cells or PBMC of Bcr-Abl negative CML patients or of normal donors was further confirm by immunoblot detection of caspase 3 activation (FIG. 3d). NaChl inhibits phosphorylation of Bcr-Abl protein tyrosine kinase without affecting the expression of Abl protein level. This is evident by flow cytometric detection of phospho-c-Abl status (FIG. 4a), and by immunoblot detection (FIG. 4b, upper panels). Expression of Abl protein was analysed also by flow cytometry (FIG. 4c) and immunoblot (FIG. 4b, middle panels). Our finding, therefore, indicates that sodium chlorogenate inhibits phosphorylation of Abl protein tyrosine kinase including Bcr-Abl kinase leading to apoptosis of cells. The IC₅₀ values for chlorogenic acid and sodium chlorogenate on K562 cells is 13.5 and 27.0 $\mu\text{M}/10^4$ cells respectively FIGS. 2C & 2D. With respect to acute toxicity of sodium chlorogenate in mouse model, the compound is non-toxic upto the dose level of 2 gm/kg body weight in oral route.

[0074] Results of Example 10:

[0075] FIG. -4d shows that chlorogenic acid (Chl, Panel-B) can fit into the binding pocket of Abi kinase in the inactive conformation in a position similar to that of Gleevec (Panel-A) and in the active conformation (Panel-D) similar to that of PD173955 (Panel-C). Empirical energies associated with the docking of the ligand into the binding pockets of the active and inactive conformations of the kinase are negative and comparable to those of other small molecule inhibitors e.g. Gleevec and PD173955 indicating stable complex formation. Binding energies of Chl in charged and neutral forms are different and the magnitude of electrical interactions depends on the electrical state of the molecule unlike the neutral inhibitors. Modeling studies indicate that Chl can bind to both the active and inactive conformations of the kinase like PD173955. Chl forms a number of hydrogen bonds with the surrounding residues as found in the complex of Gleevec while keeping some of the hydrophobic interactions intact. In comparison to PD173955, Chl forms higher number hydrogen bonds while maintaining similar number of hydrophobic contacts. It has been found that the aromatic hydroxyl groups of Chl forms a network of hydrogen bonds in the binding pocket suggesting the importance of these groups in the complex formation.

1. A pharmaceutical composition useful for treating chronic myeloid leukemia where Bcr-Abl kinase is constitutively expressed in animals and humans, said composition comprising an effective amount of analogs of chlorogenic acid and/or its salts along with pharmaceutically acceptable additives.

2. A composition as claimed in claim 1, wherein said composition is also useful for diseases caused by over expression of Abl type of kinase

3. The composition as claimed in claim 1, wherein the analogs of chlorogenic is selected from a group consisting of neochlorogenic acid (5-O-caffeoyl quinic acid), cryptochlorogenic acid (4-O-Caffeoyl quinic acid), 3-O-(3'-methylcaffeoyl) quinic acid and 5-O-(Caffeoyl-4'-methyl) quinic acid.

4. The composition as claimed in claim 1, wherein the analogs of chlorogenic acid are obtained either from natural sources or synthetically prepared.

5. The composition as claimed in claim 1, wherein the salt of chlorogenic acid analog is selected from sodium, potassium and ammonium.

6. The composition as claimed in claim 1 wherein, the additive is selected from a group consisting of nutrients such as proteins, carbohydrates, sugars, talc, magnesium stearate, cellulose, calcium carbonate, starch-gelatin paste and/or pharmaceutically acceptable carriers, excipient, diluents or solvents.

7. The composition as claimed in claim 1 is administered through oral, intravenous, intramuscular or subcutaneous routes.

8. The composition as claimed in claim 1 is administered at a dose level ranging between 1 and 20 mg per kg body weight/day.

9. The composition as claimed in claim 1, which is administered for at least four weeks and up to twelve weeks.

10. The composition as claimed in claim 1, wherein said composition is also useful for relapsed conditions of CML.

11. The composition as claimed in claim 1 wherein, the said composition inhibits the growth of leukemic cell types K562 and Molt-4.

12. The composition as claimed in claim 1 IC₅₀ value for in vitro activity against K562 cells of concentration of 10^4 /well is up to 27.0 nanomole/ml.

13. Use of pharmaceutical composition as claimed in claim 1 for the treatment of chronic myeloid leukemia in a subject where Bcr-Abl kinase is constitutively expressed in animals and humans, said composition comprising an effective amount of analogs of chlorogenic acid and/or its salts along with pharmaceutically acceptable additives.

14. A use as claimed in claim 13 wherein the subject is selected from a mammal preferably a human being.

15. A use as claimed in claim 13, wherein said composition is also useful for diseases caused by over expression of Abl type of kinase.

16. A use as claimed in claim 13, wherein the analogs of chlorogenic is selected from a group consisting of neochlorogenic acid (5-O-caffeoyl quinic acid), cryptochlorogenic acid (4-O-Caffeoyl quinic acid), 3-O-(3'-methylcaffeoyl) quinic acid and 5-O-(Caffeoyl-4'-methyl) quinic acid.

17. A use as claimed in claim 13, wherein the analogs of chlorogenic acid are obtained either from natural sources or synthetically prepared.

18. A use as claimed in claim 13, wherein the salt of chlorogenic acid analog is selected from sodium, potassium and ammonium.

19. A use as claimed in claim 13 wherein, the additive is selected from a group consisting of nutrients such as proteins, carbohydrates, sugars, talc, magnesium stearate, cellulose, calcium carbonate, starch-gelatin paste and/or pharmaceutically acceptable carriers, excipient, diluents or solvents.

20. A use as claimed in claim 13, wherein said composition is administered through oral, intravenous, intramuscular or subcutaneous routes.

21. A use as claimed in claim 13 wherein the said composition is administered at a dose level ranging between 1 and 20 mg per kg body weight/day.

22. A use as claimed in claim 13 wherein the said composition is administered for at least four weeks and up to twelve weeks.

23. A use as claimed in claim 13 wherein said composition is also useful for relapsed conditions of CML.

24. A use as claimed in claim 13 wherein, the said composition inhibits the growth of leukemic cell types K562 and Molt-4.

25. A use as claimed in claim 13 wherein IC_{50} value of the composition for in vitro activity against K562 cells of concentration of 10^4 /well is up to 27.0 nanomole/ml.

26. A method of treating chronic myeloid leukemia CML where Bcr-Abl kinase is constitutively expressed in animals and humans, said method comprising administering a pharmaceutical composition comprising an effective amount of analogs of chlorogenic acid and/or its salts along with pharmaceutically acceptable additives.

27. A method as claimed in claim 26, wherein the said composition is also useful for diseases caused by over expression of Abl type of kinase.

28. The method as claimed in claim 26, wherein the analogs of chlorogenic is selected from a group consisting of neochlorogenic acid (5-O-caffeoyl quinic acid), cryptochlorogenic acid (4-O-Caffeoyl quinic acid), 3-O-(3'-methylcaffeoyl) quinic acid and 5-O-(Caffeoyl-4'-methyl) quinic acid.

29. The method as claimed in claim 26, wherein the analogs of chlorogenic acid are obtained either from natural sources or synthetically prepared.

30. The method as claimed in claim 26, wherein the salt of chlorogenic acid analog is selected from sodium, potassium and ammonium.

31. The method as claimed in claim 26, wherein, the additive is selected from a group consisting of nutrients such as proteins, carbohydrates, sugars, talc, magnesium stearate, cellulose, calcium carbonate, starch-gelatin paste and/or pharmaceutically acceptable carriers, excipient, diluents or solvents.

32. The method as claimed in claim 26, wherein the said composition is administered through oral, intravenous, intramuscular or subcutaneous routes.

33. The method as claimed in claim 26, wherein the said composition is administered at a dose level ranging between 1 and 20 mg per kg body weight/day.

34. The method as claimed in claim 26, wherein the said composition is administered for at least four weeks and up to twelve weeks.

35. The method as claimed in claim 26, wherein the said composition is also useful for relapsed conditions of CML.

36. The method as claimed in claim 26 wherein, the said composition inhibits the growth of leukemic cell types K562 and Molt-4.

37. The method as claimed in claim 26, wherein IC_{50} value of the composition for in vitro activity against K562 cells of concentration of 10^4 /well is up to 27.0 nanomole/ml.

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