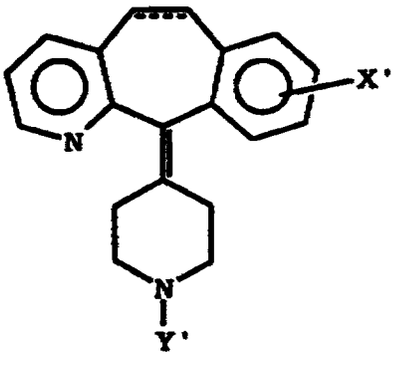
REPLACED SHEETS SG
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☐ Abstract : paged ..... to paged .....

WO 96/30017 PCT/US96/03306

TRICYCLIC 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. 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.

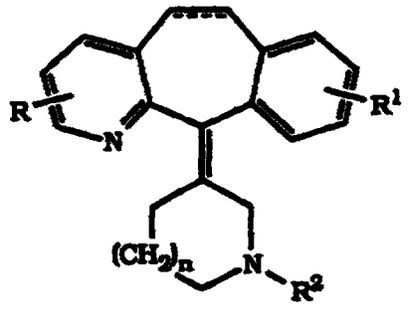
SUMMARY OF THE INVENTION

This invention provides a method for inhibiting farnesyl protein transferase (FPT) using the tricyclic compounds

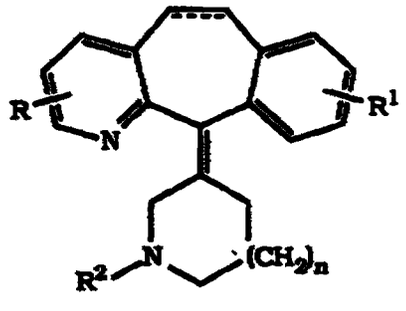
described below which: (i) potently inhibit FPT, but not geranylgeranyl protein transferase I, in vitro; (ii) block the phenotypic change induced by a form of transforming Ras which is a farnesyl acceptor but not by a form of transforming Ras engineered to be a geranylgeranyl acceptor; (iii) block intracellular 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 also provides a method for inhibiting the abnormal growth of cells, including transformed cells, by administering an effective amount of a compound of the present invention. Abnormal growth of cells means cell growth independent of normal regulatory mechanisms (e.g., loss of contact inhibition), including 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.

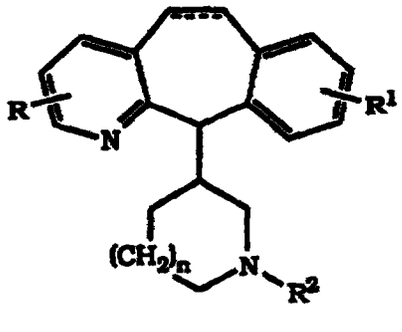
This invention provides compounds of the formula (Ia), (Ib) and (Ic)



(Ia),



(Ib)



(Ic)

wherein:

R and R^1 are independently selected from H, (C_1-C_6)alkyl, halogeno, OH, (C_1-C_6)alkoxy; NH_2; (C_1-C_6)alkylamino; di[(C_1-C_6)alkyl]amino; CF_3; SO_3H; CO_2R^3; NO_2; SO_2NH_2; and $CONHR^4$;

R^2 is $R^5C(O)$-, $R^5CH_2C(O)$-, $R^5C(R^6)_2C(O)$-, R^5SO_2-, $R^5CH_2SO_2$-, $R^5SCH_2C(O)$-, $R^5OC(O)$-, $R^5NHC(O)$-, $R^5C(O)C(O)$- or $R^5OC(S)$-;

R^3 is (C_1-C_6)alkyl, aryl;

R is (C_1-C_6)alkyl;

R^5 is (C_1-C_6)alkyl, aryl, aryl(C_1-C_6)alkyl, aryl(C_2-C_6)alkenyl, heteroaryl, heteroaryl(C_1-C_6)alkyl, heteroaryl(C_2-C_6)alkenyl or heterocycloalkyl;

Each R^6 independently represents (C_1-C_6)alkyl, or both R^4 groups together with the carbon atom to which they are attached comprise a (C_3-C_7)carbocyclic ring;

n is 0 or 1; and

the dotted line represents an optional double bond;

and pharmaceutically acceptable salts thereof.

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

As used herein, the following terms are used as defined below unless otherwise indicated:

"alkyl", including the alkyl portions of alkoxy, alkylamino and dialkylamino, means a straight or branched carbon chain containing from one to twenty carbon atoms, preferably one to six carbon atoms;

"alkenyl" means an alkyl group containing one or two double bonds;

"heterocycloalkyl" means a saturated carbocylic ring containing from 3 to 7 carbon atoms, preferably from 4 to 6 carbon atoms, which carbocyclic ring is interrupted by 1 to 3 heteroatoms selected from O, S and N, and includes heterocycloalkyls such as 2- or 3-tetrahydrofuranyl, 2-, 3- or 4-tetrahydropyranyl, 2- or 3- tetrahydrothienyl, 2-, 3- or 4-

piperidinyl, 2- or 3-pyrrolidinyl, 2- or 3-piperazinyl and 2- or 3-dioxanyl;

"aryl" represents a carbocyclic aromatic group containing from 6 to 10 carbon atoms, such as phenyl or naphthyl, said carbocyclic group being optionally substituted with 1-3 substituents selected from halogeno, (C_1-C_6)alkyl, hydroxy, (C_1-C_6)alkoxy, phenoxy, CF_3, amino, alkylamino, dialkylamino, $CH_3C(O)NH$-, $CH_3C(O)O$-, NO_2 and -$COOR^8$, wherein R^8 is H or (C_1-C_6)alkyl;

"halogeno" means fluoro, chloro, bromo and iodo; and

"heteroaryl" means a cyclic aromatic group, containing 5 to 10 ring members, comprising 2 to 9 carbon atoms and 1 to 3 heteroatoms selected from O, S, N and N→O, wherein N→O represents an N-oxide, and includes heteroaryls such as 2-, 3- or 4-pyridyl, 2-, 3- or 4- pyridyl N-oxide, imidazolyl, pyrazolyl, triazolyl, thienyl and furanyl, which heteroaryl group is optionally substituted by 1 to 3 substituents selected from halogeno, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, amino, alkylamino, dialkylamino, $C_6H_5C(O)NHCH_2$- and -$COOR^8$, wherein R^8 is H or (C_1-C_6)alkyl.

Lines drawn into the ring systems indicate that the indicated bond may be attached to any of the substitutable ring carbon atoms.

Certain compounds of the invention may exist in different isomeric forms (e.g., enantiomers and diastereoisomers). 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, ethanol and the like are equivalent to the unsolvated forms for purposes of the invention.

Certain tricyclic compounds will be acidic in nature, e.g. those compounds which possess a carboxyl or phenolic hydroxyl group. These compounds may form pharmaceutically acceptable salts. Examples of such salts may include sodium, potassium, calcium, aluminum, gold and silver salts. Also contemplated are salts formed with pharmaceutically acceptable amines such as

ammonia, alkyl amines, hydroxyalkylamines, N-methylglucamine and the like.

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.

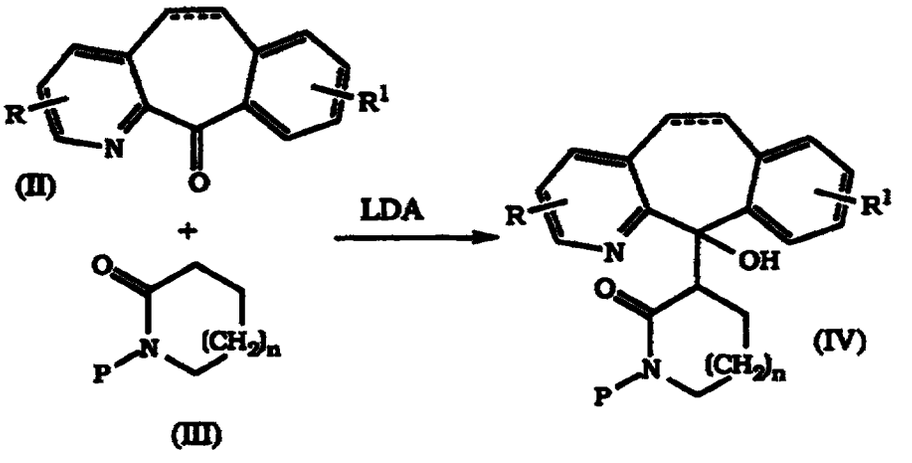
All such acid and base salts are intended to be pharmaceutically acceptable salts within the scope of the invention and all acid and base salts are considered equivalent to the free forms of the corresponding compounds for purposes of the invention.

The following compounds and reagents are referred to herein the abbreviations indicated: trifluoroacetic anhydride (TFAA); 4-dimethylaminopyridine (DMAP); methanol (MeOH; ethanol (EtOH); diethyl ether (Et_2O); triethylamine (Et_3N); ethyl acetate (EtOAc); acetic acid (HOAc); m-chloroperbenzoic acid (MCPBA); dicyclohexylcarbodiimide (DCC); 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide hydrochloride (DEC); 1-hydroxy-benzotriazole (HOBT); N-methylmorpholine (NMM); dimethyl-formamide (DMF)

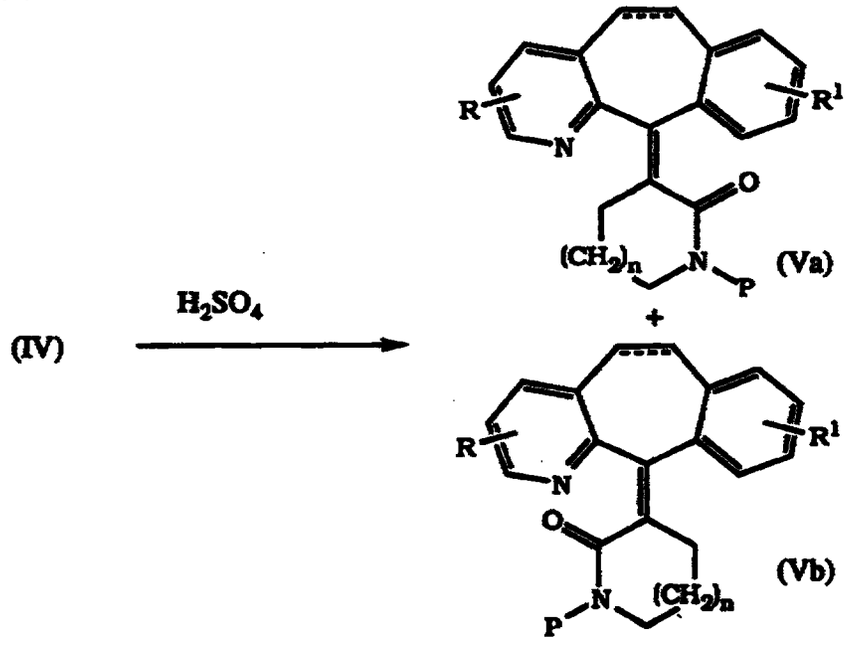
Compounds of the formula (Ia) and (Ib) can be prepared by the process shown in Reaction Scheme 1.

REACTION SCHEME 1

Step (a)

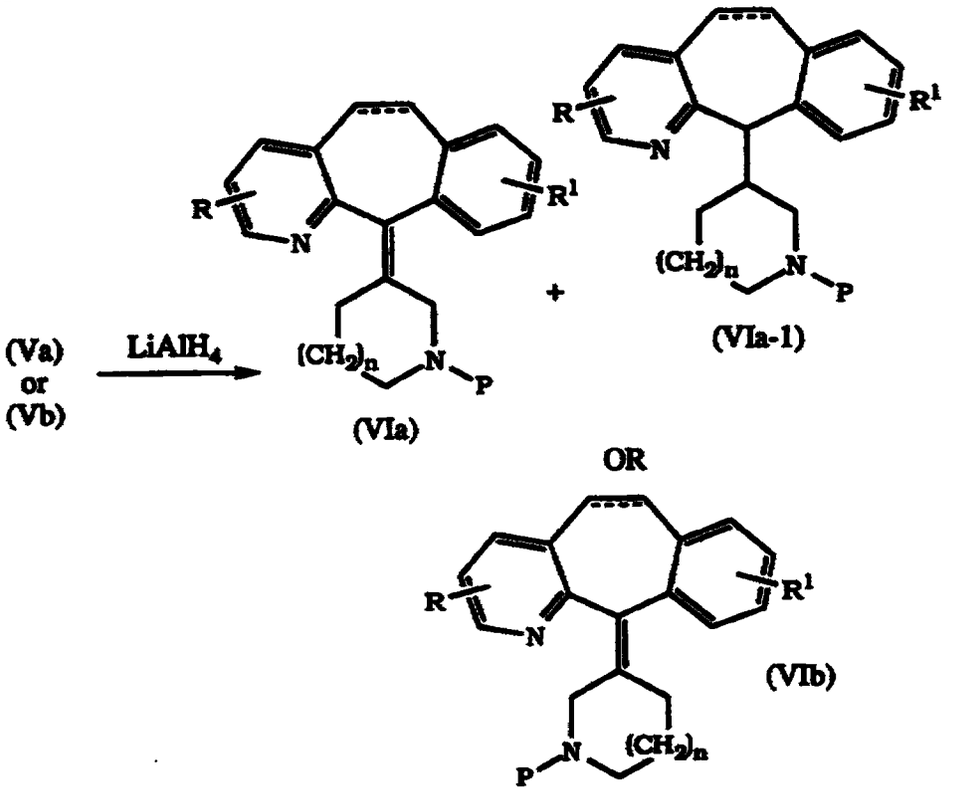


Step (b):

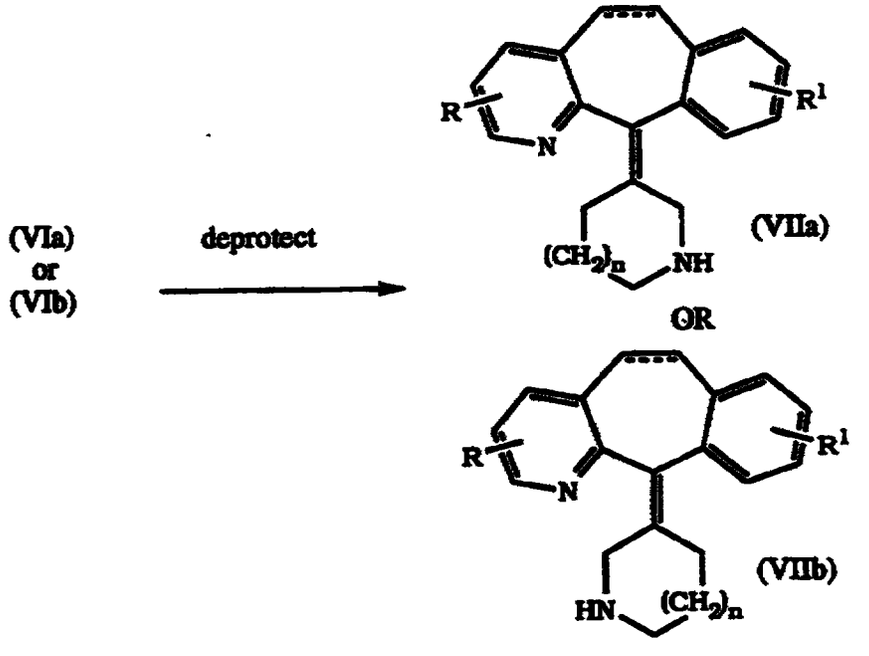


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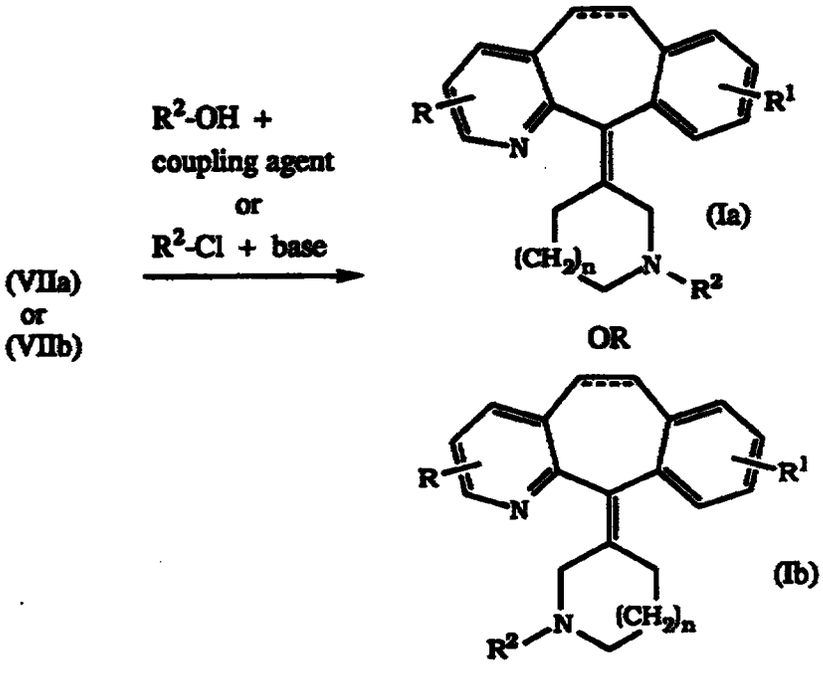
Step (c):


(Va)
or
(Vb)
LiAlH4
(VIa)
(VIa-1)
OR
(VIb)

Step (d):


(VIa)
or
(VIb)
deprotect
(VIIa)
OR
(VIIb)

Step (e):



In Step (a) of Reaction Scheme 1, A protected lactam of the formula (III), wherein P is an amine protecting group, such as CH_3, benzyl or $C_6H_5SO_2$-, and n is as defined above, is treated with LDA, then reacted with a ketone of the formula (II) wherein R and R^1 are as defined above and the dotted line represents an optional double bond, at -100° to 0°C, preferably at -80° to -20°C, to form an alcohol of the formula (IV).

In Step (b) the alcohol (IV) from Step (a) is dehydrated by treating with concentrated H_2SO_4 to form a mixture of isomeric compounds (Va) and (Vb). The compounds (Va) and (Vb) are separated, e.g. by column chromatography, and a single isomer (Va) or (Vb) is used in Step (c).

In Step (c) the compound (Va) or (Vb) is treated with $LiAlH_4$, at -40° to 40°C, preferably at -10° to 20°C, and most preferably at about 0°C, in a suitable solvent, such as THF or Et_2O, to form a mixture compounds of the formula (VIa) and (VIa-1), or a compound of the formula (VIb), respectively.

In Step (d) the compound (VIa) or (VIb) is deprotected using reagents and reaction conditions appropriate for the specific protecting group (P), such as those described in Greene, *et al.*, "Protective Groups in Organic Synthesis, 2nd Ed.", pages

315-385, John Wiley & Sons, New York (1991), to form an amine of the formula (VIIa) or (VIIb), respectfully.

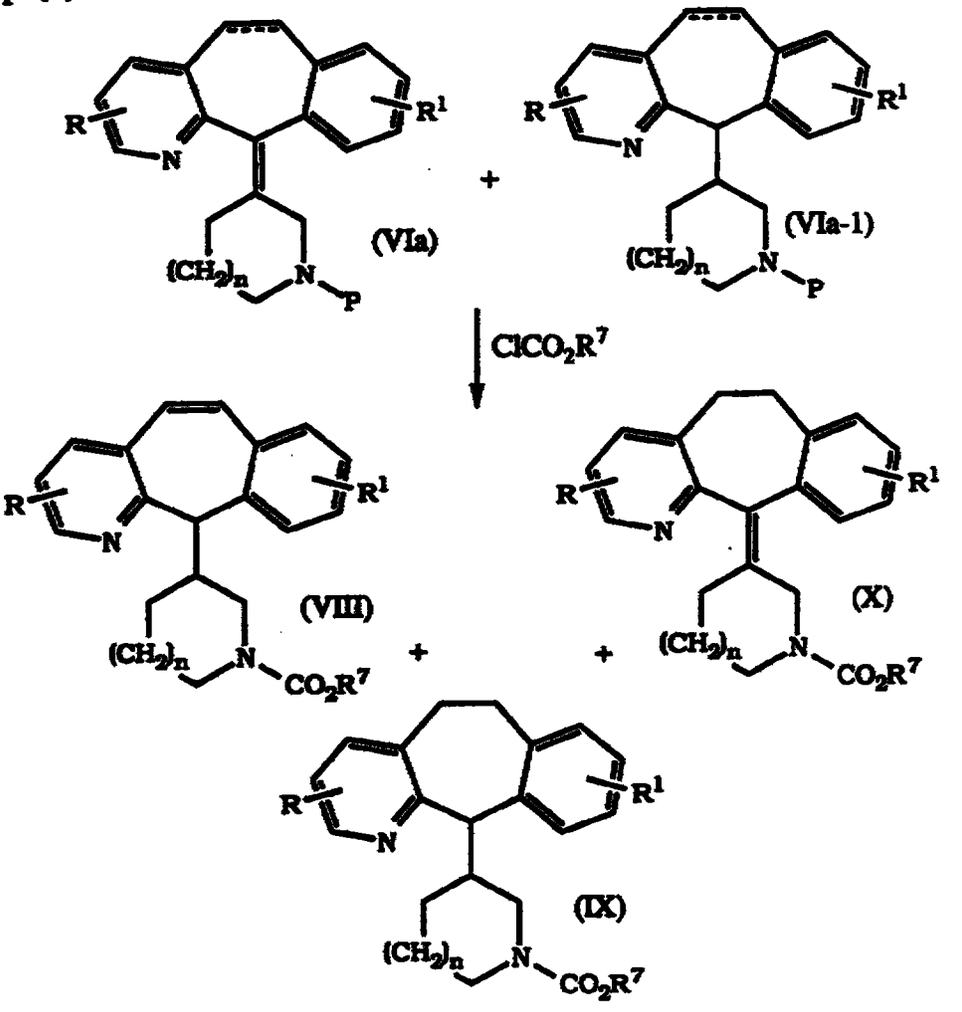
In Step (e) the amine (VIIa) or (VIIb) is reacted with a compound of the formula R^2-OH, wherein R^2 is as defined above, in a suitable solvent, such as DMF or CH_2Cl_2, in the presence of a coupling agent, such as DCC or DEC, to form a compound of the formula (Ia) or (Ib), respectfully.

Alternatively, the amine (VIIa) or (VIIb) is reacted with a compound of the formula R^2-Cl, wherein R^2 is as defined above, in the presence of a tertiary amine base, such as DMAP or pyridine, to form a compound of formula (Ia) or (Ib), respectfully.

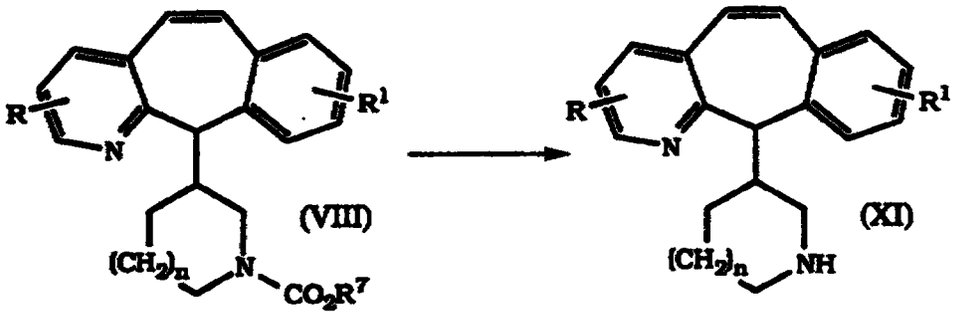
Compounds of the formula (Ic) can be prepared by the process shown in Reaction Scheme 2.

REACTION SCHEME 2

Step (a):

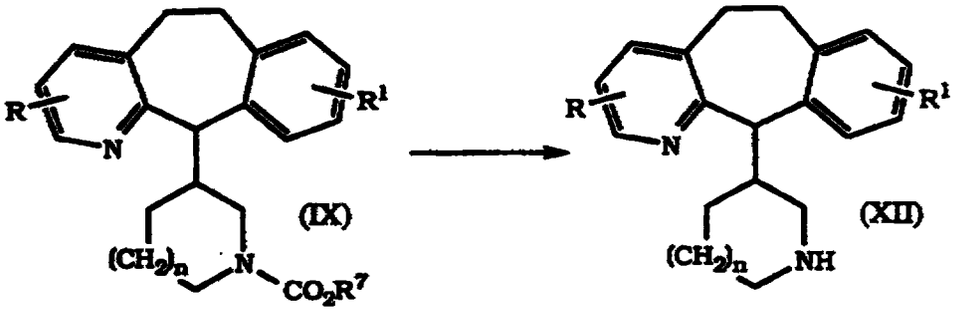


Step (b):



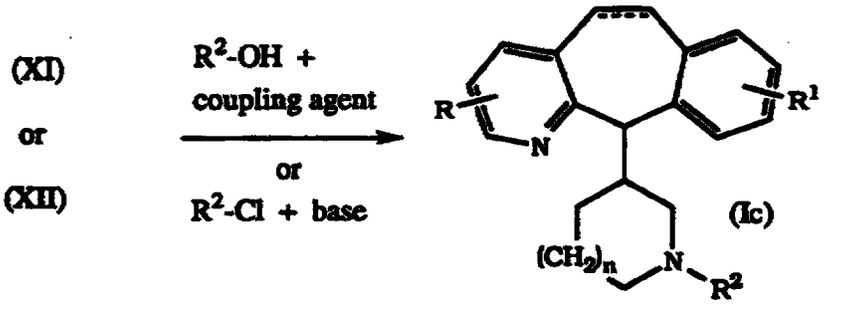
(VIII) (XI)

OR



(IX) (XII)

Step (c):



In Step (a) of Reaction Scheme 2 the mixture of compounds of the formula (VIa) and (VIa-1) from Step (c) of Reaction Scheme 1, wherein P is CH_3, and R, R^1 and n are as defined above, and the optional double bond is not present, is reacted with a compound of the formula $ClCO_2R^7$, wherein R^7 is $(C_1$-$C_6)$alkyl, (i.e. an alkyl chloroformate), preferably ethyl chloroformate, in the presence of a tertiary amine base, preferably Et_3N, in a suitable solvent, such as toluene, at 40° to 110°C, preferably at 70° to 90°C, to form a mixture of compounds (VIII), (IX) and (X). (Compounds (VIII) and (X) are fromed from compound (VIa) while compound (IX) is formed from compound (VIa-1).) Compounds (VIII), (IX) and (X) are separated, e.g. by chromatography.

In Step (b) a compound of the formula (VIII) or (IX) is reacted with concentrated HCl at 40° to 110°C, preferably at 70° to 90°C, to form an amine of the formula (XI) or (XII).

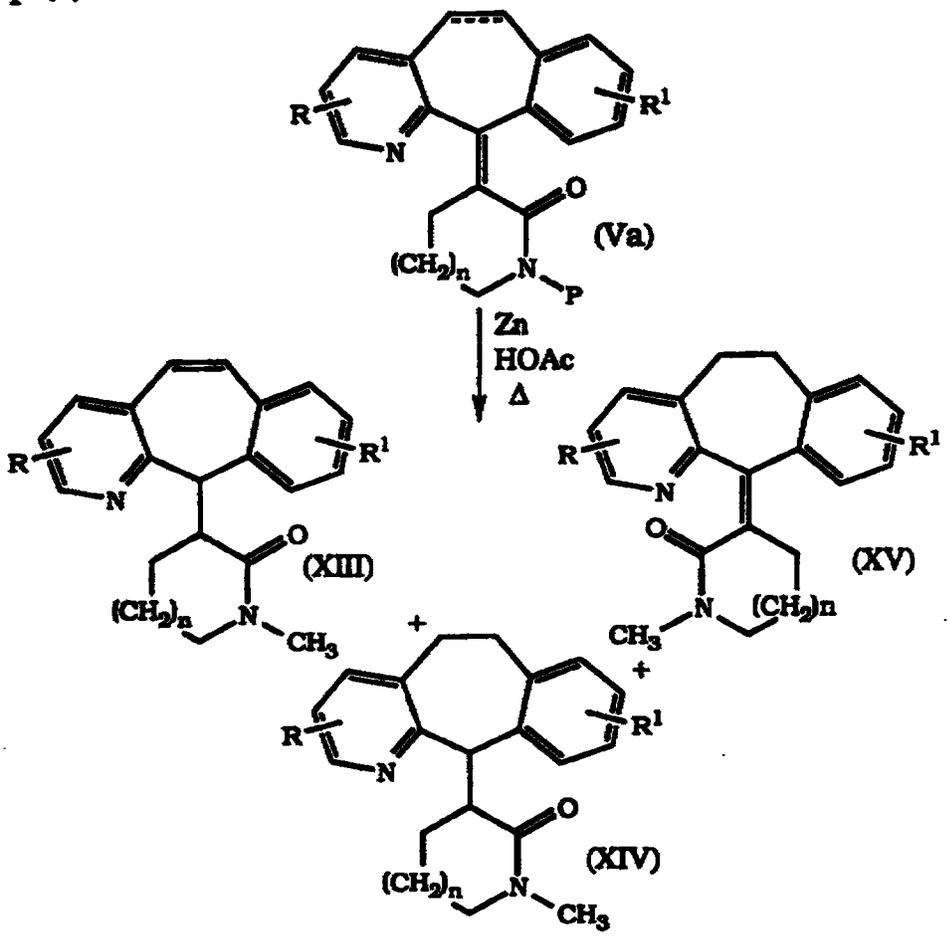
Alternatively, in Step (b) a compound of the formula (VIII) or (IX) is reacted with a hydroxide base, such as NaOH or KOH, preferably KOH, in the presence of a suitable solvent, such as a mixture of a C_1-C_6 alcohol and water, preferably a mixture of EtOH and water or iPrOH and water, at 40° to 100°C, preferably at 50° to 80°C, to form a compound of the formula (XI) or (XII), respectively.

In Step (c) a compound of the formula (XI) or (XII) is reacted with either R^2OH and a coupling agent, or R^2Cl and a base, via substantially the same procedures as described for Scheme 1, Step (e), to form a compound of the formula (Ic).

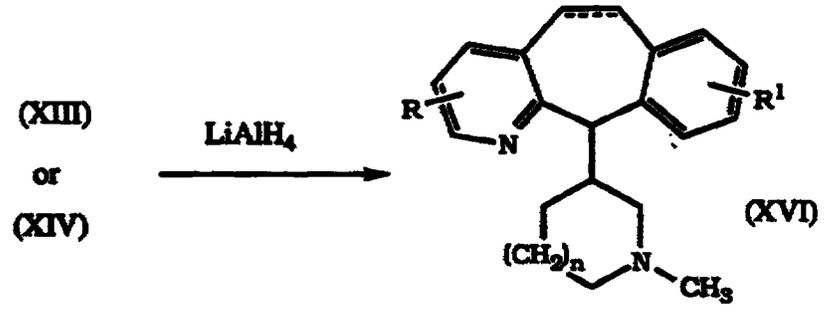
An alternative process for preparing compounds of the formula (Ic) is described in Reaction Scheme 3.

REACTION SCHEME 3

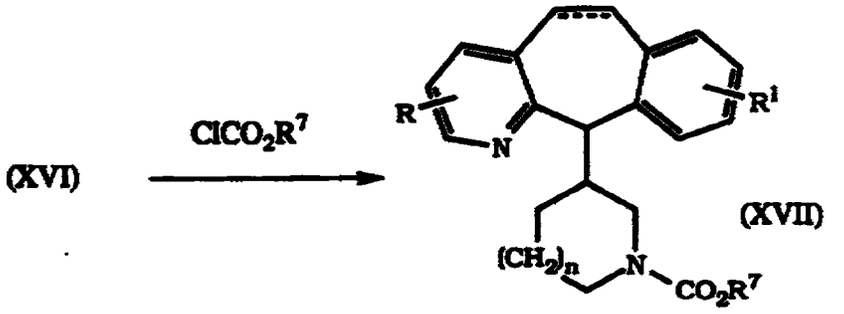
Step (a):



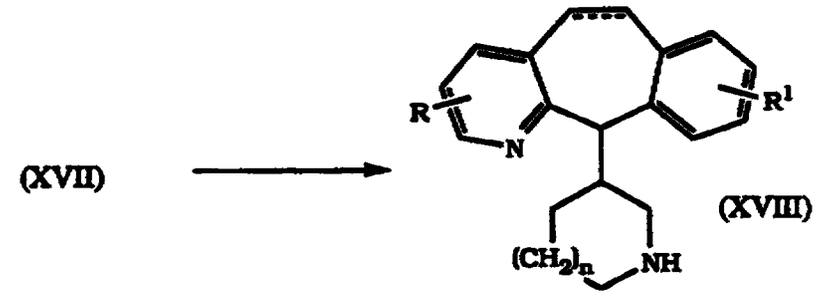
Step (b):



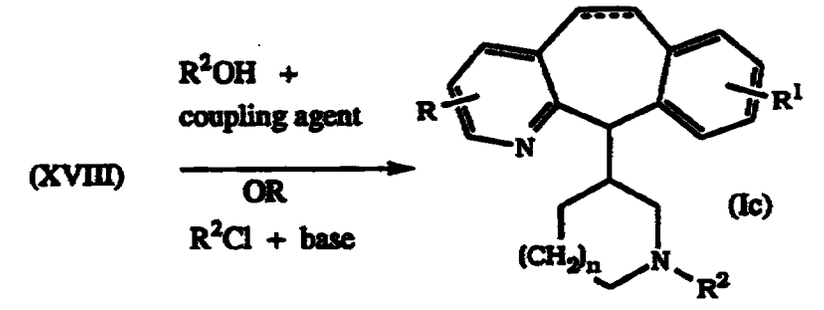
Step (c):



Step (d):



Step (e):



In Step (a) of Reaction Scheme 3, a compound of the formula (Va) from Step (b) of Reaction Scheme 1, wherein P is CH_3, and R, R^1 and n are as defined above, and the optional double bond is not present, is reacted with Zn powder and glacial HOAc at 80° to 120°C, preferably at about 100°C, to form a mixture of compounds (XIII), (XIV) and (XV). Compounds (XIII), (XIV) and (XV) are separated, e.g. by chromatography.

In Step (b) of Reaction Scheme 3, a compound of the formula (XIII) or (XIV) is reduced by treating with a hydride reducing agent, such as LiAlH, via substantially the same procedure as described for Step (c) of Reaction Scheme 1 to form a compound of the formula (XVI), wherein R, R^1 and n are as

defined above, and the dotted line represents an optional double bond.

In Step (c), a compound of the formula (XVI) is treated with a compound of the formula $ClCO_2R^7$, wherein R^7 is as defined above, via substantially the same procedure as described for Step (a) of Reaction Scheme 2 to form a compound of the formula (XVII).

In Step (d), a compound of the formula (XVII) is hydrolyzed via substantially the same procedure as described for Step (b) of Reaction Scheme 2 to form an amine of the formula (XVIII).

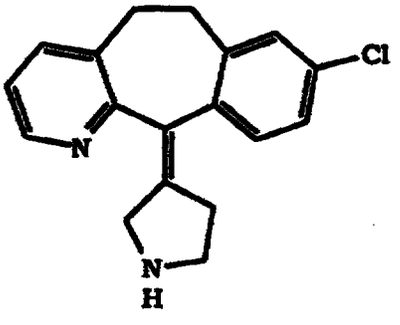
In Step (e), an amine of the formula (XVIII) is reacted with either R^2OH and a coupling agent, or R^2Cl and a base, via substantially the same procedures as described for Reaction Scheme 1, Step (e), to form a compound of the formula (Ic).

Starting ketones of the formula (II) and starting compounds of the formula (III) are known or can be prepared via known methods. Compounds of the formula R^2OH, R^2Cl and $ClCO_2R^7$ are known and are either commercially available or can be prepared via established methods.

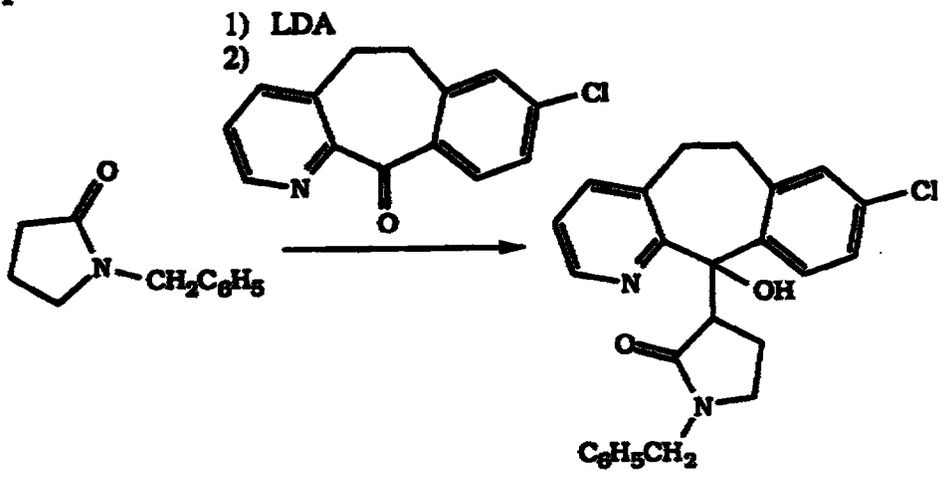
In the above processes, it is sometimes desirable and/or necessary to protect certain R^1, R^2, R^3 and R^4 etc., groups during the reactions. Conventional protecting groups are operable as described in Greene, *et al.*, "Protective Groups In Organic Synthesis, 2nd Ed.", John Wiley & Sons, New York, (1991). For example, see Table 1 on page 60 of WO 95/10516.

Compounds useful in this invention are exemplified by the following preparative examples, which should not be construed to limit the scope of the present invention.

PREPARATION 1



Step A:

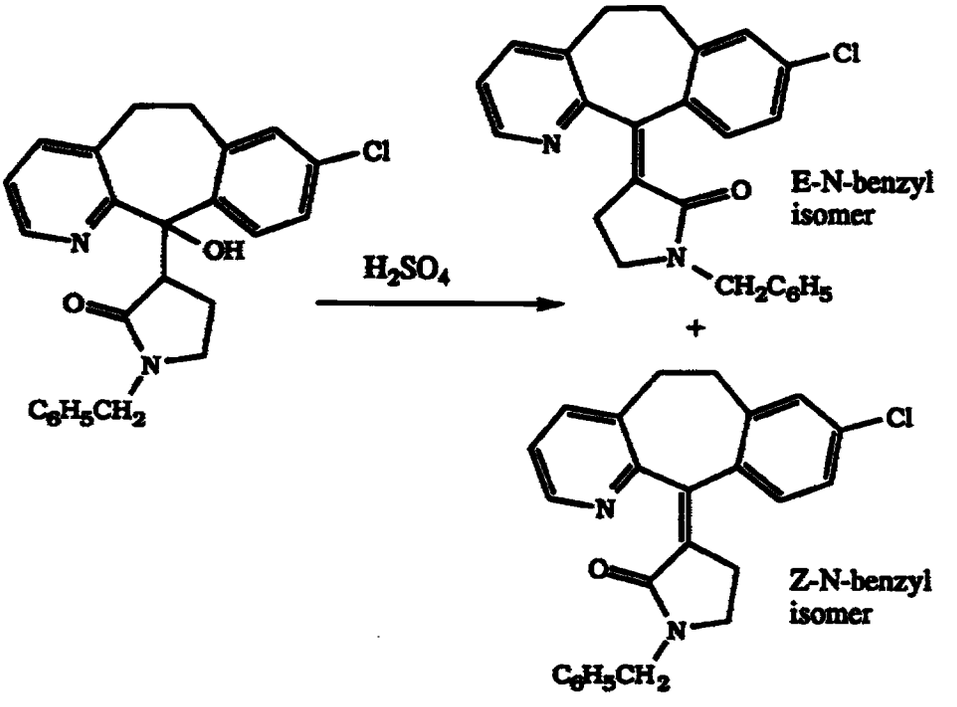


In a flame dried 2-neck flask combine THF (400mL) and dry diisopropyl amine (0.135 mol, 13.74 g, 19.0 mL). Cool the solution to -78°C and slowly add (dropwise) n-BuLi (2 M, 0.134 mol, 67 mL) over 5 min. Stir the resulting mixture at -78°C for 45 min, then slowly add (dropwise) a THF solution of the lactam (0.123 mol, 21.6 g, 20 mL) over 5 min. Stir the reaction mixture at -78°C for 1h, then raised to 0°C for 1h, to give an opaque red solution. Cool the reaction mixture back down to -78°C and add a THF solution of the ketone (0.123 mol, 30 g in 300 mL THF) via cannula. When the reaction is complete by TLC analysis (after about 2 hours), raise the temperature to -50°C for 30 min, then add saturated NH_4Cl (aqueous) to quench. Dilute the mixture with additional H_2O and extract repeatedly with EtOAc. Combine the extracts and wash with brine. Dry the extracts over Na_2SO_4, filter, and concentrate *in vacuo* to give a mixture of diastereomeric alcohols. Heat the mixture in EtOAc to give 19.9 g (42% yield) of the upper Rf diastereomer as a solid. Chromatograph (silica gel, 2% THF:CH_2Cl_2 increasing gradually to 5% THF:CH_2Cl_2) the material obtained from the mother liquor to give 21.3 g (45 % yield) of the lower Rf diastereomer as a solid, and 2.9 g of unreacted ketone.

Analytical data for the upper Rf diastereomer: MS (CI, M+H) = 385, MP 164-166°C. Combustion Analysis Calc: C, 71.51; H, 5.76; N, 6.67; Cl, 8.44. Found: C, 71.55; H, 5.58; N, 6.67; Cl, 8.49.

Analytical data for the lower R_f diastereomer: MS (CI, M+H) = 385, Combustion Analysis Calc: C, 71.51; H, 5.76; N, 6.67; Cl, 8.44. Found: C, 71.46; H, 5.57; N, 6.66; Cl, 8.40.

Step B:

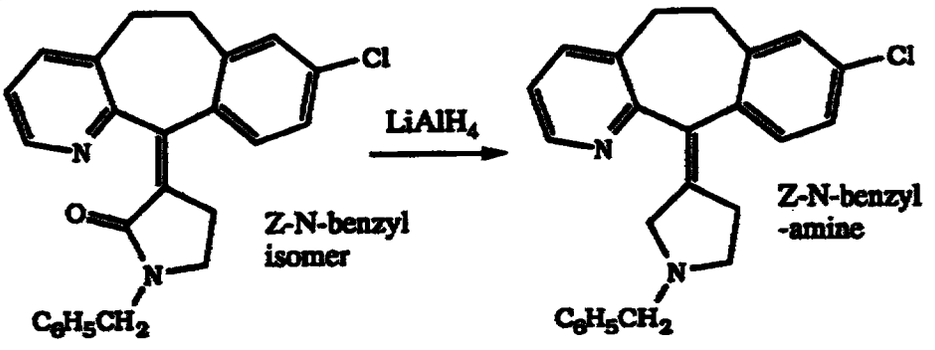


Combine 1.0 g (2.38 mmol) of the upper R_f diatereomer from Step A and concentrated H_2SO_4 at room temperature. Heat the mixture to 60°C for 1.5 h, then cool to room temperature and poured into crushed ice. Basify the resulting solution to a pH of about 10 with 10% NaOH (aqueous) and extract repeatedly with CH_2Cl_2 (or EtOAc). Combine the extracts, wash the extracts with brine, then dry over Na_2SO_4, filtered and concentrate *in vacuo* to a residue. Chromatograph (silica gel, 5% acetone:CH_2Cl_2 increasing gradually to 5% MeOH:CH_2Cl_2) to give 200 mg of the E-N-benzyl isomer, and 600 mg of the Z-N-benzyl isomer as solids.

Analytical data for the E-N-benzyl isomer: MS (CI, M+H) = 401, MP 178-180.5°C. Combustion Analysis Calc: C, 74.90; H, 5.28; N, 699; Cl, 8.84.

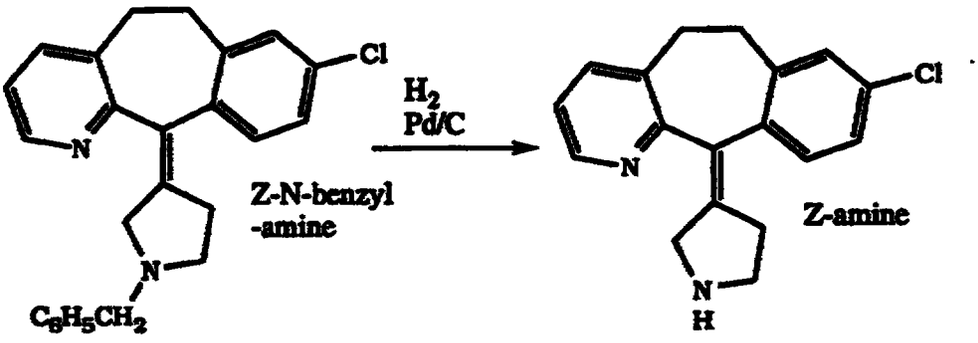
Analytical data for the Z-benzyl isomer: MS (CI, M+H) = 401, Combustion Analysis Calc; C, 74.90; H, 5.28; N, 6.99; Cl, 8.84. Found: C, 74.78; H, 5.41; N, 6.97; Cl, 8.82.

Step C:



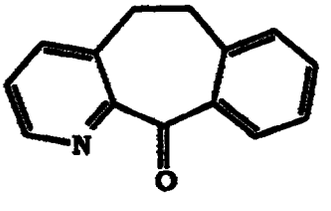
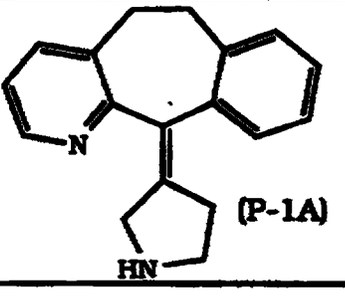
Combine the Z-N-benzyl isomer from Step B and 5 mL of THF under a N_2 atmosphere. Cool the solution to 0°C, and add 70 mg of $LiAlH_4$ (1.867 mmol) in portions. Stir the mixture for about 30 min. at 0°C, then quench with EtOAc and MeOH. Filter through celite® to remove the aluminum salts, concentrate the filtrate and add 5% NaOH (aqueous). Extract the aqueous portion with EtOAc:THF (9:1), combine the organic phases and wash with brine. Dry over Na_2SO_4, filter and concentrate the filtrate *in vacuo* to a residue. Chromatograph (silica gel, 10% acetone:hexane increasing gradually to 20% acetone:hexane) to give 175 mg (51% yield) of the Z-N-benzylamine. Analytical data for the Z-N-benzylamine: MS (CI, M+H) = 387. High resolution MS Calc. for $C_{25}H_{24}N_2Cl$: 387.1628. Found: 387.1609.

Step D:

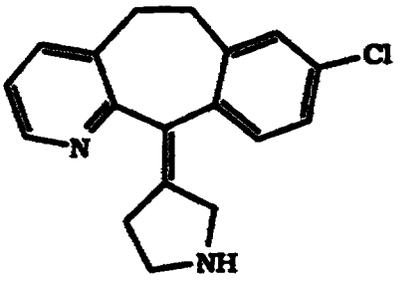


Combine 500 mg of the Z-N-benzylamine from Step C (1.29 mmol), MeOH (20 mL), HOAc (5 mL), 1,4-cyclohexadiene (5 mL) and 210 mg of 10% Pd/C under N_2 atmosphere. Carefully heat the mixture to 70°C at which time hydrogen evolution began. After 1 hour and add hydrogen and continue heating at about 40°C for an additional 1h. Filter the mixture through celite®, and concentrate the filtrate *in vacuo* to a residue. Add toluene and concentrate *in vacuo* again to remove residual HOAc. Chromatograph (silica gel, 5% MeOH:CH_2Cl_2 increasing gradually to 10% MeOH:CH_2Cl_2:1% NH_4OH) to give 221 mg (58 % yield) of the Z-amine product (P-1). Analytical data for Z-amine: MS (CI, M+H) = 296.

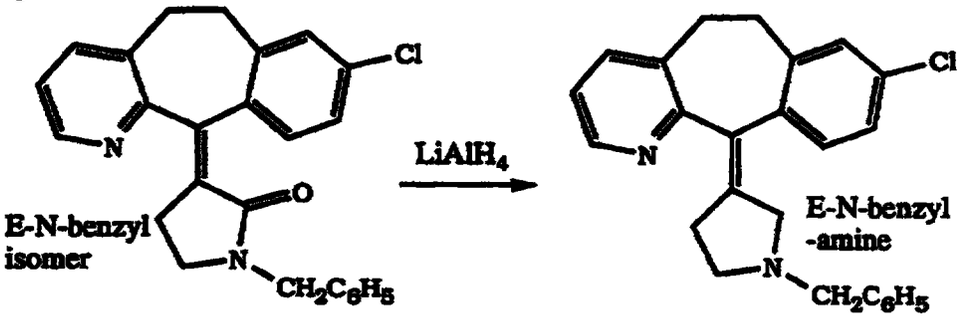
Using the starting ketone indicated and following substantially the same procedure as described in Preparation 1, the following amine was prepared:

Starting Ketone	Amine
	 (P-1A)

PREPARATION 2



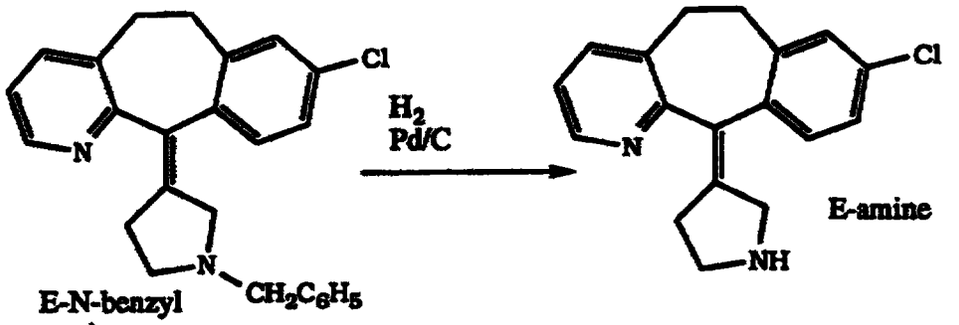
Step A



Combine 1.04 g of $LiAlH_4$ (27.7 mmol) and 75 mL of Et_2O under a N_2 atmosphere. Cool the mixture to 0°C, and add a THF solution of 2.20 g (5.49 mmol) of the E-N-benzyl isomer from Step B of Preparation 1, via syringe. After 120 min., quench the reaction mixture with EtOAc and MeOH, followed by the addition of 1% NaOH (aqueous). Extract the aqueous portion with EtOAc (4 X 75 mL), then with EtOAc:THF (4:1), and combine the extracts Wash the extracts with brine, dry over $MgSO_4$, filter and concentrate *in vacuo* to a residue. Chromatograph (silica gel, 15% acetone:EtOAc increasing gradually to 5% MeOH:EtOAc) to give 1.08 g (51% yield) of the E-N-benzylamine product.

Analytical data for the E-N-benzylamine: MS (CI, M+H) = 387, Combustion Analysis Calc: C, 77.61; H, 5.99; N, 7.24; Cl, 9.16. Found: C, 77.80; H, 6.07; N, 7.20; Cl, 8.94.

Step B:

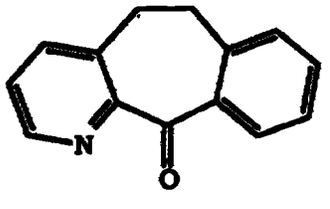
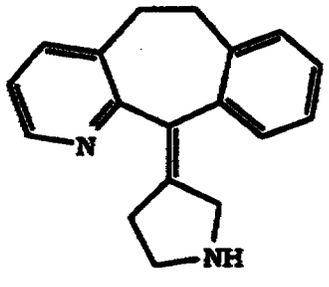


E-N-benzyl -amine

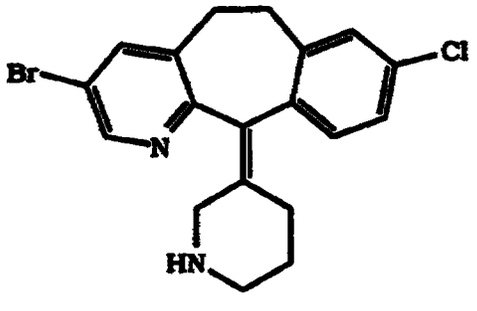
E-amine

The E-N-benzylamine from Step A is hydrogenated using Pd/C via essentially the same procedure as described for the Z-isomer in Step D of Preparation 1 to give the E amine product (P-2).

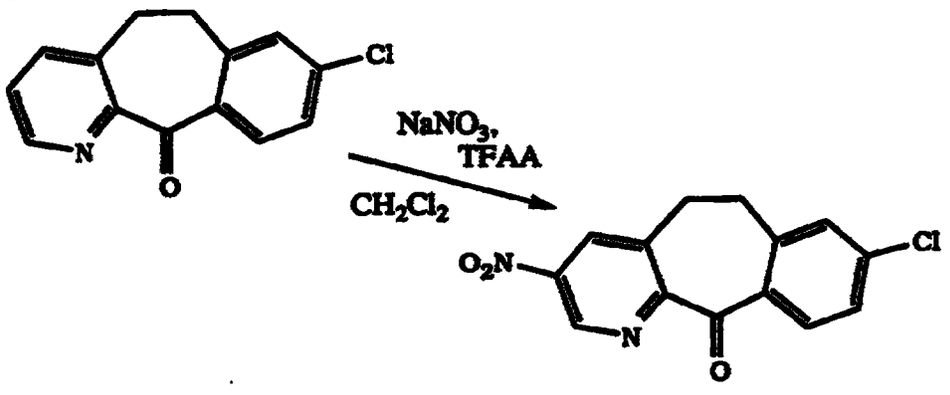
Using the starting ketone indicated and following substantially the same procedure as described in Preparation 2, the following amine was prepared:

Starting Ketone	Amine
	 (P-2A)

PREPARATION 3

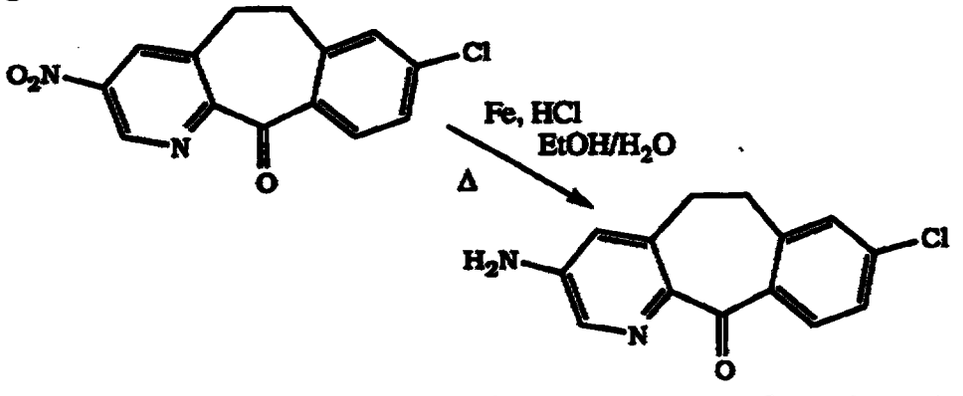


Step A:



Combine 10 g (41.03 mmol) of the ketone and 100 mL of CH_2Cl_2 and cool to -5°C. Add 7.0 mL (49.5 mmol) of TFAA, then add 3.7 g (43.53 mmol) $NaNO_3$ to the stirred mixture. Allow the mixture to warm to 20°C and stir for 30 hours. Cool the mixture to 0°C and slowly add a solution of 30 mL of concentrated NH_4OH (aqueous) in 100 mL of water. Stir for 30 min. then add 300 mL of CH_2Cl_2 and 200 mL of water. Separate the layers and dry the organic phase over $MgSO_4$. Filter and concentrate *in vacuo* to a solid residue. Stir the solid in 100 mL of hot MeOH for 30 min. then allow the mixture to cool to room temperature. Filter, wash the solid with 20 mL of MeOH and dry under vacuum (0.2 mm Hg) at room temperature to give 4.9 g (41.4% yield) of the nitroketone product.

Step B:

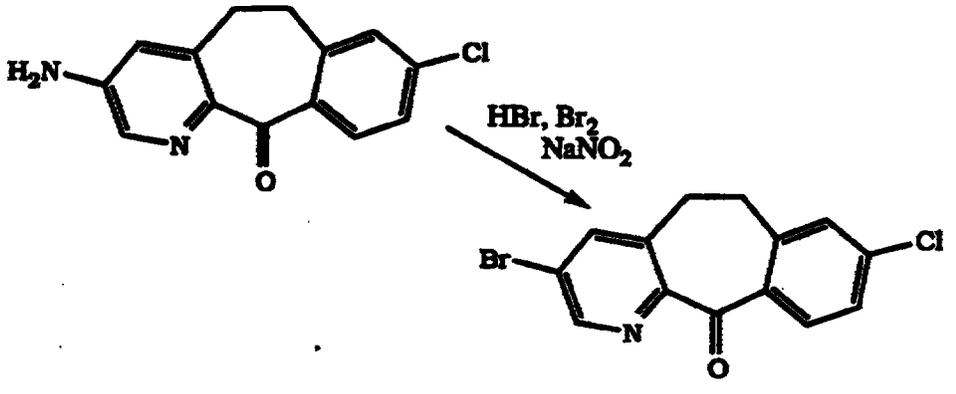


Combine 5 g (17.3 mmol) of the nitroketone from Step A, 140 mL of EtOH and 15 mL of water at room temperature, then add 3 g (54.5 mmol) of Fe powder. Add 1 mL of concentrated HCl and heat the mixture at reflux for 4 hours. Cool the mixture to room temperature and concentrate *in vacuo* to a volume of about

20 mL. Add 100 mL of water, 200 mL of CH_2Cl_2 and 30 mL of 20% NaOH (aqueous). Separate the layers and extract the aqueous phase with 200 mL of CH_2Cl_2. Combine the organic extracts, filter and wash with 100 mL of water. Dry over $MgSO_4$ then concentrate *in vacuo* to a residue. Stir the residue in a mixture of 20 mL of acetone and 100 mL Et_2O to form a solid. Filter and wash the solid with 20 mL of Et_2O, then dry *in vacuo* at 20°C to give 4.0 g (89.5% yield) of the aminoketone product.

Analytical data for the aminoketone: m.p.=199°-200°C; MS (CI) = 259, 261; Combustion analysis: calc. - C, 64.99; H, 4.28; N, 10.83, found - C, 64.79; H, 4.41; N, 10.58.

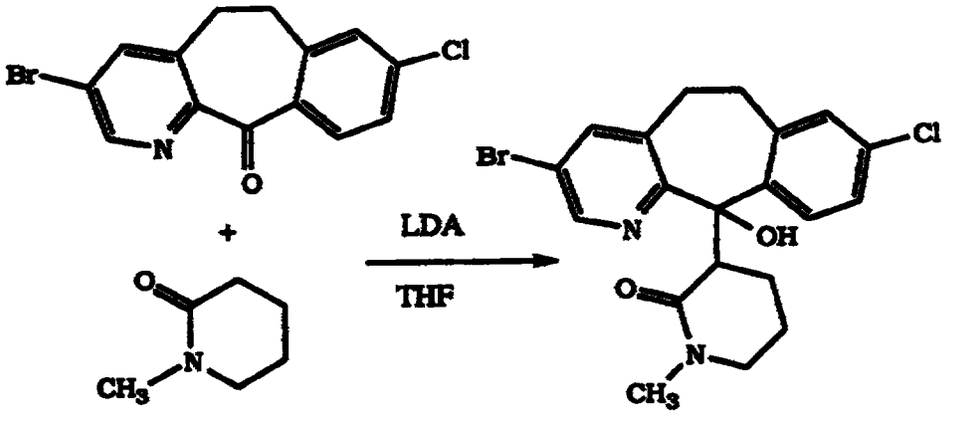
Step C:



Combine 10 g (0.386 mole) of the aminoketone from Step B and 300 mL of 48% HBr at -5°C, then add 9.0 mL (1.74 mole) of Br_2 and stir at -5°C for 20 min. Slowly add (dropwise) a solution of 10.5 g (1.52 mole) $NaNO_2$ in 25 mL of water, keeping the temperature at -5°C. Stir for 1 hour at -5°C, allow the mixture to warm to 20°C over 1 hour and stir at 20°C for 4 hours. Pour the mixture into 300 g of ice, and add 40% NaOH (aqueous) to the ice cold mixture to adjust to pH = 14. Extract with CH_2Cl_2 (2 X 300 mL), combine the extracts and dry over $MgSO_4$. Filter and concentrate *in vacuo* to a residue. Chromatograph the residue (silica gel, 25% EtOAc/hexanes) to give 8.7 g (69.9% yield) of the bromoketone product.

Analytical data for the bromoketone: MS (CI) = 322, 324.

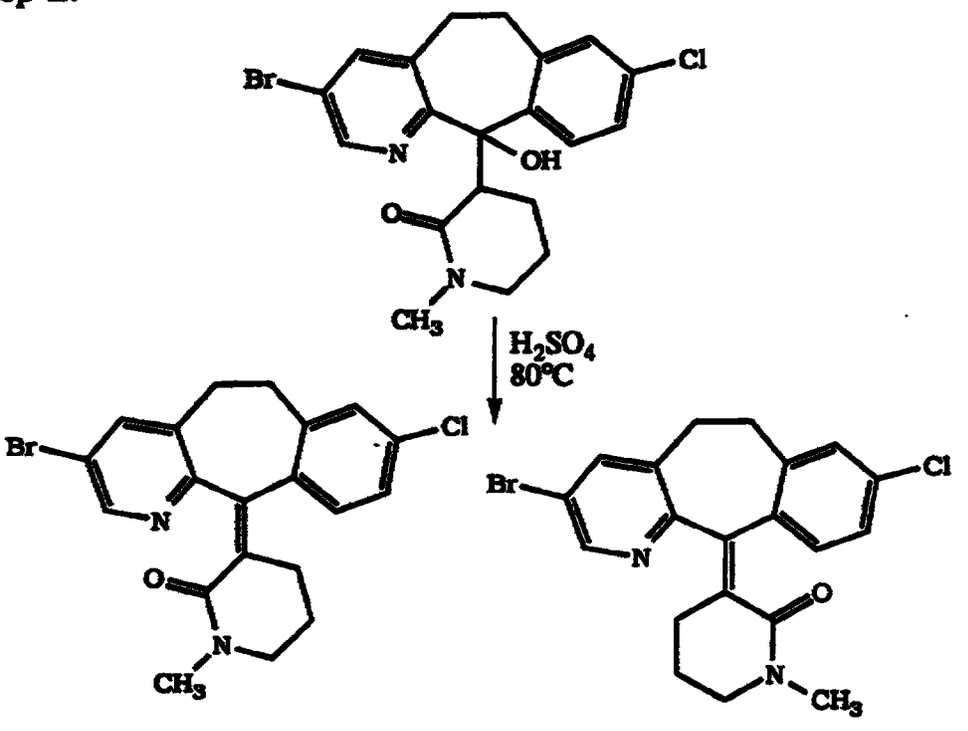
Step D:



Slowly add (dropwise) 18 mL (45.0 mmol) of 2.5 M n-butyllithium in hexanes to a solution of 7.0 mL (49.41 mmol) diisopropylamine in 100 mL of THF at -78°C. Stir at -78°C for 15 min. then add 7.0 mL (64 mmol) of N-methyl-2-piperidone. Stir the mixture at -78°C for 30 min. then warm to -5°C over a 1 hour period. Cool to -78°C and slowly add (dropwise) a solution of 12 g (37.2 mmol) of the bromoketone from Step C in 200 mL of dry THF. Stir the mixture at -78°C for 1 hour, then warm to -10°C over 1.5 hours. Add 25 mL of water and concentrate *in vacuo* to remove about 200 mL of the solvent. Extract with 600 mL of CH_2Cl_2 and 300 mL of brine, and dry the organic extract over $MgSO_4$. Filter, concentrate *in vacuo* to a residue and stir the residue in a mixture of 30 mL of acetone and 20 mL of Et_2O to form a solid. Filter, wash the solid with 10 mL of Et_2O and dry at 20°C, 0.2 mm Hg, overnight to give 11.89 g of the product as a mixture of diastereomers. Chromatograph (silica gel, 25% EtOAc/hexanes) the mother liquor and Et_2O wash to give an additional 1.0 g of the product (79.56% total yield).

Analytical data for the product of Step D: MS (CI, M+H) = 437; combustion analysis: calc. - C, 55.12; H, 4.62; N, 6.43, found - C, 54.70; H, 4.57; N, 6.26.

Step E:

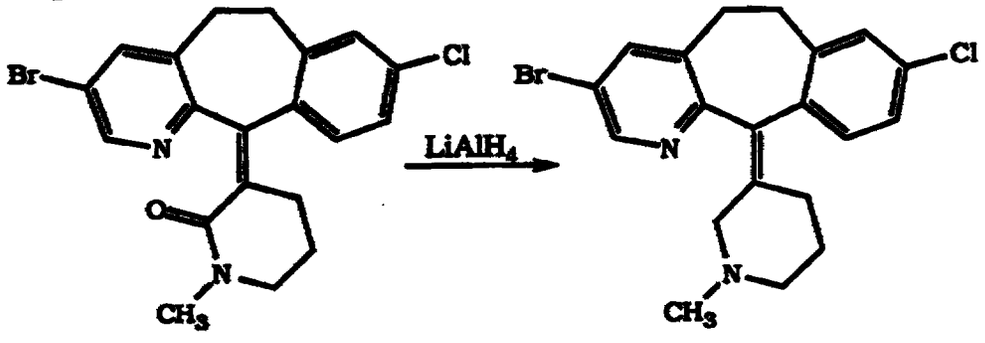


Combine 11.4 g (26.1 mmol) of the product from Step D and 100 mL of concentrated H_2SO_4 and heat to 80°C for 4 hours. Cool the mixture to 20°C, pour into 300 g of ice and add 50% NaOH (aqueous) to the ice cold mixture to adjust to pH = 14. Filter to collect the resulting solid, wash the solid with 300 mL of water, then dry at 20°C, 0.2 mm Hg, overnight. Chromatograph the solid (silica gel, 2% MeOH/EtOAc) to give 4.48 g of the Z-isomer and 4.68 g of the E-isomer of the product (total yield 84%).

Analytical data for Z-isomer: MS (CI, M+H) = 417, 419; combustion analysis: calc. - C, 57.50; H, 4.34; N, 6.70, found - C, 57.99; H, 4.76; N, 6.66.

Analytical data for E-isomer: MS (CI, M+H) = 417, 419; combustion analysis: calc. - C, 57.50; H, 4.34; N, 6.70, found - C, 57.23; H, 4.43; N, 6.65.

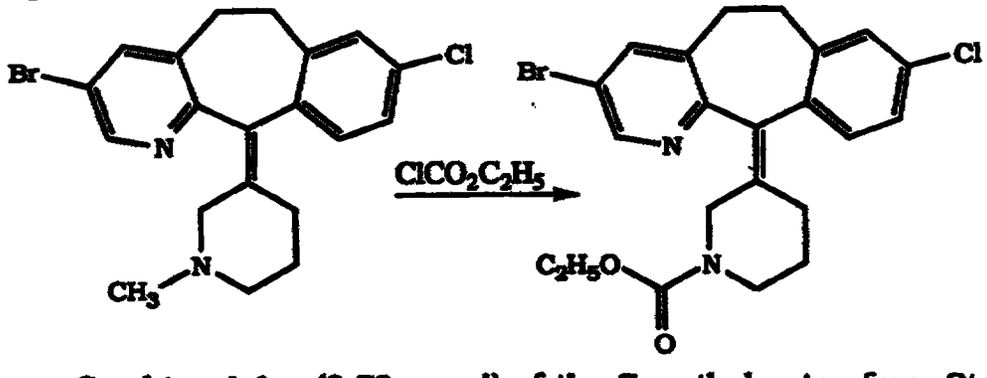
Step F:



Combine 1.0 g (2.39 mmol) of the Z-isomer product from Step E and 10 mL of dry THF at -10°C and add 110 mg (2.78 mmol) of $LiAlH_4$. Stir the mixture at -10° to -5°C for 2 hours, then add 2 mL of EtOAc folowed by 20 mL of 10% potassium sodium tartrate tetrahydrate (aqueous), 5 mL of 10% NaOH (aqueous) and 150 mL of CH_2Cl_2. Separate the layers and dry the organic phase over $MgSO_4$. Filter and concentrate *in vacuo* to a residue. Chromatograph the residue (silica gel, first 25% EtOAc/hexanes, then 3% MeOH/EtOAc containing concentrated 1% NH_4OH) to give 480 mg (50% yield) of the Z-methylamine product.

Analytical data for the Z-methylamine: m.p.= 160°-161°C; MS (CI, M+H) = 403, 405; combustion analysis: calc. - C, 59.49; H, 4.99; N, 6.94, found - C, 59.75; H, 5.43; N, 6.79.

Step G:

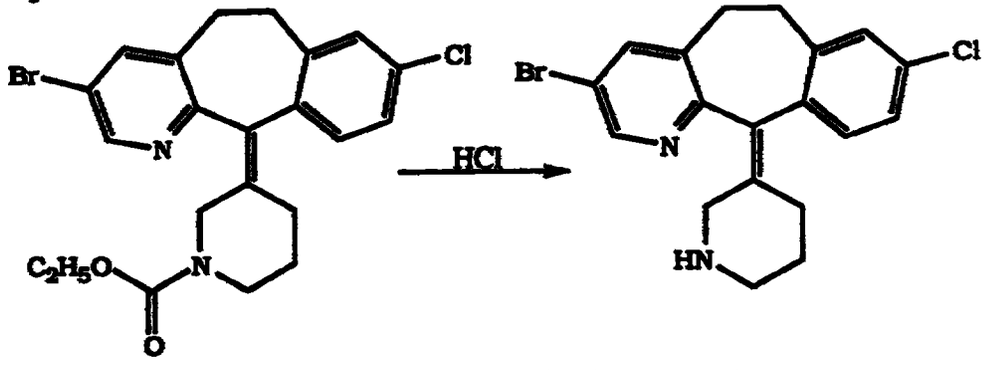


Combine 1.1 g (2.72 mmol) of the Z-methylamine from Step F and 20 mL of toluene at 0°C and add 1.0 mL (10.4 mmol) of $ClCO_2C_2H_5$. Add 1.0 mL (13.6 mmol) of Et_3N and heat the mixture to 70°C for 3 hours. Cool the mixture and concentrate *in vacuo* to a residue. Extract the residue with 50 mL of CH_2Cl_2 and wash the extract with 30 mL of water. Dry over $MgSO_4$, filter and

concentrate *in vacuo* to a residue. Chromatograph the residue (silica gel, 20% EtOAc/hexanes) to give the crude product. Crystallize from a mixture of EtO and CH_2Cl_2 to give 510 mg (40.8% yield) of the Z-ethylcarbamate product.

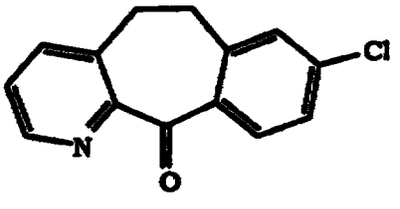
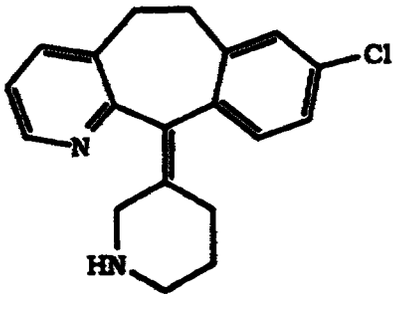
Analytical data for the Z-ethylcarbamate: m.p. = 182°-183°C; MS (CI, M+H) = 461, 463; combustion analysis: calc.- C, 57.29; H, 4.80; N, 6.06, found - C, 57.38; H, 4.72; N, 6.08.

Step H:

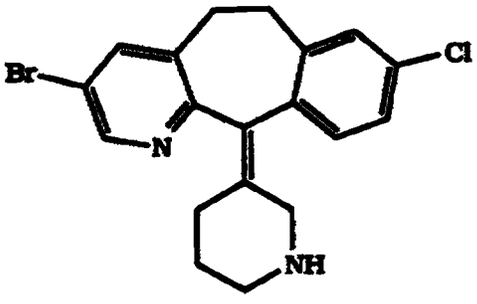


Combine 400 mg (0.866 mmol) of the Z-ethylcarbamate from Step G and 5 mL of concentrated HCl and heat at 100°C overnight. Cool to 0°C and slowly add 30% NaOH (aqueous) to basify the mixture. Extract with CH_2Cl_2 (2 X 250 mL) and dry the extract over $MgSO_4$. Filter and concentrate *in vacuo* to give 320 mg (94.86% yield) of the Z-amine product (P-3). Analytical data for the Z-amine (P-3): MS (FAB, M+H)= 389, 391.

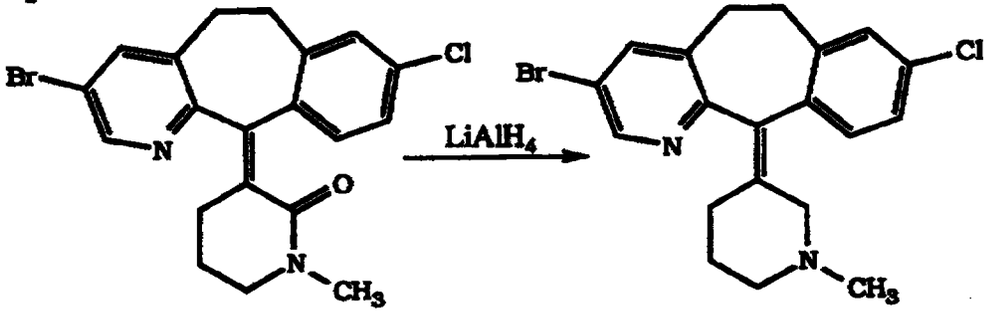
Using the starting ketone indicated and following substantially the same procedure as described in Steps D to H of Preparation 3, the following amine was prepared:

Starting Ketone	Amine
	 (P-3A), m.p. = 169°-170°C

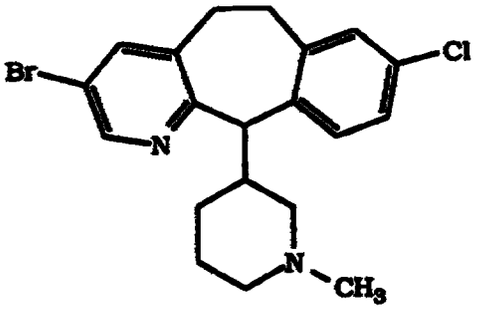
PREPARATION 4



Step A:



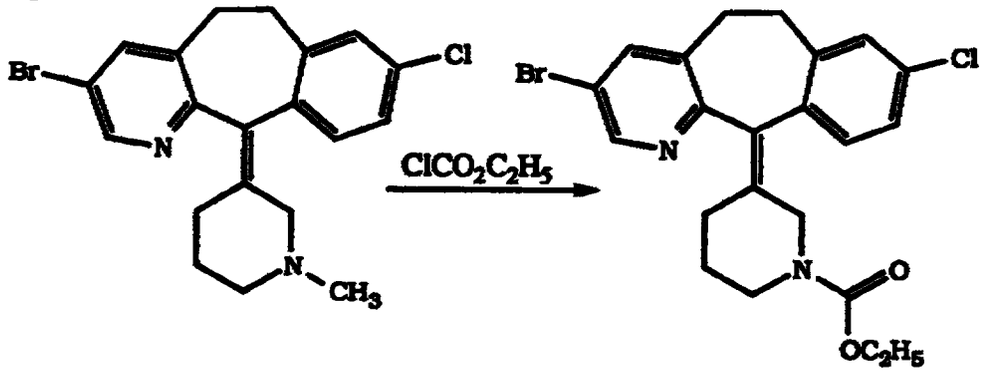
Combine 3.4 g (8.15 mmol) of the E-isomer product from Step E of Preparation 3 and 40 mL of dry THF at -5°C and add 470 mg (11.9 mmol) of $LiAlH_4$. Stir the mixture at 0°C for 5 hours, then add 5 mL of water, 20 mL of 10% potassium sodium tartrate tetrahydrate (aqueous), 5 mL of 10% NaOH (aqueous) and 150 mL of CH_2Cl_2. Separate the layers and dry the organic phase over $MgSO_4$. Filter and concentrate *in vacuo* to a residue. The residue is a mixture of the product compound and a compound of the formula



Chromatograph the residue (silica gel, 2% MeOH/EtOAc) to give 1.3 g (40% yield) of the E-methylamine product.

Analytical data for the E-methylamine: m.p.= 140°-141°C; MS (CI, M+H) = 403, 405; combustion analysis: calc. - C, 59.49; H, 4.99; N, 6.94, found - C, 59.11; H, 4.75; N, 6.98.

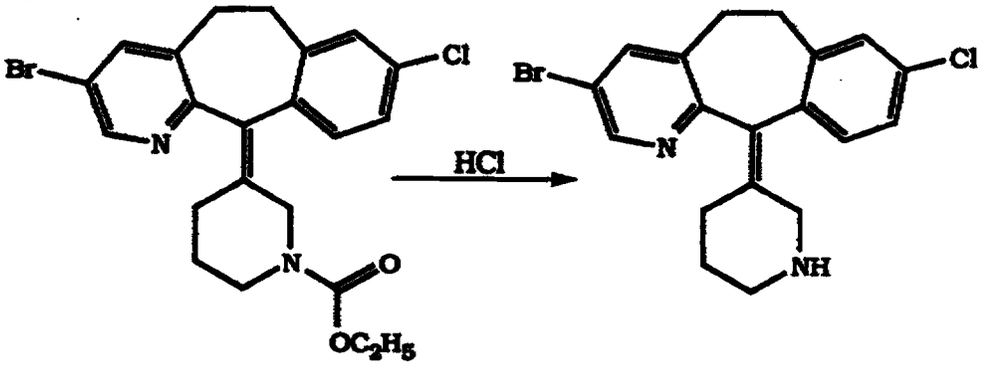
Step B:



Using 0.4 g (0.99 mmol) of the E-methylamine from Step A, 15 mL of toluene, 0.5 mL (5.2 mmol) of $ClCO_2C_2H_5$, and 0.5 mL (6.8 mmol) of Et_3N, and substantially the same procedure as described in Preparation 3, Step G, 230 mg (51.1% yield) of the E-ethylcarbamate product is prepared.

Analytical data for the E-ethylcarbamate: m.p. = 186°-187°C; MS (CI, M+H) = 463, 464; combustion analysis: calc.- C, 57.29; H, 4.80; N, 6.06, found - C, 57.43; H, 5.11; N, 6.09.

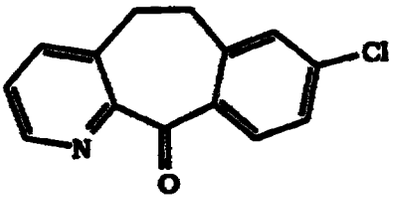
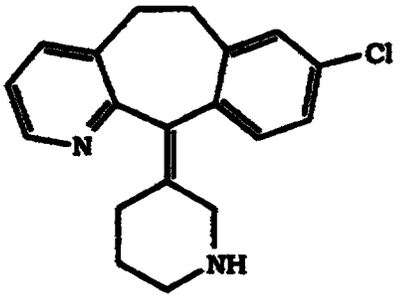
Step C:



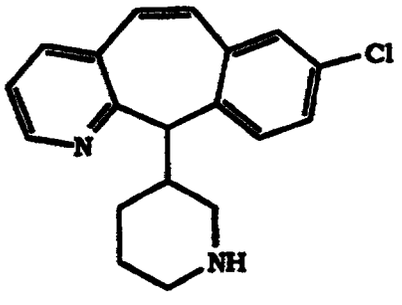
The E-ethylcarbamate from Step B is converted to the E-amine (P-4) in 97.8% yield using substantially the same procedure as described in Preparation 3, Step H.

Analytical data for the E-amine (P-4): m.p. = 166°-167°C; MS (CI)= 389, 391; combustion analysis: calc. - C, 57.88; H, 4.66; N, 7.10, found - C, 57.63; H, 4.61; N, 7.03.

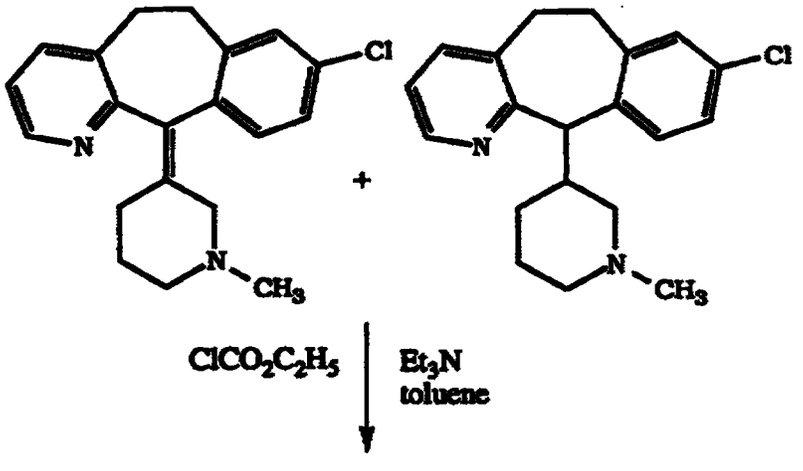
Using the starting ketone indicated to prepare the appropriate E-isomer via the procedures described in Steps D and E of Preparation 3, Steps A-E, the following amines were prepared via substantially the same procedure as described in Steps A-C of Preparation 4:

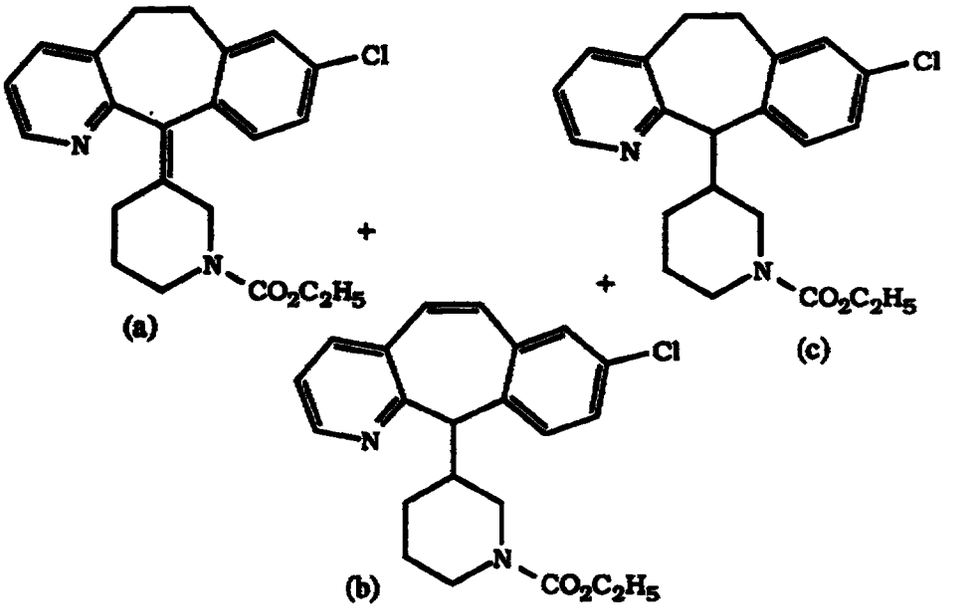
Starting Ketone	Amine
	 (P-4A), m.p. = 140°-141°C

PREPARATION 5



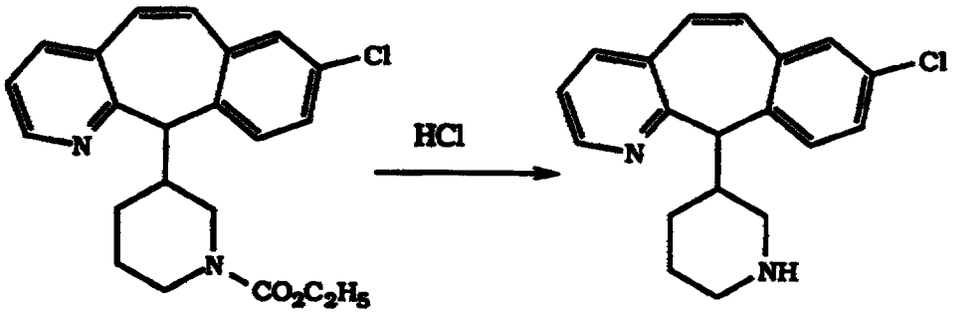
Step A:





Combine 20 g (61.5 mmol) of the crude (no chromatography) E-methyl-amine obtained from Preparation 4, Step A (using the appropriate starting ketone), and 200 mL of toluene at 0°C, and add 20 mL (208 mmol) of $ClCO_2C_2H_5$. Add 20 mL (272 mmol) of Et_3N, then heat to 80°C and stir for 4 hours. Cool to room temperature and concentrate *in vacuo* to a residue. Extract the residue with 300 mL of CH_2Cl_2, wash the extract with 200 mL of water, then dry over $MgSO_4$. Filter, concentrate *in vacuo* to a residue, then chromatograph the residue (silica gel, 70% EtOAc/hexanes) to give 5.0 g of product (a), 4.2 g of product (b), and 300 mg of product (c). Analytical data: MS (Cl, M+H) product (a) = 383, product (b) = 383, product (c) = 385.

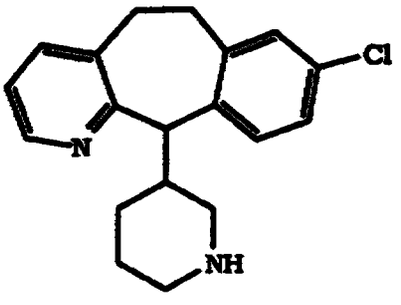
Step B:



Combine 4.0 g (10.4 mmol) of product (b) from Step A and 20 mL of concentrated HCl and heat at 80°C overnight. Cool to 20°C, basify to pH = 14 with 20% NaOH (aqueous), and extract with 200 mL of CH_2Cl_2. Wash the extract with 25 mL of water,

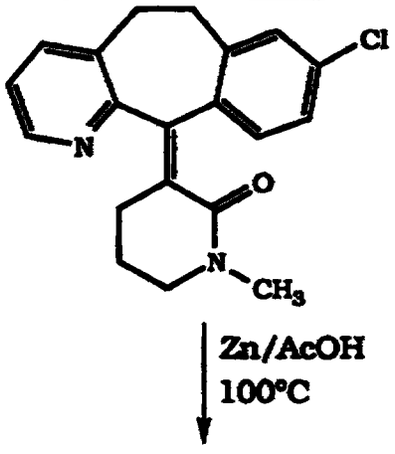
dry over $MgSO_4$, filter and concentrate *in vacuo* to a residue. Chromatograph the residue (silica gel, 10% MeOH/EtOAc + 2% NH_4OH (aqueous)), then triturate with 15 mL of acetone/Et_2O to give 1.96 g (60.5% yield) of the amine product (P-5). Analytical data for amine (P-5): m.p.=157°-158°C; MS (CI, M+H)=311, 313.

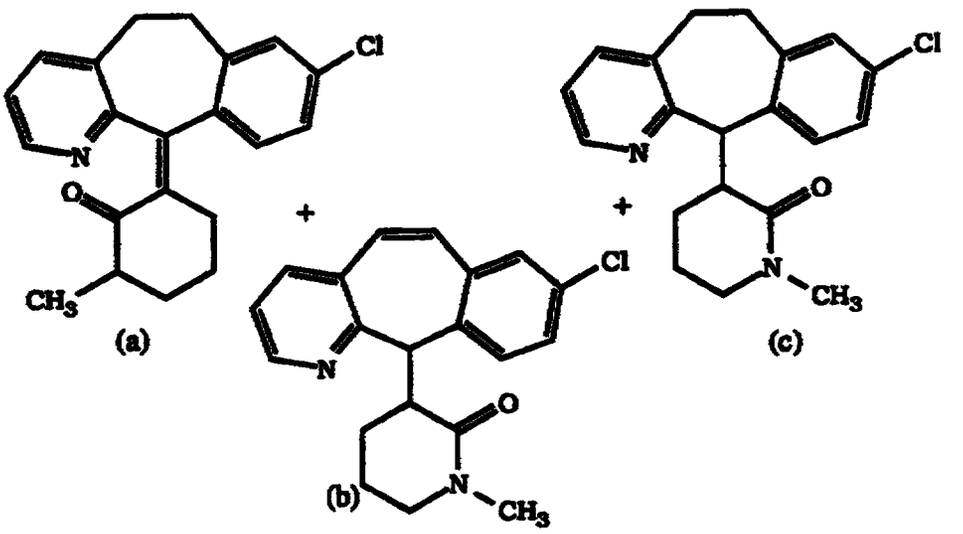
PREPARATION 6



Combine 400 mg (1.03 mmol) of product (c) from Preparation 5, Step A, and 5 mL of EtOH at 20°C, then add a solution of 0.23 g (4.15 mmol) KOH in 10 mL of water. Heat the mixture at reflux for 3 days, cool to room temperature and concentrate *in vacuo* to a residue. Extract the residue with 80 mL of CH_2Cl_2 and wash the extract with 50 mL of water. Dry over $MgSO_4$, filter and concentrate *in vacuo* to a residue. Chromatograph the residue (silica gel, 10% MeOH/EtOAc + 2% NH_4OH (aqueous), then triturate with 10 mL of Et_2O to give 200 mg (61.5% yield) of the amine (P-6). Analytical data for the amine (P-6): MS (CI, M+H) = 313, 315.

PREPARATION 7



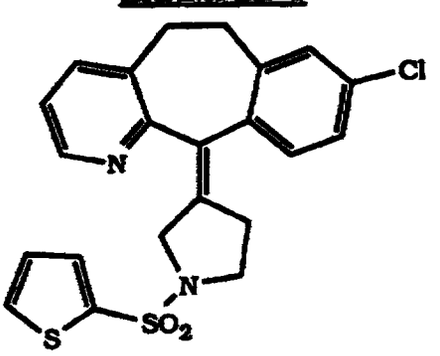


Combine 1.0 g (2.95 mmol) of E-isomer product (obtained using the appropriate ketone) from Preparation 3, Step E, 30 mL of glacial HOAc and 1.0 g (15.29 mmol) of Zn powder and heat the mixture at 100°C overnight. Filter through celite®, wash the filter cake with 20 mL of glacial HOAc, then concentrate the filtrate *in vacuo* to a residue. Basify the residue with 15 mL of concentrated NH_4OH (aqueous), add 50 mL of water and extract with CH_2Cl_2 (2 X 100 mL). Dry the combined extracts over $MgSO_4$, filter and concentrate *in vacuo* to a residue. Chromatograph the residue (silica gel, 5% MeOH/EtOAc + 2% concentrated NH_4OH (aqueous)) to give three products: 300 mg (30% yield) of the product (a); 250 mg (25% yield) of the product (b); and 250 mg (25% yield) of the product (c).

Analytical data for product (c): m.p. = 172°-173°C, MS (CI, M+H) = 341, 343.

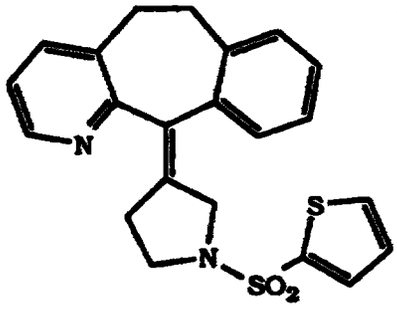
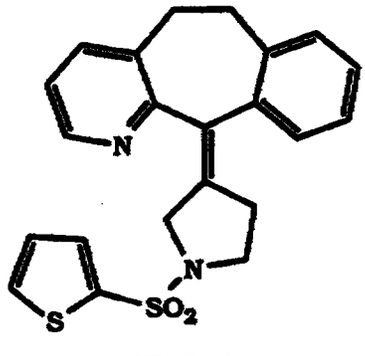
Analytical data for product (b): m.p. = 142°-144°C, MS (CI, M+H) = 339, 341.

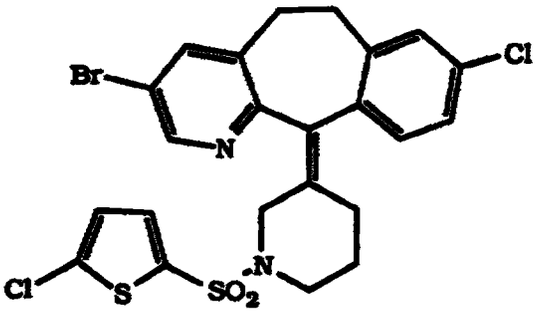
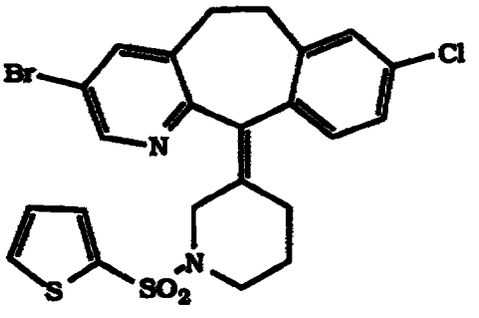
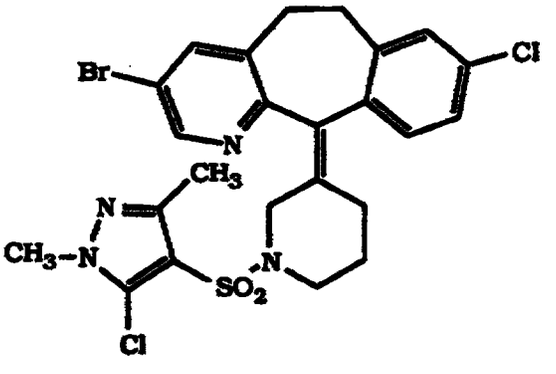
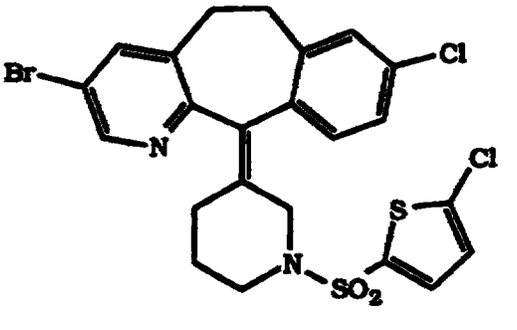
EXAMPLE 1

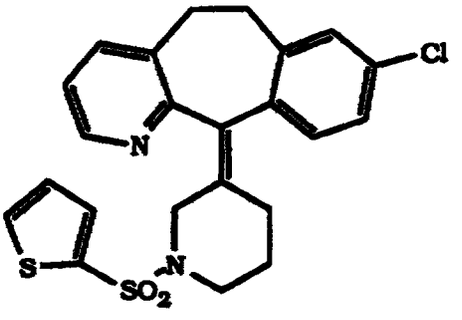
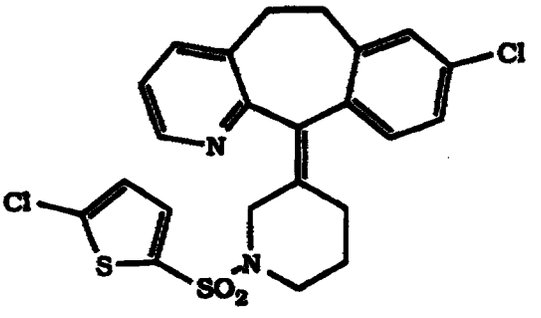
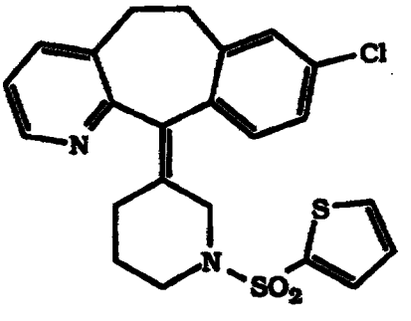
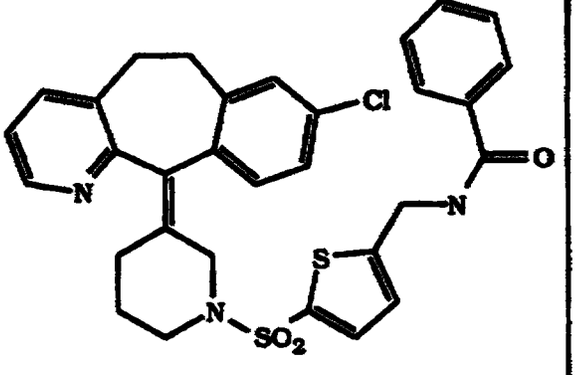


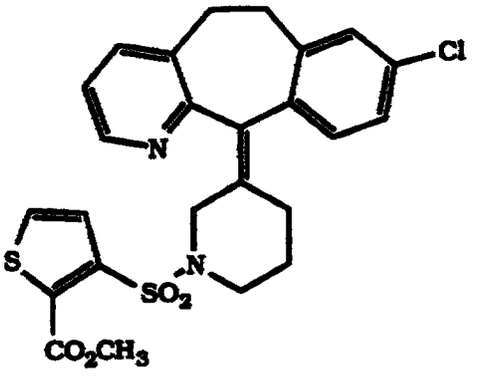
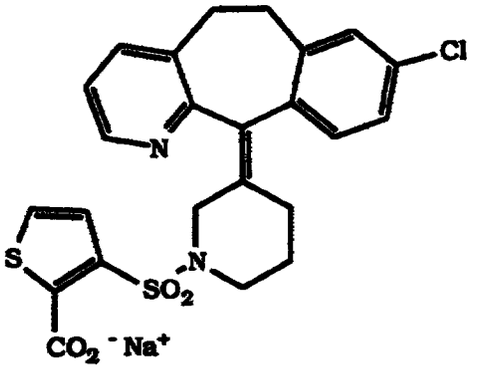
Combine 115 mg of the Z-amine product (P-1) from Preparation 1 (0.389 mmol), 5 mL of pyridine (5 mL) and a catalytic amount (15 mg) of DMAP under a N_2 atmosphere. Cool the solution to 0°C and add 175 mg of 2-thienylsulfonyl chloride (0.961 mmol). Stir for 10 min. at 0°C, then warm to room temperature and stir for 17 hours. Quench the reaction mixture by the adding a solution of $NaHCO_3$ (aqueous), and extract the aqueous layer with EtOAc-THF (20:1). Combine the extracts, wash with brine, dry over Na_2SO_4, filter and concentrate *in vacuo* to a residue. Chromatograph (silica gel, 25% EtOAc:hexane increasing gradually to 35% EtOAc:hexane) to give 65 mg (39% yield) of the Z-N-(2-thienyl)sulfonamide product (E-1). Analytical data for the Z-N-(2-thienyl)sulfonamide: MS (CI, M+H) = 443.

Using the appropriate sulfonyl chloride and the amine indicated, and following substantially the same procedure as described for Example 1, the following sulfonamide compounds were prepared:

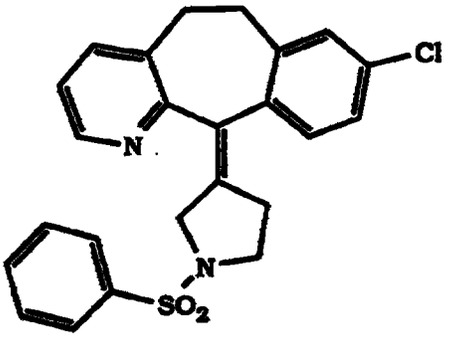
Amine	Amide	Analytical Data
P-2A	 (E-1A)	MS (FAB, M+H) = 443
P-1A	 (E-1B)	MS (CI, M+H) = 409

P-3	 (E-1C)	m.p. = 165°-167°C MS (CI, M+H) = 569, 571
P-3	 (E-1D)	m.p. = 183°-184°C MS (CI, M+H) = 535, 537
P-3	 (E-1E)	m.p. = 251°-252°C MS (CI) = 583, 585
P-4	 (E-1F)	m.p. = 171°-172°C MS (CI) 569, 571

P-3A	 (E-1G)	MS (CI, M+H) = 457, 459
P-3A	 (E-1H)	m.p. = 154°-155°C MS (CI) = 452, 454
P-4A	 (E-1J)	m.p. = 254-255°C MS (CI, M+H) = 457, 459
P-4A	 (E-1K)	MS (CI, M+H) = 590, 592

P-3A	 (E-1M)	m.p. = 134°-136°C MS (CI, M+H) = 515, 517
P-3A	 (E-1N)	m.p. = 220°C (dec.) MS (FAB, M+H) = 501, 503

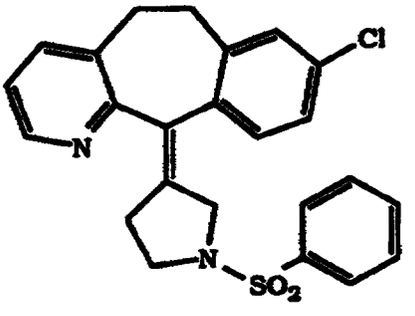
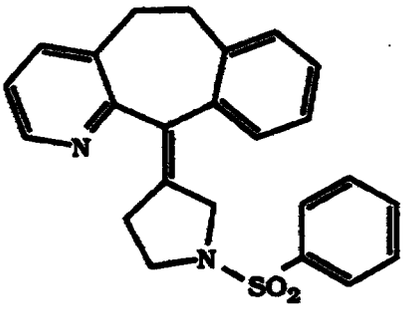
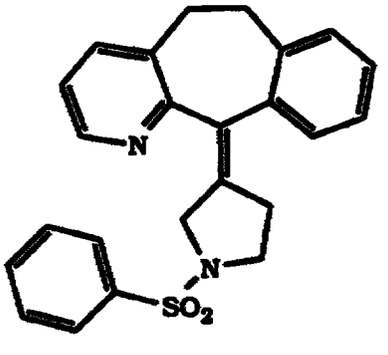
EXAMPLE 2

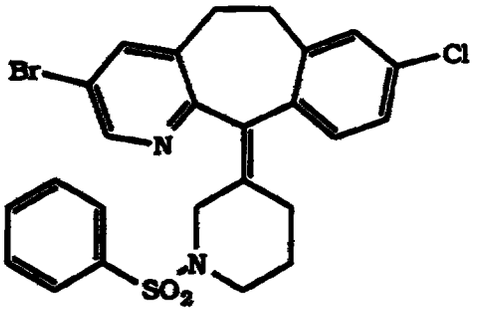
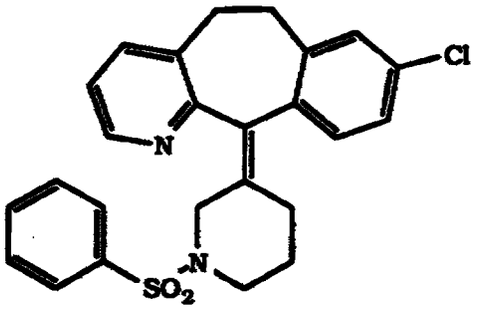
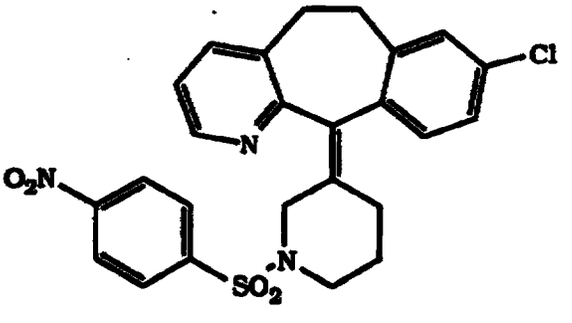
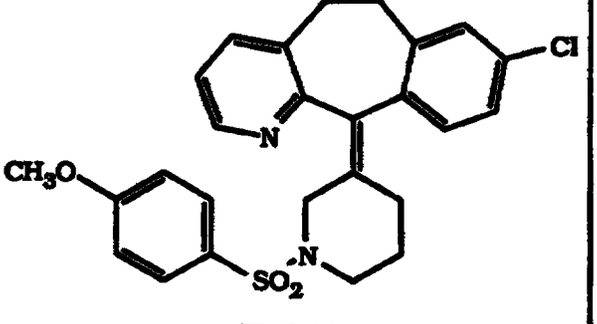


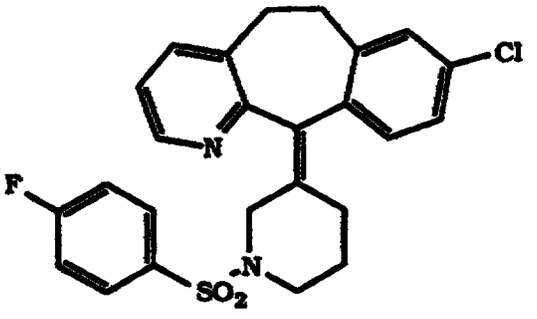
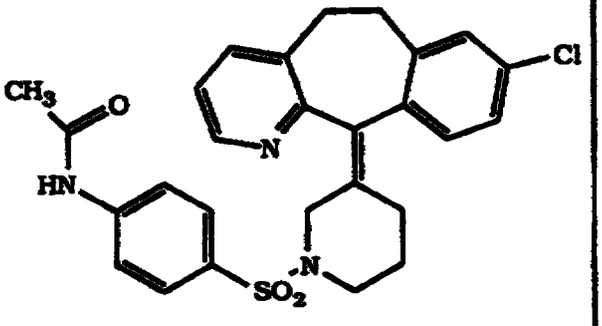
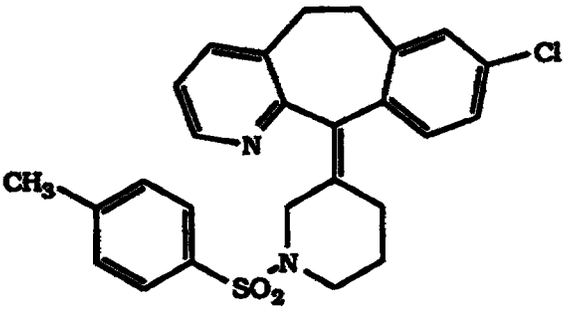
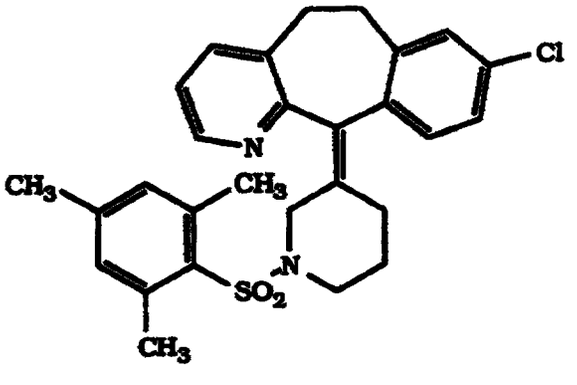
Combine 110 mg of the Z-amine product (P-1) from Preparation 1 (0.339 mmol), 5 mL of pyridine and a catalytic amount (10 mg) of DMAP under a N_2 atmosphere. Cool the solution to 0°C and add $C_6H_5SO_2Cl$ (1.17 mmol, 207 mg). Stir the mixture for 10 min at 0°C, then warm to room temperature and stir for 17h. Add a solution of $NaHCO_3$ (aqueous) to quench the reaction mixture, then extract the aqueous layer with EtOAc-THF (20:1). Combine the extracts, wash with brine, dry over Na_2SO_4, filter and concentrate *in vacuo* to a residue. Chromatograph

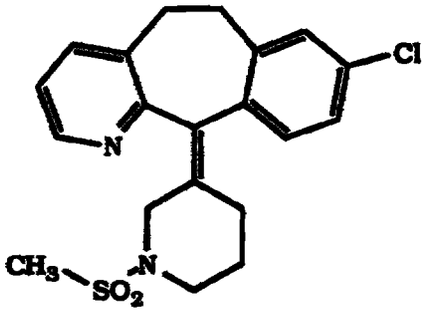
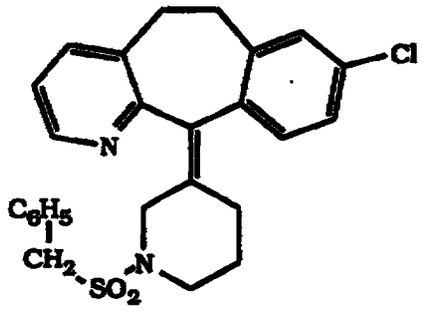
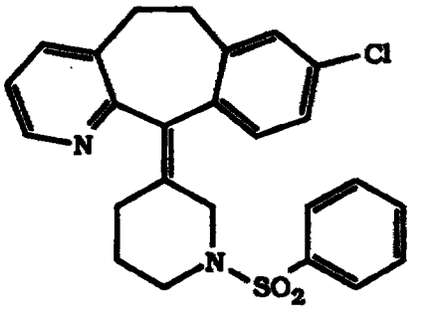
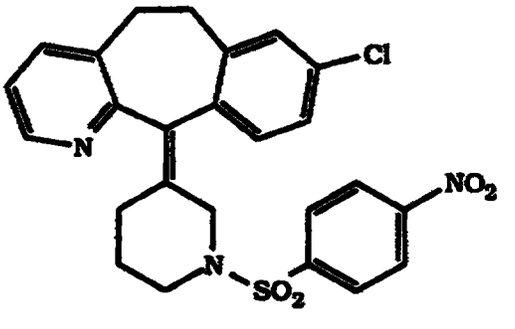
(silica gel, 25% EtOAc:hexane increasing gradually to 35% EtOAc:hexane) to give 80 mg (54 % yield) of the Z-benzene-sulfonamide product (E-2). Analytical data for the Z-N-benzenesulfonamide: MS (CI, M+H) = 437.

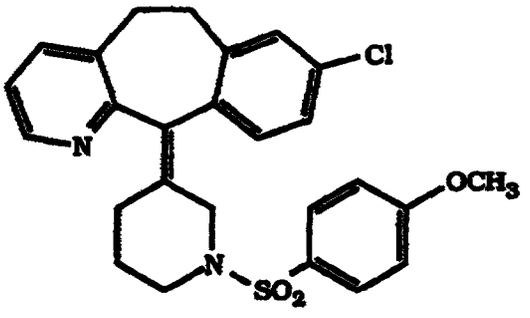
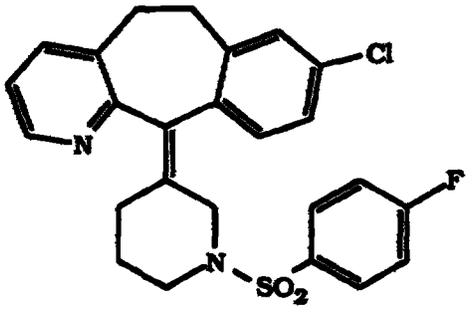
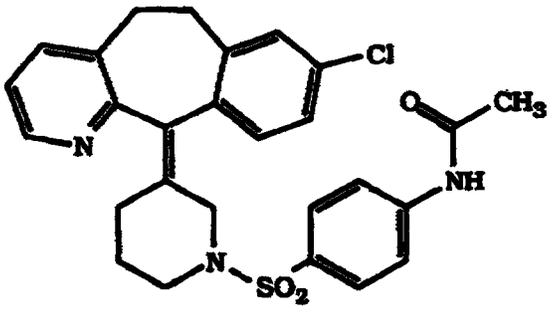
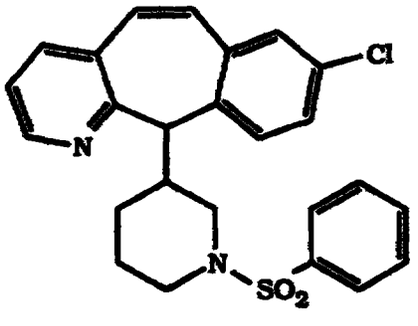
Using the appropriate sulfonyl chloride and the amine indicated, and following substantially the same procedure as described for Example 2, the following sulfonamide compounds were prepared:

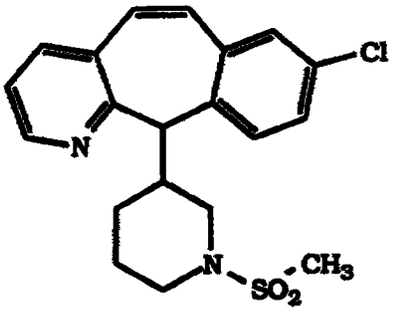
Amine	Amide	Analytical Data
P-2	 (E-2A)	MS (FAB, M+H) = 437
P-2A	 (E-2B)	
P-1A	 (E-2C)	MS (CI, M+H) = 403

P-3	 (E-2D)	m.p. = 184°-185°C MS (CI, M+H) = 529, 531
P-3A	 (E-2E)	MS (CI) = 451, 453
P-3A	 (E-2F)	m.p. = 178°-179°C MS (CI, M+H) = 496
P-3A	 (E-2G)	m.p. = 160°-161°C MS (CI) = 481, 483

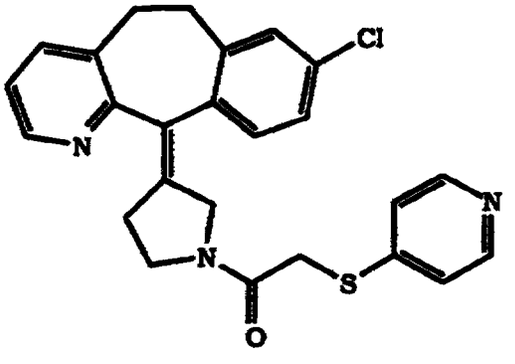
P-3A	 (E-2H)	m.p. = 173°-174°C MS (CI, M+H) = 469, 471
P-3A	 (E-2J)	m.p. = 162°-163°C MS (CI) = 508, 510
P-3A	 (E-2K)	MS (CI) = 465, 467
P-3A	 (E-2L)	m.p. = 227°-229°C MS (CI) = 493, 495

P-3A	 (E-2M)	m.p. = 189°-190°C MS (CI, MH) = 389, 391
P-3A	 (E-2N)	m.p. = 198°-199°C MS (CI) = 465, 467
P-4A	 (E-2P)	m.p. = 235°-236°C MS (CI, M+H) = 451, 453
P-4A	 (E-2Q)	m.p. = 232°-233°C MS (CI, M+H) = 496, 498

P-4A	 (E-2R)	m.p. = 168°-169°C MS (CI, M+H) = 481, 483
P-4A	 (E-2S)	m.p. = 154°-155°C MS (CI, M+H) = 469, 471
P-4A	 (E-2T)	m.p. = 147°-149°C MS (CI, M+H) = 508, 510
P-5	 (E-2U)	m.p. = 178°-179°C MS (FAB, M+H) = 451, 453

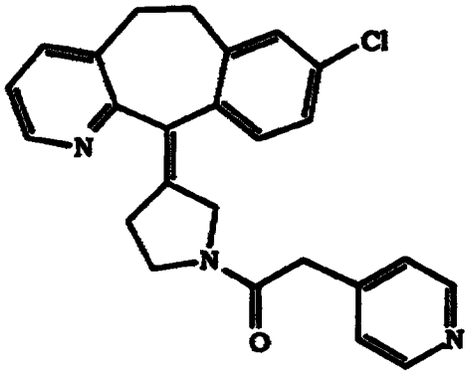
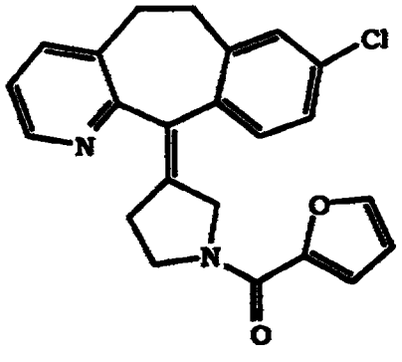
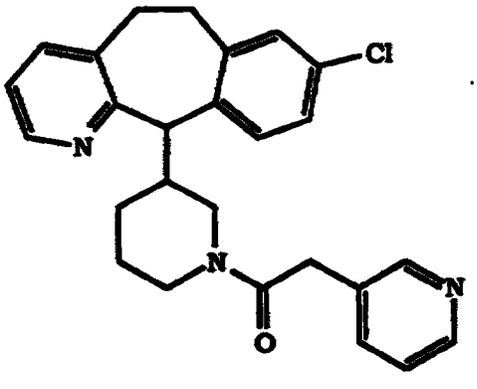
P-5	 (E-2V)	M.P. = 231°-232°C MS (CI, M+H) = 389, 391

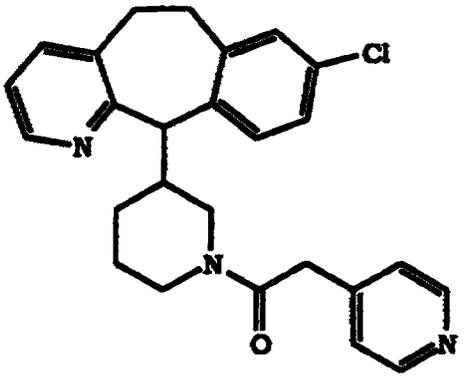
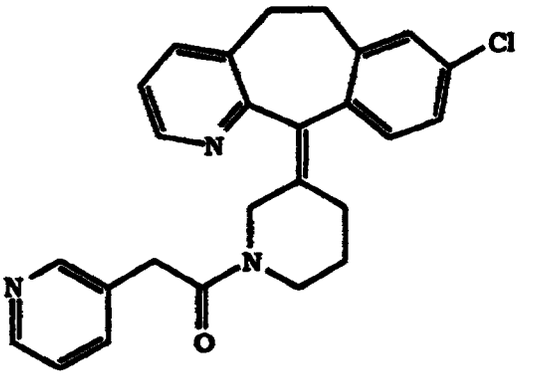
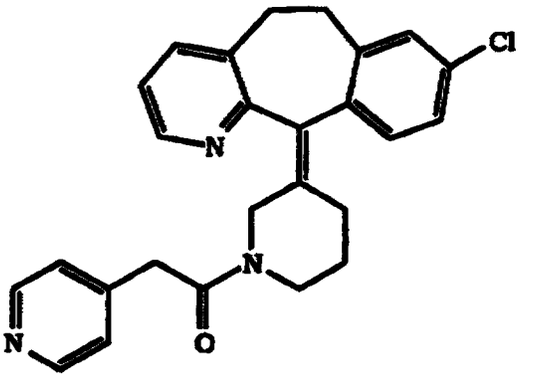
EXAMPLE 3

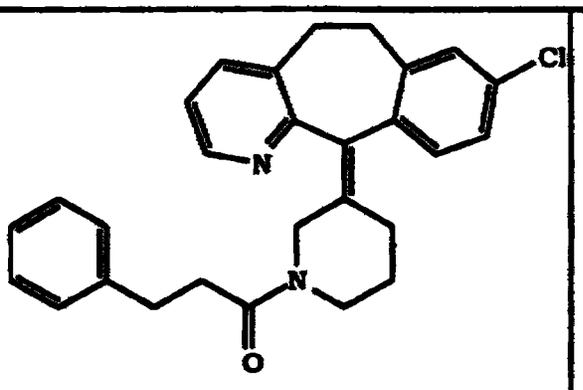
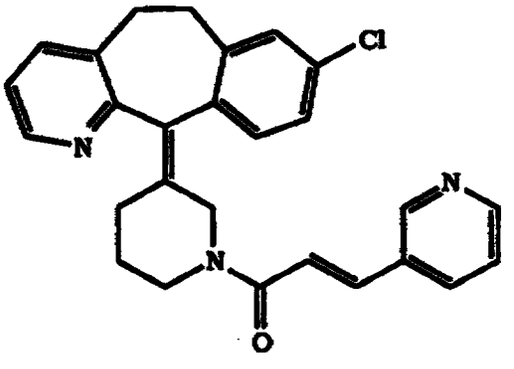
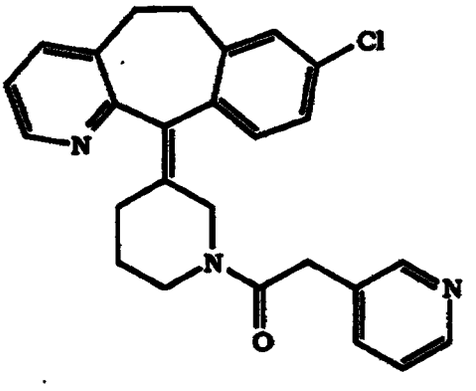


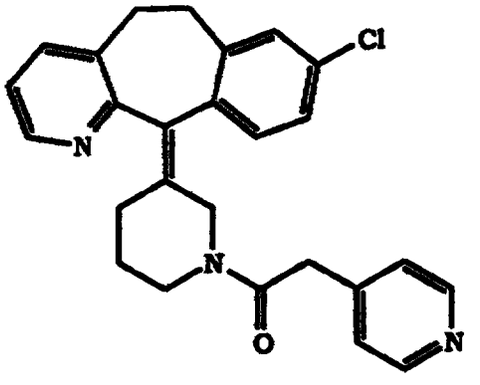
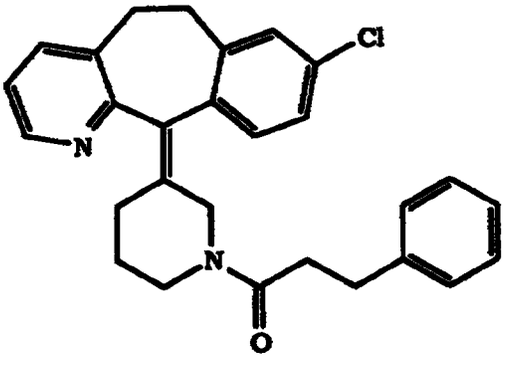
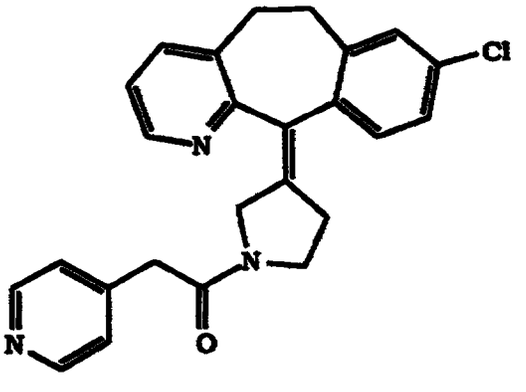
Combine 80 mg of the E-amine product (P-2) from Preparation 2 (0.270 mmol) 3 mL of DMF and 2 mL of NMM under a N_2 atmosphere. Cool the mixture to 0°C and add 110 mg of HOBT (0.888 mmol), 250 mg of DEC (1.31 mmol), and 0.651 mmol of (4-pyridylthio)acetic acid. After 30 min., warm to room temperature and stir for 24 hours. Concentrate *in vacuo* to a residue, dilute the residue with $NaHCO_3$ (aqueous), and extract with CH_2Cl_2. Combine the extracts, wash with brine, dry over Na_2SO_4, filter and concentrate *in vacuo* to give a residue. Decolorize with activated carbon and chromatograph (silica gel, 5% MeOH:CH_2Cl_2 increasing gradually to 10% MeOH:CH_2Cl_2) to give 45 mg (37% yield) of the E-(4-pyridylthio)amide product (E-3). Analytical data for the E-(4-pyridylthio)amide: MS (CI, M+H) = 448.

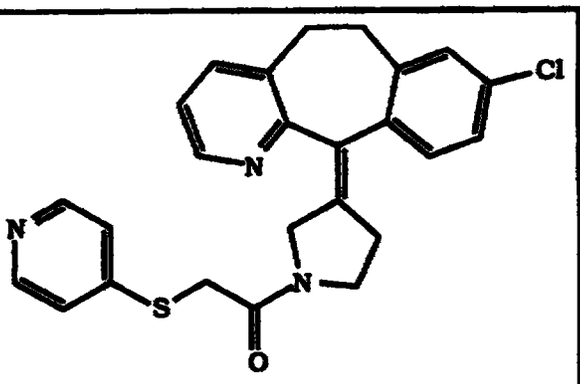
Using the appropriate carbocylic acid and the amine indicated, and following substantially the same procedure as described for Example 3, the following amide compounds were prepared:

Amine	Amide	Analytical Data
P-2	 (E-3A)	MS (CI, M+H) = 415
P-2	 (E-3B)	MS (CI, M+H) = 391
P-6	 (E-3C)	MS (CI, M+H) = 432, 434

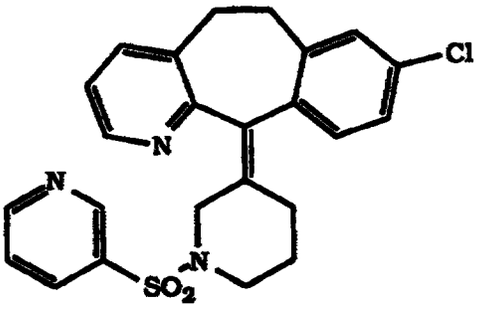
P-6	 (E-3D)	MS (CI, M+H) = 432, 434
P-3A	 (E-3E)	MS (CI, M+H) = 430, 432
P-3A	 (E-3F)	MS (CI, M+H) = 430, 432

P-3A	 (E-3G)	MS (CI, M+H) = 443, 445
P-4A	 (E-3H)	m.p. = 165°-166°C MS (CI, M+H) = 442, 444
P-4A	 (E-3J)	m.p. = 157°-158°C MS (CI, M+H) = 430, 432

P-4A	 (E-3K)	MS (CI, M+H) = 430, 432
P-4A	 (E-3L)	MS (CI, M+H) = 443, 445
P-1	 (E-3M)	MS (CI, M+H) = 416

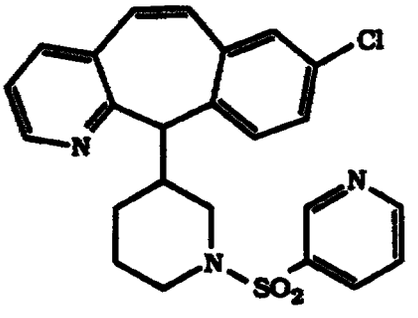
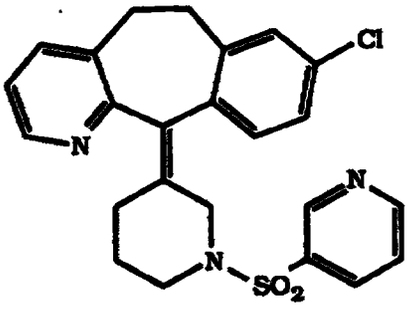
P-1	 (E-3N)	MS (CI, M+H) = 448

EXAMPLE 4

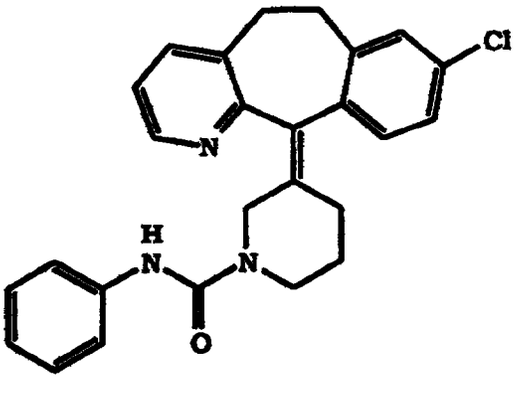


Combine 100 mg (0.626 mmol) of 3-pyridinesulfonic acid and 3 mL of anhydrous pyridine at 0°C and add 100mg (0.406 mmol) of 4-nitrobenzenesulfonyl chloride. Add 5 mg of DMAP and stir the mixture at 0°C for 7 hours. Add 80 mg (0.258 mmol) of the Z-amine (P-3A) from Preparation 3 and stir the mixture for 1 hour at 0°C, then overnight at 20°C. Add 50 mL of CH_2Cl_2 and 20 mL of water, separate the layers, and wash the organic phase with water. Dry over $MgSO_4$, filter and concentrate *in vacuo* to a residue. Chromatograph the residue (silica gel, 5% MeOH/EtOAc + 1% concentrated NH_4OH (aqueous)), crystallize from 10 mL of Et_2O and dry the resulting solid at 60°C *in vacuo* to give 180 mg (68.9% yield) of the Z-3-pyridylsulfonamide product (E-4). Analytical data for the Z-3-pyridylsulfonamide (E-4): m.p. = 158°-159°C; MS (CI) = 452, 454.

Using the the E- or Z-amine indicated, and following substantially the same procedure as described for Example 4, the following sulfonamide compounds were prepared:

Amine	Amide	Analytical Data
P-5	 (E-4A)	m.p. = 178°-179°C MS (CI, M+H) = 452, 454
P-4A	 (E-4B)	m.p. = 214°-215°C MS (CI, M+H) = 452, 454

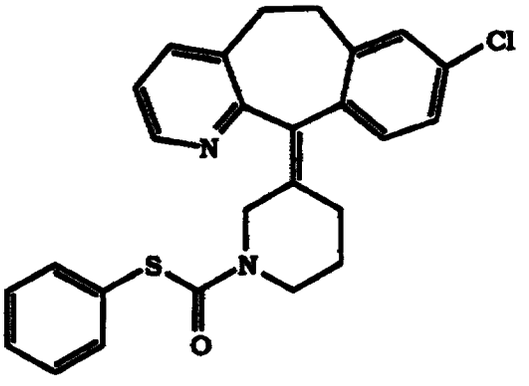
EXAMPLE 5



Combine 70 mg (0.225 mmol) of Z-amine (P-3A) from Preparation 3, 0.2 mL (1.53 mmol) of $C_6H_5N{=}C{=}O$ and 15 mL of CH_2Cl_2 at 0°C, add 0.2 mL (2.72 mmol) of Et_3N and stir at 20°C overnight. Add 20 mL of water and 25 mL of CH_2Cl_2, separate the layers and dry the organic phase over $MgSO_4$. Filter, concentrate *in vacuo* to a residue, chromatograph the residue (silica gel, 20% EtOAc/hexanes) and crystallize from 10 mL of Et_2O. Dry the resulting solid *in vacuo* at 20°C to give 75 mg (78% yield) of the

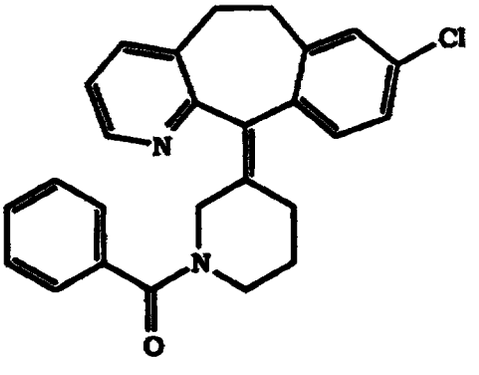
Z-phenylurea product (E-5). Analytical data for the Z-phenylurea (E-5): m.p. = 184°-185°C; MS (CI, M+H) = 430, 432.

EXAMPLE 6



Combine 25 mg (0.08 mmol) of the Z-amine (P-3A) from Preparation 3, 0.2 mL (2.72 mmol) of Et_3N and 2 mL of anhydrous pyridine at 0°C and add 0.2 g (1.13 mmol) of phenyl chlorothioformate. Add 5 mg (0.04 mmol) of DMAP and stir the mixture overnight. Concentrate *in vacuo* to a residue and partition the residue between 25 mL of EtOAc and 20 mL of water. Dry the organic phase over Na_2SO_4, filter and concentrate *in vacuo* to a residue. Chromatograph the residue (silica gel, 5% MeOH/EtOAc), triturate with hexanes and dry the resulting solid at 20°C *in vacuo* to give 30 mg (83.6% yield) of the Z-phenylthiocarbamate product (E-6). Analytical data for the Z-phenylthiocarbamate (E-6): m.p. = 187°-188°C; MS (CI) = 447.

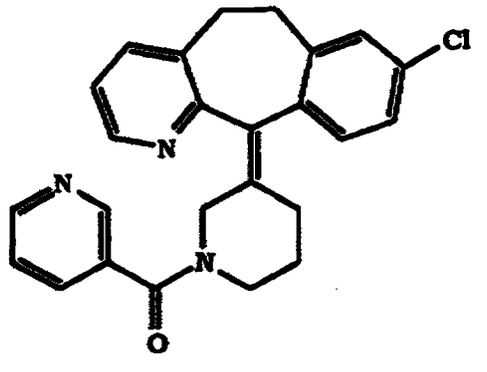
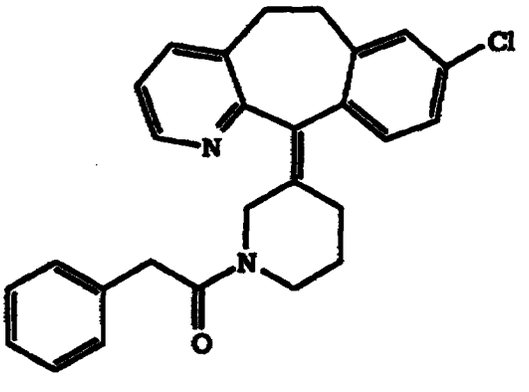
EXAMPLE 7



Combine 40 mg (0.129 mmol) of the Z-amine (P-3A) from Preparation 3, 0.5 mL (0.391 mmol) of benzoyl chloride and 5 mL of anhydrous pyridine at 0°C, add 2 mg of DMAP, then stir the mixture overnight at 20°C. Add 20 mL of CH_2Cl_2 and 10 mL of water, separate the layers and wash the organic phase with 20 mL

of brine. Dry the organic phase over $MgSO_4$, filter and concentrate *in vacuo* to a residue. Chromatograph the residue (silica gel, 5% MeOH/EtOAc + 1% concentrated NH_4OH (aqueous)), recrystallize the resulting solid from acetone/hexanes and dry the at 60°C *in vacuo* to give the Z-phenylamide product (E-7). Analytical data for the Z-phenylamide (E-7): m.p. = 215°-216°C; MS (CI, M+H) = 415, 417.

Using the appropriate acid chloride and the E- or Z-amine indicated, and following substantially the same procedure as described for Example 7, the following amide compounds were prepared:

Amine	Amide	Analytical Data
P-3A	 (E-7A)	MS (CI, M+H) = 416, 418
P-3A	 (E-7B)	MS (CI, M+H) = 429, 431

ASSAYS

FPT IC_{50} (inhibition of farnesyl protein transferase, in vitro enzyme assay), GGPT IC_{50} (inhibition of geranylgeranyl protein transferase, in vitro enzyme assay), COS Cell IC_{50} (Cell-Based Assay) and Cell Mat Assay were determined following the assay procedures in WO 95/10516.

TABLE 2 - FPT INHIBITION

COMPOUND	FPT IC_{50} (μM)	COS IC_{50} (μM)
E-1	0.01-10	-----
E-1A	-----	-----
E-1B	0.01-10	-----
E-2	0.01-10	0.01-10
E-2A	-----	-----
E-2B	-----	-----
E-2C	0.01-10	-----
E-3	0.01-10	-----
E-3A	10-100	-----
E-3B	10-100	-----
E-3C	0.01-10	-----
E-3D	10-100	-----
E-3J	0.01-10	-----
E-3K	10-100	-----
E-3L	10-100	-----
E-3H	-----	-----
E-2P	0.01-10	-----
E-4B	0.01-10	-----
E-2Q	10-100	-----
E-1J	0.01-10	-----
E-2R	10-100	-----
E-2T	10-100	-----
E-2S	10-100	-----
E-1K	10-100	-----
E-3E	10-100	-----
E-3F	10-100	-----
E-7A	10-100	-----
E-7B	10-100	-----
E-6	10-100	-----
E-2E	0.01-10	0.01-10
E-3G	10-100	-----
E-2M	10-100	-----
E-7	>100	-----
E-1G	0.01-10	-----

E-2J	10-100	-----
E-2H	10-100	-----
E-2G	10-100	-----
E-2F	10-100	-----
E-4	0.01-10	-----
E-1H	0.01-10	-----
E-2K	10-100	-----
E-2L	10-100	-----
E-2N	10-100	-----
E-5	10-100	-----
E-1D	0.01-10	-----
E-2D	0.01-10	-----
E-2U	0.01-10	-----
E-2V	10-100	-----
E-4A	0.01-10	-----
E-3N	0.01-10	-----
E-3M	10-100	-----

TABLE 2

COMPARISON OF FPT INHIBITION AND GGPT INHIBITION

COMPOUND	ENZYME INHIBITION FPT IC_{50} μM	ENZYME INHIBITION GGPT IC_{50} μM
E-2E	0.01-10	7.4 mM
E-1G	0.01-10	<13

TABLE 3

INHIBITION OF TUMOR CELL GROWTH - MAT ASSAY

COMPOUND	INHIBITION OF TUMOR CELL GROWTH (IC_{50} μM)	INHIBITION OF NORMAL CELL GROWTH (IC_{50} μM)
E-2E	<3.1	>50
E-1G	12.5	>25
E-2H	12.5	>25

RESULTS

1. Enzymology:

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 2.

The data also demonstrate that compounds of the invention are poorer inhibitors of geranylgeranyl protein transferase (GGPT) assayed using Ras-CVLL as isoprenoid acceptor. Tested compounds were inactive or weakly active as geranylgeranyl transferase inhibitors at 20 μg/ml. This selectivity is important for the therapeutic potential of the compounds used in the methods of this invention, and increases the potential that the compounds will have selective growth inhibitory properties against Ras-transformed cells.

2. Cell-Based: COS Cell and Cell Mat Assays

Immunoblot analysis of the Ras protein expressed in Ras-transfected COS cells indicated that the farnesyl transferase inhibitors of this invention inhibit Ras-CVLS processing, causing accumulation of unprocessed Ras (Table 2). For example, compounds E-2 and E-2E inhibit Ras-CVLS processing with IC_{50} values of >5 and 2.5 μM, respectively. These results show that the compounds inhibit farnesyl protein transferase in intact cells and indicate their potential to block cellular transformation by activated Ras oncogenes.

Compounds of this invention also inhibited the growth of Ras-transformed tumor cells in the Mat assay. For example, compound E-2E inhibited with an IC_{50} value of <3.1 μM. This compound only displayed cytotoxic activity against the normal cell monolayer at higher concentrations (IC_{50} of >50 μM).

In Vivo Anti-Tumor Studies:

The anti-tumor activity of compounds of the present invention can also be determined by the method described in WO 95/10516.

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

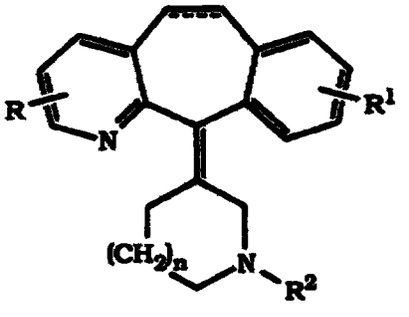
Method of Manufacture

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.

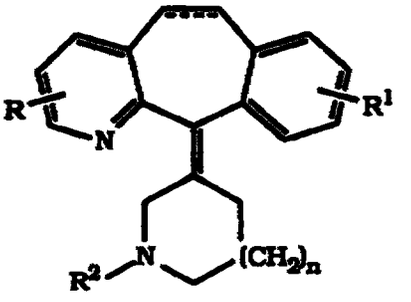
While the present invention has been described in conjunction with the specific embodiments set forth above, many alternatives, modifications and variations thereof will be apparent to those of ordinary skill in the art. All such alternatives, modifications and variations are intended to fall within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

WHAT IS CLAIMED IS:

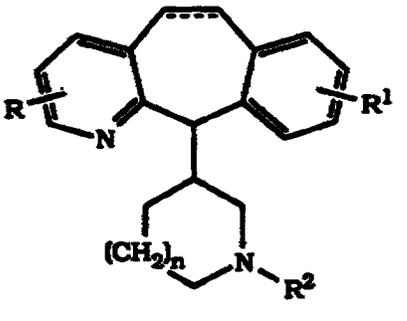
1. A method for inhibiting the abnormal growth of cells comprising administering an effective amount of a compound of formula (Ia), (Ib) or (Ic)



(Ia),



(Ib)



(Ic)

wherein:

R and R^1 are independently selected from H, $(C_1$-$C_6)$alkyl, halogeno, OH, $(C_1$-$C_6)$alkoxy; NH_2; $(C_1$-$C_6)$alkylamino; di[$(C_1$-$C_6)$alkyl]amino; CF_3; SO_3H; CO_2R^3; NO_2; SO_2NH_2; and $CONHR^4$;

R^2 is $R^5C(O)$-, $R^5CH_2C(O)$-, $R^5C(R^6)_2C(O)$-, R^5SO_2-, $R^5CH_2SO_2$-, $R^5SCH_2C(O)$-, $R^5OC(O)$-, $R^5NHC(O)$-, $R^5C(O)C(O)$- or $R^5SC(O)$-;

R^3 is $(C_1$-$C_6)$alkyl, aryl;

R^4 is $(C_1$-$C_6)$alkyl;

R^5 is $(C_1$-$C_6)$alkyl, aryl, aryl$(C_1$-$C_6)$alkyl, aryl$(C_2$-$C_6)$alkenyl, heteroaryl, heteroaryl$(C_1$-$C_6)$alkyl, heteroaryl$(C_2$-$C_6)$alkenyl or heterocycloalkyl;

each R^6 independently represents $(C_1$-$C_6)$alkyl, or both R^4 groups together with the carbon atom to which they are attached comprise a $(C_3$-$C_7)$carbocyclic ring;

n is 0 or 1; and

the dotted line represents an optional double bond;

and pharmaceutically acceptable salts thereof.

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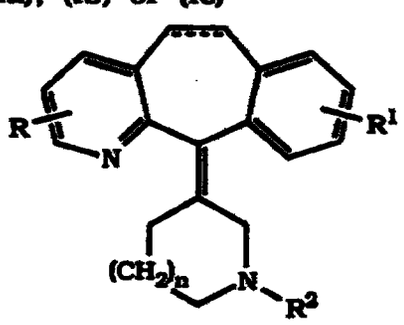
2. The method of Claim 1 wherein the cells inhibited are tumor cells expressing an activated Ras oncogene.

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.

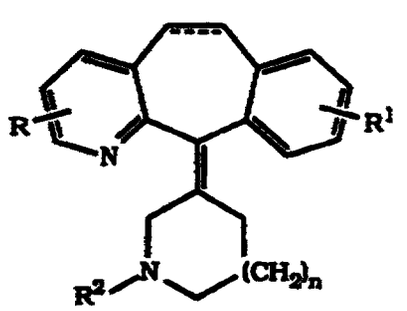
4. The method of Claim 1 wherein the inhibition of the abnormal growth of cells occurs by the inhibition of farnesyl protein transferase.

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.

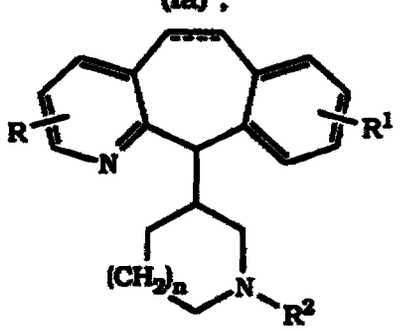
6. A compound selected from a compound of the formula (Ia), (Ib) or (Ic)



(Ia),



(Ib)



(Ic)

wherein:

R and R^1 are independently selected from H, $(C_1$-$C_6)$alkyl, halogeno, OH, $(C_1$-$C_6)$alkoxy, NH_2, $(C_1$-$C_6)$alkylamino, di$((C_1$-$C_6)$alkyl)amino, CF_3, SO_3H, CO_2R^3, NO_2, SO_2NH_2, and $CONHR^4$;

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R^2 is $R^5C(O)$-, $R^5CH_2C(O)$-, $R^5C(R^6)_2C(O)$-, R^5SO_2-, $R^5CH_2SO_2$-, $R^5SCH_2C(O)$-, $R^5OC(O)$-, $R^5NHC(O)$-, $R^5C(O)C(O)$- or $R^5SC(O)$-;

R^3 is $(C_1$-$C_6)$alkyl, aryl;

R^4 is $(C_1$-$C_6)$alkyl;

R^5 is $(C_1$-$C_6)$alkyl, aryl, aryl$(C_1$-$C_6)$alkyl, aryl$(C_2$-$C_6)$alkenyl, heteroaryl, heteroaryl$(C_1$-$C_6)$alkyl, heteroaryl$(C_2$-$C_6)$alkenyl or heterocycloalkyl;

each R^6 independently represents $(C_1$-$C_6)$alkyl, or both R^6 groups together with the carbon atom to which they are attached comprise a $(C_3$-$C_7)$carbocyclic ring;

n is 0 or 1; and

the dotted line represents an optional double bond;

and pharmaceutically acceptable salts thereof.

7. A compound of claim 6 having the structure (Ib).

8. A compound of claim 6 wherein R and R^1 are indepedently selected from H or halogeno.

9. A compound of claim 8 wherein R^2 is $R^5C(O)$-, $R^5CH_2C(O)$-, $R^5SCH_2C(O)$-, R^5SO_2-, $R^5CH_2SO_2$-, $R^5NHC(O)$- or $R^5SC(O)$-;

10. A compound of claim 9 wherein R^5 is methyl, phenyl, benzyl, 2-thienyl, 4-pyridyl, 3-pyridyl, 5-chloro-2-thienyl, p-tolyl, p-nitrophenyl, p-flurorophenyl, p-acetoxyphenyl, 5-chloro-1,3-dimethyl-4-pyrazolyl, 2,4,6-trimethylphenyl, 5-(benzoylamino-methyl)-2-thienyl, 2-methoxycarbonyl-3-thienyl, 4-pyridylthio, 2-furanyl, E-(3-pyridyl)ethenyl, p-methoxyphenyl, p-acetamido-phenyl, or the sodium salt of 2-carboxy-3-thienyl.

11. A compound of claim 7 wherein R^2 is $R^5C(O)$-, and R^5 is 2-furanyl or E-(3-pyridyl)ethenyl.

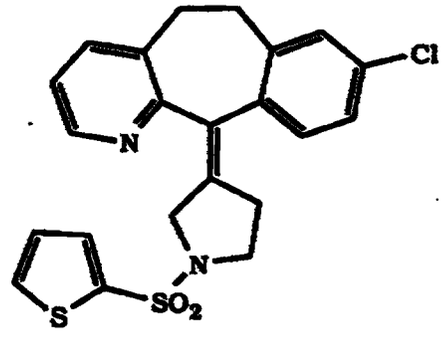
12. A compound of claim 7 wherein R^2 is $R^5CH_2C(O)$-, and R^5 is 4-pyridylthio, 4-pyridyl, 3-pyridyl or benzyl.

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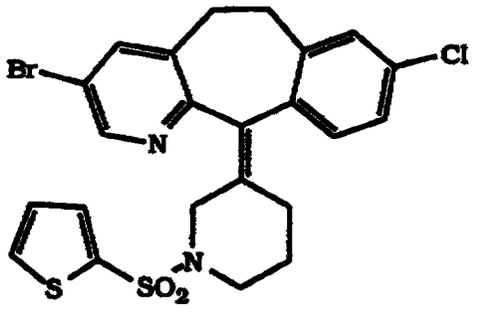
13. A compound of claim 7 wherein R^2 is R^5SO_2-, and R^5 is 2-thienyl, 5-chloro-2-thienyl, 5-chloro-1,3-dimethyl-4-pyrazolyl, 5-(benzoylaminomethyl)-2-thienyl, 2-methoxycarbonyl-3-thienyl, phenyl, p-nitrophenyl, p-methoxyphenyl, p-fluorophenyl, p-acetamidophenyl, p-tolyl, 2,4,6-trimethylphenyl, methyl, benzyl, 3-pyridyl or the sodium salt of 2-carboxy-3-thienyl.

14. A compound of claim 7 wherein R^2 is selected from $R^5CH_2SO_2$- wherein R^5 is phenyl; $R^5NHC(O)$- wherein R^5 is phenyl; $R^5SC(O)$-, wherein R^5 is phenyl; R^5SO_2- wherein R^5 is aryl or heteroaryl; or $R^5SCH_2C(O)$- wherein R^5 is heteroaryl.

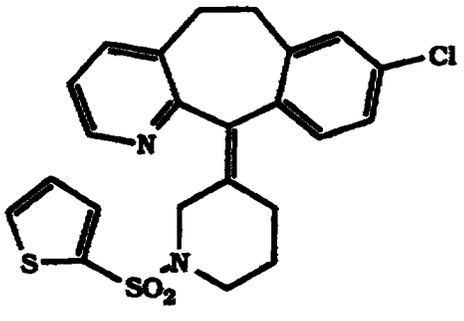
15. A compound of claim 7 having the structural formula



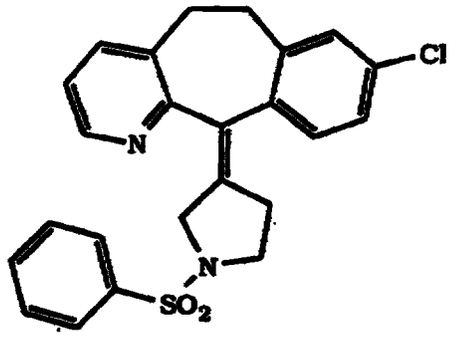
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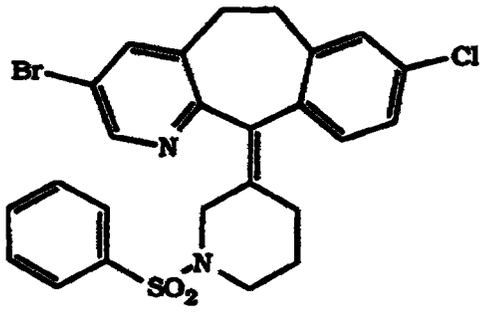
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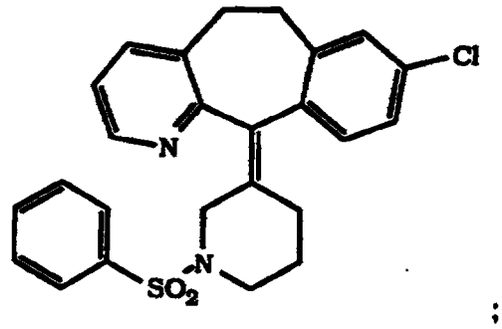
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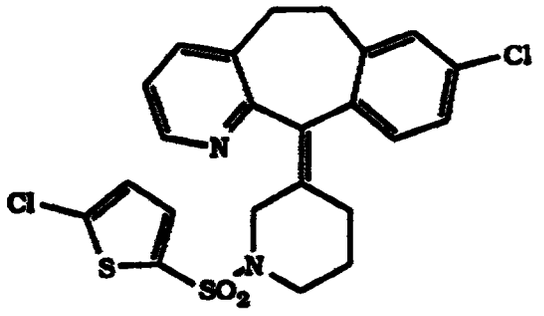


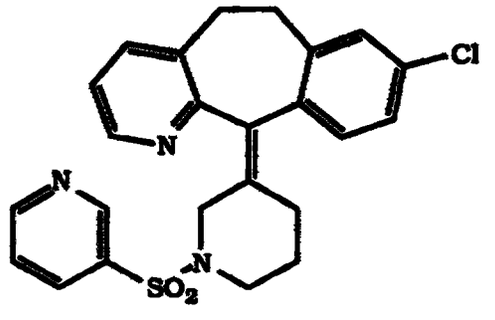
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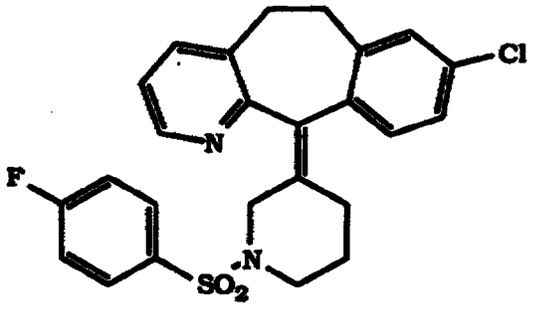
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or



16. 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.

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

18. The use of a compound of Claim 6 for inhibiting the abnormal growth of cells.

* C L M S *