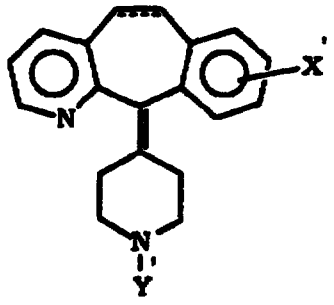
TRICYCLIC CARBAMATE COMPOUNDS USEFUL FOR INHIBITION OF G-PROTEIN FUNCTION AND FOR TREATMENT OF PROLIFERATIVE DISEASES

BACKGROUND

International Publication Number WO92/11034, published July 9, 1992, discloses a method of increasing the sensitivity of a tumor to an antineoplastic agent, which tumor is resistant to the antineoplastic agent, by the concurrent administration of the antineoplastic agent and a potentiating agent of the formula:



wherein Y' is hydrogen, substituted carboxylate or substituted sulfonyl. For example, Y' can be, amongst others, -COOR' wherein R' is C1 to C6 alkyl or substituted alkyl, phenyl, substituted phenyl, C7 to C12 aralkyl or substituted aralkyl or -2, -3, or -4 piperidyl or N-substituted piperidyl. Y' can also be, amongst others, SO_2R' wherein R' is C1 to C6 alkyl, phenyl, substituted phenyl, C7 to C12 aralkyl or substituted aralkyl. Examples of such potentiating agents include 11-(4-piperidylidene)-5H-benzo[5,6]cyclohepta[1,2-b]pyridines such as Loratadine.

To acquire transforming potential, the precursor of the Ras oncoprotein must undergo farnesylation of the cysteine residue located in a carboxyl-terminal tetrapeptide. Inhibitors of the enzyme that catalyzes this modification, farnesyl protein transferase, have therefore been suggested as anticancer agents for tumors in which Ras contributes to transformation. Mutated, oncogenic forms of ras are frequently found in many human cancers, most notably in more than 50% of colon and pancreatic carcinomas (Kohl et al., Science, Vol. 260, 1834 to 1837, 1993).

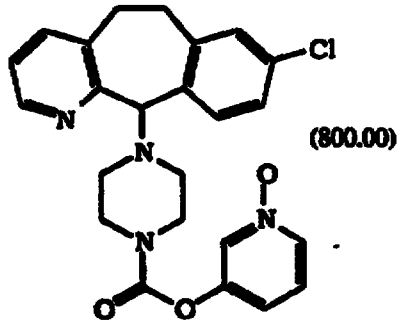
A welcome contribution to the art would be compounds useful for the inhibition of farnesyl protein transferase. Such a contribution is provided by this invention.

SUMMARY OF THE INVENTION

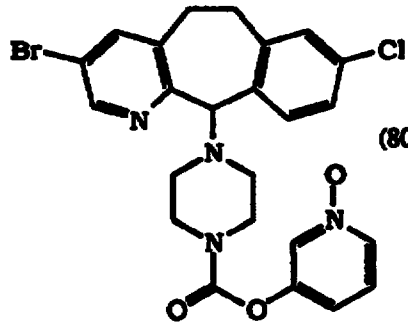
Inhibition of farnesyl protein transferase by tricyclic compounds of this invention has not been reported previously. Thus, this invention provides a method for inhibiting farnesyl protein transferase using tricyclic compounds of this invention which: (i) potently inhibit farnesyl protein transferase, but not geranylgeranyl protein transferase I, in vitro; (ii) block the phenotypic change induced by a form of transforming Ras which is a farnesyl acceptor but not by a form of transforming Ras engineered to be a geranylgeranyl acceptor; (iii) block intracellular processing of Ras which is a farnesyl acceptor but not of Ras engineered to be a geranylgeranyl acceptor; and (iv) block abnormal cell growth in culture induced by transforming Ras.

This invention provides a method for inhibiting the abnormal growth of cells, including transformed cells, by administering an effective amount of a compound of this invention. Abnormal growth of cells refers to cell growth independent of normal regulatory mechanisms (e.g., loss of contact inhibition). This includes the abnormal growth of: (1) tumor cells (tumors) expressing an activated Ras oncogene; (2) tumor cells in which the Ras protein is activated as a result of oncogenic mutation in another gene; and (3) benign and malignant cells of other proliferative diseases in which aberrant Ras activation occurs.

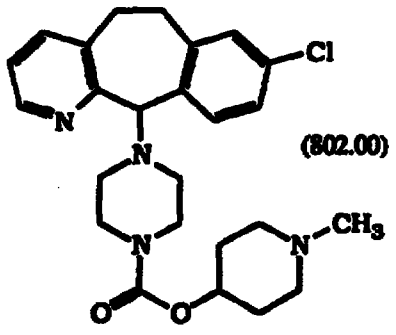
The novel compounds of this invention are:

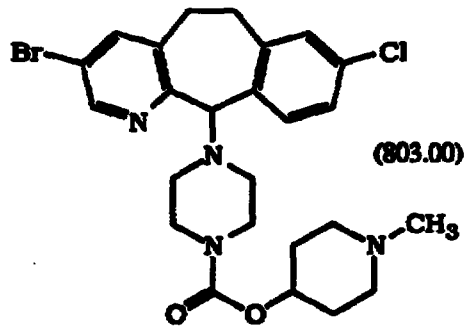


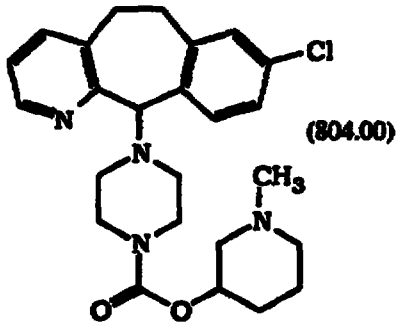
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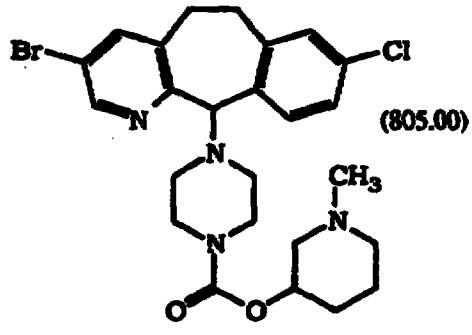


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, and

These compounds are used in the methods of this invention. Preferred compounds useful in this invention are represented by Formulas 801.00, 802.00, 803.00, 804.00 and 805.00.

This invention also provides a method for inhibiting tumor growth by administering an effective amount of the tricyclic compounds, described herein, to a mammal (e.g., a human) in need of such treatment. In particular, this invention provides a method for inhibiting the growth of tumors expressing an activated Ras oncogene by the administration of an effective amount of the above described compounds. Examples of tumors which may be inhibited include, but are not limited to, lung cancer (e.g., lung adenocarcinoma), pancreatic cancers (e.g., pancreatic carcinoma such as, for example, exocrine pancreatic carcinoma), colon cancers (e.g., colorectal carcinomas, such as, for example, colon adenocarcinoma and colon adenoma), myeloid leukemias (for example, acute myelogenous leukemia (AML)), thyroid follicular cancer, bladder carcinoma, and myelodysplastic syndrome (MDS).

It is believed that this invention also provides a method for inhibiting proliferative diseases, both benign and malignant, wherein Ras proteins are aberrantly activated as a result of oncogenic mutation in other genes--i.e., the Ras gene itself is not activated by mutation to an oncogenic form--

with said inhibition being accomplished by the administration of an effective amount of the tricyclic compounds described herein, to a mammal (e.g., a human) in need of such treatment. For example, the benign proliferative disorder neurofibromatosis, or tumors in which Ras is activated due to mutation or overexpression of tyrosine kinase oncogenes (e.g., neu, src, abl, lck, lyn, fyn), may be inhibited by the tricyclic compounds described herein.

The compounds of this invention inhibit farnesyl protein transferase and the farnesylation of the oncogene protein Ras. This invention further provides a method of inhibiting ras farnesyl protein transferase, in mammals, especially humans, by the administration of an effective amount of the tricyclic compounds described above. The administration of the compounds of this invention to patients, to inhibit farnesyl protein transferase, is useful in the treatment of the cancers described above.

The tricyclic compounds useful in the methods of this invention inhibit abnormal cellular growth. Without wishing to be bound by theory, it is believed that these compounds may function through the inhibition of G-protein function, such as ras p21, by blocking G-protein isoprenylation, thus making them useful in the treatment of proliferative diseases such as tumor growth and cancer. Without wishing to be bound by theory, it is believed that these compounds inhibit ras farnesyl protein transferase, and thus show antiproliferative activity against ras transformed cells.

DETAILED DESCRIPTION OF THE INVENTION

Certain compounds of the invention may exist in different isomeric (e.g., enantiomers and diastereoisomers) forms. The invention contemplates all such isomers both in pure form and in admixture, including racemic mixtures. Enol forms are also included.

The compounds of the invention can exist in unsolvated as well as solvated forms, including hydrated forms, e.g., hemi-hydrate. In general, the solvated forms, with pharmaceutically acceptable solvents such as water, EtOH and the like are equivalent to the unsolvated forms for purposes of the invention.

Certain basic tricyclic compounds also form pharmaceutically acceptable salts, e.g., acid addition salts. For example, the pyrido-nitrogen atoms may form salts with strong acid, while compounds having basic substituents such as amino groups also form salts with weaker acids. Examples of suitable acids for salt formation are hydrochloric, sulfuric,

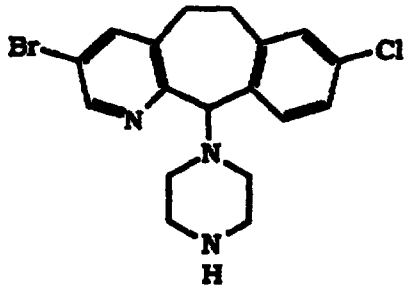
phosphoric, acetic, citric, oxalic, malonic, salicylic, malic, fumaric, succinic, ascorbic, maleic, methanesulfonic and other mineral and carboxylic acids well known to those in the art. The salts are prepared by contacting the free base form with a sufficient amount of the desired acid to produce a salt in the conventional manner. The free base forms may be regenerated by treating the salt with a suitable dilute aqueous base solution such as dilute aqueous sodium hydroxide, potassium carbonate, ammonia and sodium bicarbonate. The free base forms differ from their respective salt forms somewhat in certain physical properties, such as solubility in polar solvents, but the acid and base salts are otherwise equivalent to their respective free base forms for purposes of the invention.

All such acid and base salts are intended to be pharmaceutically acceptable salts within the scope of the invention and all acid and base salts are considered equivalent to the free forms of the corresponding compounds for purposes of the invention.

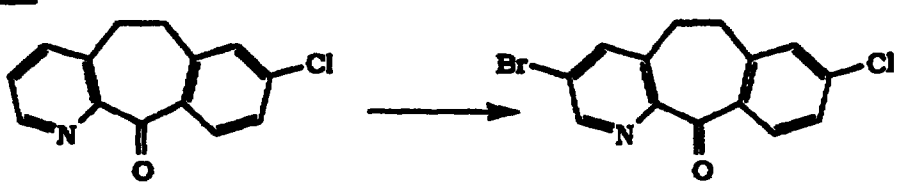
Compounds 800.00 to 805.00 may be made by the methods described in WO 95/10515, published April 20, 1995 (e.g. see the preparations described for Formula 400.00), and by the methods described in the examples below.

In the examples, MH^+ represents the molecular ion plus hydrogen of the molecule in the mass spectrum. Also, the following solvents and reagents are referred to herein by the abbreviations indicated: methanol (MeOH); ethyl acetate (EtOAc); and N,N-dimethylformamide (DMF).

PREPARATIVE EXAMPLE 1

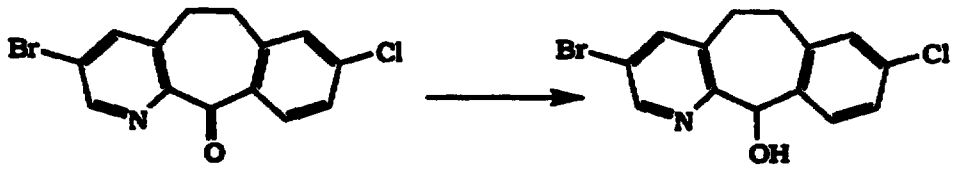


Step A:



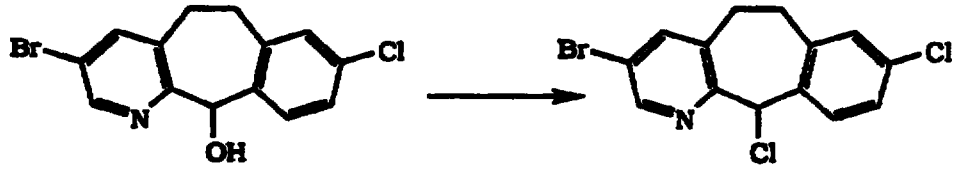
Cool 50.0 g (20.5 mmol) of 8-Chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-one to 0°C and slowly add 75 mL of sulfur monochloride over 20 minutes. Add 25 mL (48.59 mmol) of Br_2 over 15 minutes, then heat at 95°C for 20 hours. Add 12.5 mL (24.3 mmol) of Br_2 over 15 minutes and heat for 24 hours more. Cool the mixture and slowly add it to a mixture of CH_2Cl_2 and 1N NaOH (aqueous) at 0°C. Wash the organic phase with water dry over $MgSO_4$, and concentrate *in vacuo* to a residue. Chromatograph (silica gel, 500 mL of CH_2Cl_2, then 0.2%-5% (10% concentrated NH_4OH in MeOH)-CH_2Cl_2), then rechromatograph (silica gel, 3-8.5% EtOAc/hexane) to give 8.66 g of the product compound. Mass Spec.: $MH^+ = 322$

Step B:



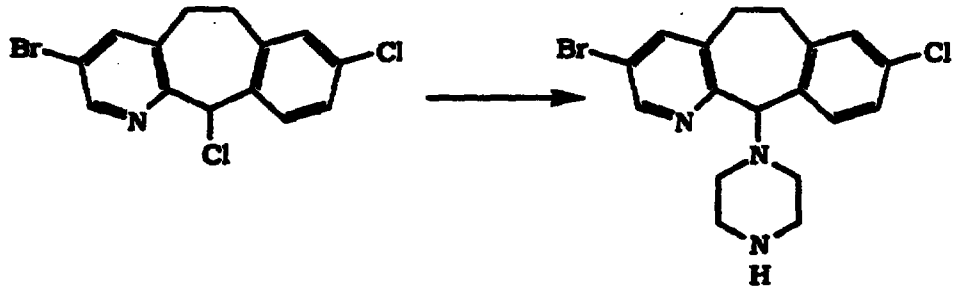
Combine 6.84 g (21.2 mmol) of the product of Step A 160.5 mL of MeOH and treat with 1.1709 g of $NaBH_4$ as described in Preparative Example 7, Step A, of WO 95/10515, to give 5.93 g of the product compound. MH^+ 326

Step C:



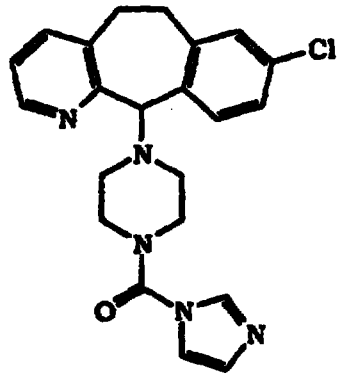
Combine 5.93 g (18.3 mmol) of the product of Step B and 116 mL of anhydrous toluene, cool to 0°C, and slowly add (dropwise) a solution of 2.465 g (33.9 mmol) of $SOCl_2$ in 23 mL of anhydrous toluene over a period of 0.5 hours. Stir at 0°C for 1.5 hours and at 0°-25°C for 2 hours, then work up as described in Preparative Example 7, Step B, of WO 95/10515, to give the product compound.

Step D:



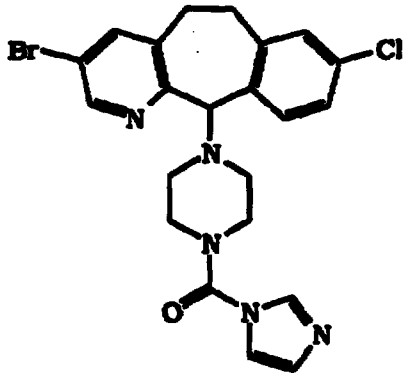
React 18.3 mmol of the product of Step C with 9.94 g (91.5 mmol) of piperazine via the procedure described in Preparative Example 7, Step C, of WO 95/10515, to give 8.0 g of the title compound. Mass Spec.: MH^+ = 394

PREPARATIVE EXAMPLE 2



Combine 10 g (31.9 mmol) of the product of Preparative Example 7, Step C, of WO 95/10515, 100 mL of dry CH_2Cl_2 and slowly (dropwise) add the solution to a mixture of 5.17 g (31.9 mmol) of carbonyldiimidazole in 150 mL of dry CH_2Cl_2 over 0.75 hours. Stir at 0°C for 2 hours, wash with water, dry over $MgSO_4$, and concentrate *in vacuo* to a residue. Chromatograph (silica gel, 2% (10% conc. NH_4OH in MeOH)/CH_2Cl_2) to give 8.71 g of the title compound. Mass Spec.: MH^+ = 408.2

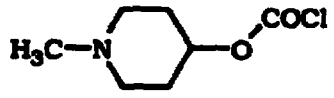
Using the product of Preparative Example 1, Step D, and essentially the same procedure as described for Preparative Example 2, the following compound is prepared:



Preparative Example 2-A

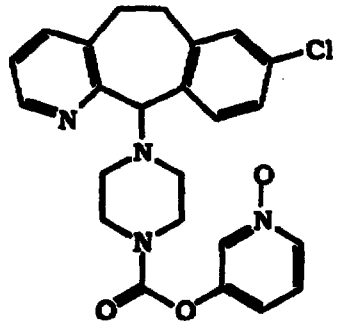
Mass Spec.: $MH^+ = 488.2$

PREPARATIVE EXAMPLE 3



Combine 10 mL of dry CH_2Cl_2 and 914.6 mL (28.1 mmol) of a 1.93 M solution of phosgene in toluene, cool to 0°C and slowly add (dropwise) a solution of 0.6484 g (5.62 mmol) of 3-hydroxy-1-N-methylpiperidine, 1.214 mL (15 mmol) of pyridine and 10 mL of dry CH_2Cl_2, over 10 min., then stir at 0°-25°C for 2 hours. Purge excess phosgene with N_2 then concentrate *in vacuo* to give the title compound.

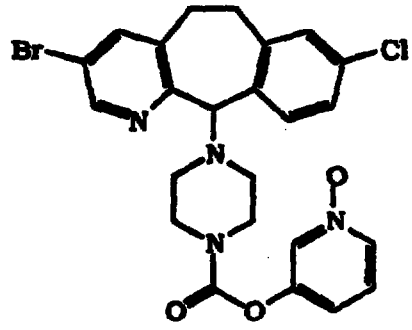
EXAMPLE 1



Combine 12 mL of dry CH_2Cl_2 and 12.58 mL (23.9 mmol) of a 28% soution of phosgene in toluene, cool to 0°C under Ar atmosphere and slowly add (dropwise) a solution of 0.5 g (1.59 mmol) of the product of Preparative Example 7, Step C, of WO 95/10515, 0.515 mL (6.36 mmol) of pyridine and 12 mL of dry CH_2Cl_2, over 0.75 hours, then warm the mixture to 12°C over 0.5 hours. Purge excess phosgene with Ar then concentrate *in vacuo* to a residue. Add 10 mL of DMF, 0.515 mL of pyridine and 0.885 g (7.95 mmol) of 3-hydroxypyridine-1-N-oxide and stir at 25°C for 18 hours. Dilute with CH_2Cl_2, wash with sat. $NaHCO_3$ (aqueous) and dry

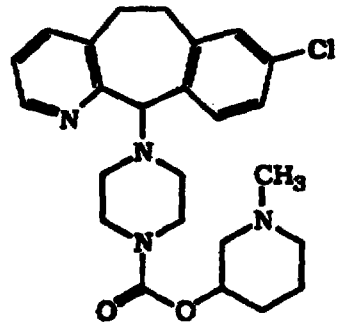
over $MgSO_4$. Concentrate *in vacuo* to a residue and chromatograph (silica gel, 1.5% (10% NH_4OH in MeOH)/CH_2Cl_2) to give 0.186 g of the title compound. Mass Spec.: $MH^+ = 451.3$

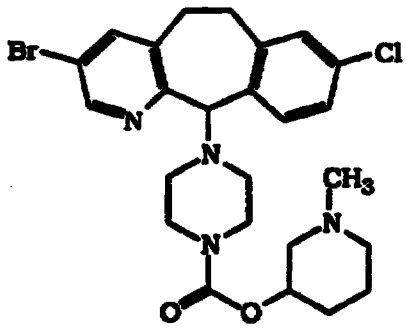
EXAMPLE 2



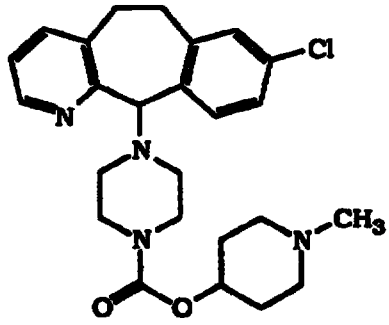
Combine 0.1 g (0.205 mmol) of the product of Preparative Example 2-A, 0.0463 g (0.205 mmol) of $ZnBr_2$, 0.0913 g (0.822 mmol) of 3-hydroxypyridine-1-N-oxide and 3 mL of dry DMF, and heat the mixture at 90°C for 51 hours, then stir at 25°C for 19 hours. Concentrate *in vacuo* to a residue and chromatograph (silica gel, 2% (10% NH_4OH in MeOH)/CH_2Cl_2) to give 0.0812 g of the title compound. Mass Spec.: $MH^+ = 531.1$

Using the starting compounds indicated and following essentially the same procedure as described for Example 2, the following compounds are obtained:

Starting Compound	Product Compound	Analytical Data
3-hydroxy-1-N-methylpiperidine and Preparative Example 2	 Example 2-A	Mass Spec.: $MH^+ = 455.25$

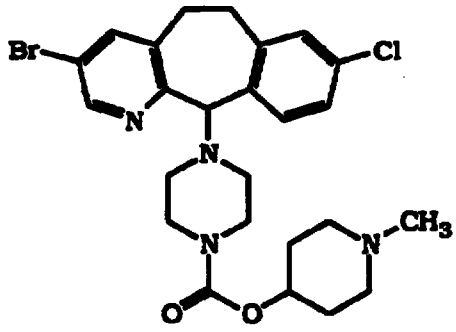
3-hydroxy-1-N-methylpiperidine and Preparative Example 2-A	 Example 2-B	Mass Spec.: MH^+ = 533.15

EXAMPLE 3



Combine 0.5 g (1.6 mmol) of the product of Preparative Example 7, Step C, of WO 95/10515, 0.849 g (4.8 mmol) of the title compound of Preparative Example 3, and 10 mL of 1:1 pyridine/CH_2Cl_2, and stir at 25°C for 19 hours. Workup as described for Example 4, of WO 95/10515, and chromatograph (silica gel, 3% (10% NH_4OH in MeOH)/CH_2Cl_2) to give 0.5231 g of the title compound. Mass Spec.: MH^+ = 455.25

Using the starting compound indicated and following essentially the same procedure as described for Example 3, the following compound is obtained:

Starting Compound	Product Compound	Analytical Data
Preparative Example 1	 Example 3-A	Mass Spec.: MH^+ = 533.15

FPT IC_{50} (inhibition of Farnesyl Protein Transferase by in vitro enzyme assays) and COS IC_{50} (Cell-Based Assay) were determined using the methods described in WO 95/10515. The results are given in Table 1 below. Cell Mat Assays and in vivo anti-tumor studies could be done by the methods described in WO 95/10515.

TABLE 1
FPT INHIBITION

COMPOUND	FPT IC_{50} (μM)	COS IC_{50} (μM)
(800.00) Example 1	0.01-10	-----
(801.00) Example 2	0.01-10	-----
(802.00) Example 3	10-100	----
(803.00) Example 3-A	0.01-10	10-100
(804.00) Example 2-A	0.01-10	-----
(805.00) Example 2-B	0.01-10	-----

The data demonstrate that the compounds of the invention are inhibitors of Ras-CVLS farnesylation by partially purified rat and human brain farnesyl protein transferase (FPT). The data also show that there are compounds of the invention which can be considered as potent (IC_{50} <10 μM) inhibitors of Ras-CVLS farnesylation by partially purified rat brain farnesyl protein transferase (FPT)--see Table 1.

For preparing pharmaceutical compositions from the compounds described by this invention, inert, pharmaceutically acceptable carriers can be either solid or liquid. Solid form preparations include powders, tablets, dispersible granules, capsules, cachets and suppositories. The powders and tablets may be comprised of from about 5 to about 70 percent active ingredient. Suitable solid carriers are known in the art, e.g. magnesium carbonate, magnesium stearate, talc, sugar, lactose. Tablets, powders, cachets and capsules can be used as solid dosage forms suitable for oral administration.

For preparing suppositories, a low melting wax such as a mixture of fatty acid glycerides or cocoa butter is first melted, and the active ingredient is dispersed homogeneously therein as by stirring. The molten homogeneous mixture is then poured into convenient sized molds, allowed to cool and thereby solidify.

Liquid form preparations include solutions, suspensions and emulsions. As an example may be mentioned water or water-propylene glycol solutions for parenteral injection.

Liquid form preparations may also include solutions for intranasal administration.

Aerosol preparations suitable for inhalation may include solutions and solids in powder form, which may be in combination with a pharmaceutically acceptable carrier, such as an inert compressed gas.

Also included are solid form preparations which are intended to be converted, shortly before use, to liquid form preparations for either oral or parenteral administration. Such liquid forms include solutions, suspensions and emulsions.

The compounds of the invention may also be deliverable transdermally. The transdermal compositions can take the form of creams, lotions, aerosols and/or emulsions and can be included in a transdermal patch of the matrix or reservoir type as are conventional in the art for this purpose.

Preferably the compound is administered orally.

Preferably, the pharmaceutical preparation is in unit dosage form. In such form, the preparation is subdivided into unit doses containing appropriate quantities of the active component, e.g., an effective amount to achieve the desired purpose.

The quantity of active compound in a unit dose of preparation may be varied or adjusted from about 0.1 mg to 1000 mg, more preferably from about 1 mg. to 300 mg, according to the particular application.

The actual dosage employed may be varied depending upon the requirements of the patient and the severity of the condition being treated. Determination of the proper dosage for a particular situation is within the skill of the art. Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under the circumstances is reached. For convenience, the total daily dosage may be divided and administered in portions during the day if desired.

The amount and frequency of administration of the compounds of the invention and the pharmaceutically acceptable salts thereof will be regulated according to the judgment of the attending clinician considering such factors as age, condition and size of the patient as well as severity of the symptoms being treated. A typical recommended dosage regimen is

oral administration of from 10 mg to 2000 mg/day preferably 10 to 1000 mg/day, in two to four divided doses to block tumor growth. The compounds are non-toxic when administered within this dosage range.

The following are examples of pharmaceutical dosage forms which contain a compound of the invention. The scope of the invention in its pharmaceutical composition aspect is not to be limited by the examples provided.

Pharmaceutical Dosage Form Examples

EXAMPLE A

Tablets

No.	Ingredients	mg/tablet	mg/tablet
1.	Active compound	100	500
2.	Lactose USP	122	113
3.	Corn Starch, Food Grade, as a 10% paste in Purified Water	30	40
4.	Corn Starch, Food Grade	45	40
5.	Magnesium Stearate	3	7
	Total	300	700

Method of Manufacture

Mix Item Nos. 1 and 2 in a suitable mixer for 10–15 minutes. Granulate the mixture with Item No. 3. Mill the damp granules through a coarse screen (e.g., 1/4", 0.63 cm) if necessary. Dry the damp granules. Screen the dried granules if necessary and mix with Item No. 4 and mix for 10–15 minutes. Add Item No. 5 and mix for 1–3 minutes. Compress the mixture to appropriate size and weigh on a suitable tablet machine.

EXAMPLE B - Capsules

No.	Ingredient	mg/capsule	mg/capsule
1.	Active compound	100	500
2.	Lactose USP	106	123
3.	Corn Starch, Food Grade	40	70
4.	Magnesium Stearate NF	7	7
	Total	253	700

Method of Manufacture

Mix Item Nos. 1, 2 and 3 in a suitable blender for 10-15 minutes. Add Item No. 4 and mix for 1-3 minutes. Fill the mixture into suitable two-piece hard gelatin capsules on a suitable encapsulating machine.

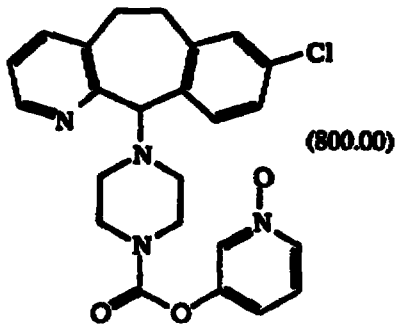
While the present invention has been described in conjunction with the specific embodiments set forth above, many alternatives, modifications and variations thereof will be apparent to those of ordinary skill in the art. All such alternatives, modifications and variations are intended to fall within the spirit and scope of the present invention.

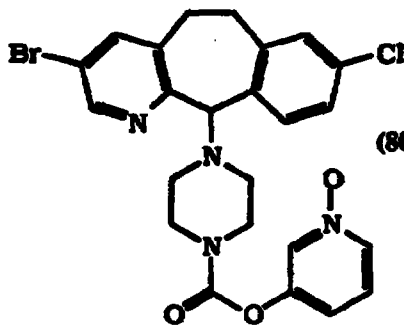
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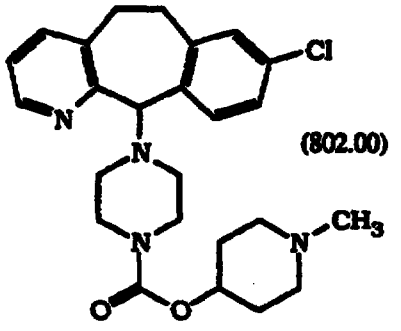
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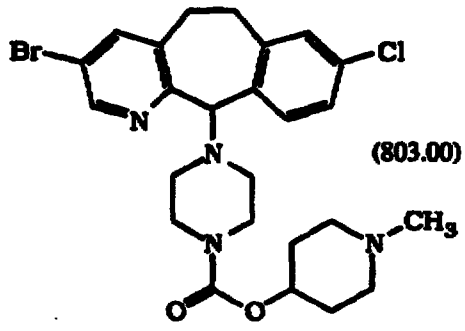
WHAT IS CLAIMED IS:

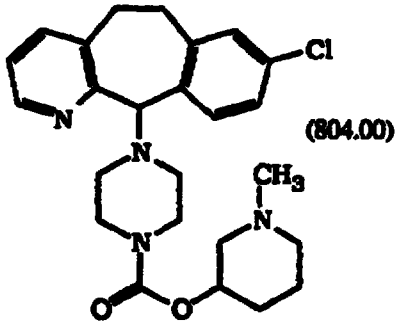
1. A method for inhibiting the abnormal growth of cells comprising administering an effective amount of a compound selected from:

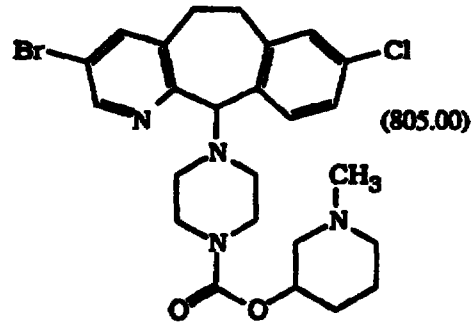

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, or

2. The method of Claim 1 wherein the cells inhibited are tumor cells expressing an activated Ras oncogene.

3. The method of Claim 2 wherein the cells inhibited are pancreatic tumor cells, lung cancer tumor cells, epidermal carcinoma tumor cells, myeloid leukemia tumor cells, thyroid follicular tumor cells, myelodysplastic cells, bladder carcinoma tumor cells or colon tumor cells.

4. The method of Claim 1 wherein the inhibition of the abnormal growth of cells occurs by the inhibition of farnesyl protein transferase.

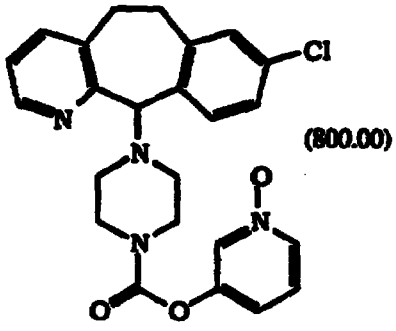
* C L M S *

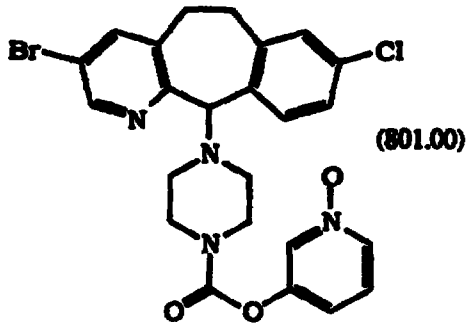
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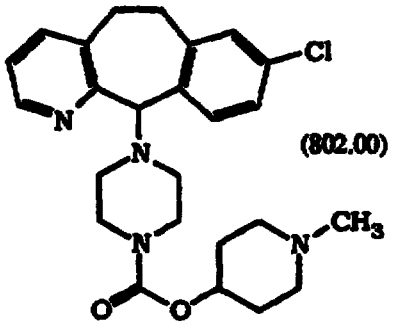
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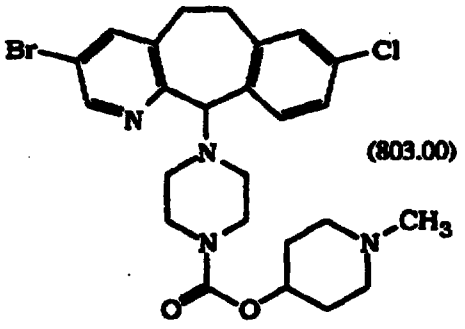
5. The method of Claim 1 wherein the inhibition is of tumor cells wherein Ras protein is activated as a result of oncogenic mutation in genes other than the Ras gene.

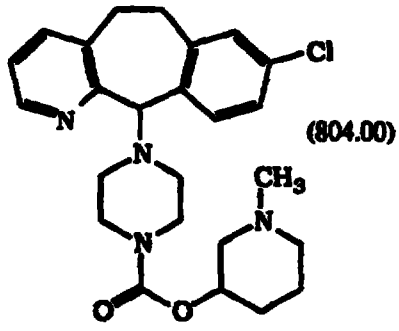
6. A compound selected from a compound of the formula:

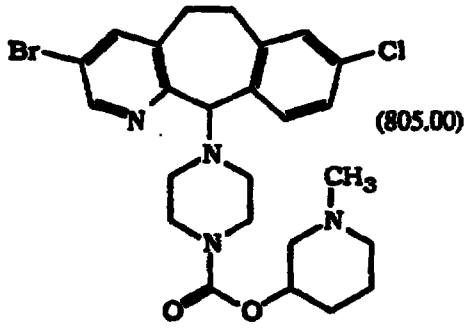

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,or

7. A pharmaceutical composition, for use in inhibiting the growth of abnormal cells, comprising a pharmaceutically acceptable carrier and an effective amount of a compound of Claim 6.

8. The use of a compound of Claim 6 for the manufacture of a medicament for use in inhibiting the growth of abnormal cells.

9. The use of a compound of Claim 6 in inhibiting the growth of abnormal cells