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(71) Applicant *(for all designated States except US)*: **MERCK & CO., INC.** [US/US]; 126 East Lincoln Avenue, Rathway, NJ 07065-0907 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **BELL, Ian, M.** [GB/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(74) Common Representative: **MERCK & CO., INC.**; 126 East Lincoln Avenue, Rathway, NJ 07065-0907 (US).

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(54) Title: INHIBITORS OF PRENYL-PROTEIN TRANSFERASE

(57) Abstract: The present invention is directed to spirocyclic compounds which inhibit prenyl-protein transferase and the prenylation of the oncogene protein Ras. The invention is further directed to chemotherapeutic compositions containing the compounds of this invention and methods for inhibiting prenyl-protein transferase and the prenylation of the oncogene protein Ras.

TITLE OF THE INVENTION
INHIBITORS OF PRENYL-PROTEIN TRANSFERASE

BACKGROUND OF THE INVENTION

The Ras proteins (Ha-Ras, Ki4a-Ras, Ki4b-Ras and N-Ras) are part of a signalling pathway that links cell surface growth factor receptors to nuclear signals initiating cellular proliferation. Biological and biochemical studies of Ras action indicate that Ras functions like a G-regulatory protein. In the inactive state, Ras is bound to GDP. Upon growth factor receptor activation Ras is induced to exchange GDP for GTP and undergoes a conformational change. The GTP-bound form of Ras propagates the growth stimulatory signal until the signal is terminated by the intrinsic GTPase activity of Ras, which returns the protein to its inactive GDP bound form (D.R. Lowy and D.M. Willumsen, *Ann. Rev. Biochem. 62*:851-891 (1993)). Mutated *ras* genes (Ha-*ras*, Ki4a-*ras*, Ki4b-*ras* and N-*ras*) are found in many human cancers including colorectal carcinoma, exocrine pancreatic carcinoma, and myeloid leukemias. The protein products of these genes are defective in their GTPase activity and constitutively transmit a growth stimulatory signal.

Ras must be localized to the plasma membrane for both normal and oncogenic functions. At least 3 post-translational modifications are involved with Ras membrane localization, and all 3 modifications occur at the C-terminus of Ras. The Ras C-terminus contains a sequence motif termed a "CAAX" or "Cys-Aaa^1-Aaa^2-Xaa" box (Cys is cysteine, Aaa is an aliphatic amino acid, the Xaa is any amino acid) (Willumsen *et al., Nature 310*:583-586 (1984)). Depending on the specific sequence, this motif serves as a signal sequence for the enzymes farnesyl-protein transferase or geranylgeranyl-protein transferase type I, which catalyze the alkylation of the cysteine residue of the CAAX motif with a C_{15} or C_{20} isoprenoid, respectively. (S. Clarke., *Ann. Rev. Biochem. 61*:355-386 (1992); W.R. Schafer and J. Rine, *Ann. Rev. Genetics 30*:209-237 (1992)). The term prenyl-protein transferase may be used to refer generally to farnesyl-protein transferase and geranylgeranyl-protein transferase type I. The Ras protein is one of several proteins that are known to undergo post-translational farnesylation. Other farnesylated proteins include the Ras-related GTP-binding proteins such as Rho, fungal mating factors, the nuclear lamins, and the gamma subunit of transducin. James, et al., *J. Biol. Chem. 269*, 14182 (1994) have identified a peroxisome associated protein Pxf which is also farnesylated.

James, et al., have also suggested that there are farnesylated proteins of unknown structure and function in addition to those listed above.

Inhibition of farnesyl-protein transferase has been shown to block the growth of Ras-transformed cells in soft agar and to modify other aspects of their transformed phenotype. It has also been demonstrated that certain inhibitors of farnesyl-protein transferase selectively block the processing of the Ras oncoprotein intracellularly (N.E. Kohl et al., *Science*, *260*:1934-1937 (1993) and G.L. James et al., *Science*, *260*:1937-1942 (1993). Recently, it has been shown that an inhibitor of farnesyl-protein transferase blocks the growth of *ras*-dependent tumors in nude mice (N.E. Kohl et al., *Proc. Natl. Acad. Sci U.S.A.*, *91*:9141-9145 (1994) and induces regression of mammary and salivary carcinomas in *ras* transgenic mice (N.E. Kohl et al., *Nature Medicine*, 1:792-797 (1995).

Indirect inhibition of farnesyl-protein transferase *in vivo* has been demonstrated with lovastatin (Merck & Co., Rahway, NJ) and compactin (Hancock et al., *ibid*; Casey *et al.*, *ibid*; Schafer et al., *Science 245*:379 (1989)). These drugs inhibit HMG-CoA reductase, the rate limiting enzyme for the production of poly-isoprenoids including farnesyl pyrophosphate. Farnesyl-protein transferase utilizes farnesyl pyrophosphate to covalently modify the Cys thiol group of the Ras CAAX box with a farnesyl group (Reiss et al., *Cell, 62*:81-88 (1990); Schaber et al., *J. Biol. Chem., 265*:14701-14704 (1990); Schafer et al., *Science, 249*:1133-1139 (1990); Manne et al., *Proc. Natl. Acad. Sci USA, 87*:7541-7545 (1990)). Inhibition of farnesyl pyrophosphate biosynthesis by inhibiting HMG-CoA reductase blocks Ras membrane localization in cultured cells. However, direct inhibition of farnesyl-protein transferase would be more specific and attended by fewer side effects than would occur with the required dose of a general inhibitor of isoprene biosynthesis.

Inhibitors of farnesyl-protein transferase (FPTase) have been described in two general classes. The first are analogs of farnesyl diphosphate (FPP), while the second class of inhibitors is related to the protein substrates (e.g., Ras) for the enzyme. The peptide derived inhibitors that have been described are generally cysteine containing molecules that are related to the CAAX motif that is the signal for protein prenylation. (Schaber *et al.*, *ibid*; Reiss *et. al.*, *ibid*; Reiss *et al.*, *PNAS*, *88*:732-736 (1991)). Such inhibitors may inhibit protein prenylation while serving as alternate substrates for the farnesyl-protein transferase enzyme, or may be purely competitive inhibitors (U.S. Patent 5,141,851, University of Texas; N.E. Kohl et al., *Science*, *260*:1934-1937 (1993); Graham, et al., *J. Med. Chem., 37*, 725 (1994)). In

general, deletion of the thiol from a CAAX derivative has been shown to dramatically reduce the inhibitory potency of the compound. However, the thiol group potentially places limitations on the therapeutic application of FPTase inhibitors with respect to pharmacokinetics, pharmacodynamics and toxicity. Therefore, a functional replacement for the thiol is desirable.

It has recently been reported that farnesyl-protein transferase inhibitors are inhibitors of proliferation of vascular smooth muscle cells and are therefore useful in the prevention and therapy of arteriosclerosis and diabetic disturbance of blood vessels (JP H7-112930).

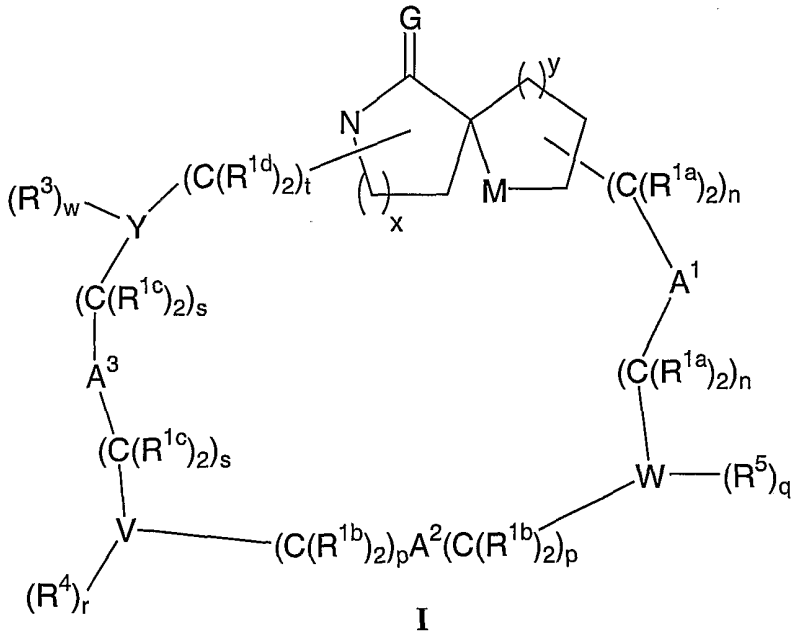
It has recently been disclosed that certain tricyclic compounds which optionally incorporate a piperidine moiety are inhibitors of FPTase (WO 95/10514, WO 95/10515 and WO 95/10516). Imidazole-containing inhibitors of farnesyl protein transferase have also been disclosed (WO 95/09001 and EP 0 675 112 A1).

It is, therefore, an object of this invention to develop compounds that do not have a thiol moiety, and that will inhibit prenyl-protein transferase and thus, the post-translational prenylation of proteins. It is a further object of this invention to develop chemotherapeutic compositions containing the compounds of this invention and methods for producing the compounds of this invention.

SUMMARY OF THE INVENTION

The present invention comprises spirocyclic compounds which inhibit prenyl-protein transferase. Further contained in this invention are chemotherapeutic compositions containing these prenyl-protein transferase inhibitors and methods for their production.

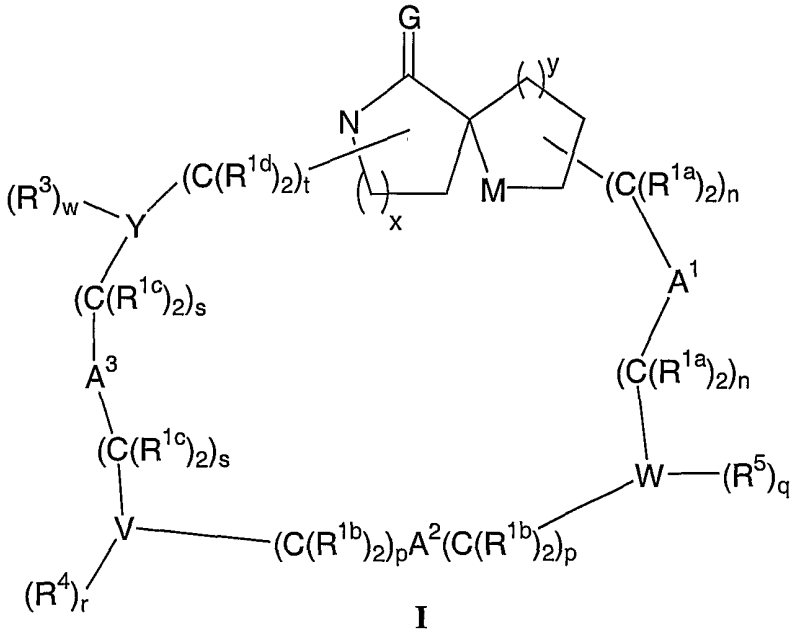
The compounds of this invention are illustrated by the formula I:



I

DETAILED DESCRIPTION OF THE INVENTION

The compounds of this invention are useful in the inhibition of prenyl-protein transferase and the prenylation of the oncogene protein Ras. In a first embodiment, the compounds of the instant invention are illustrated by the formula I:



I

wherein

M is selected from N, O or S;

A^1, A^2 and A^3 are independently selected from

1) a bond,
2) O,
3) C(O),
4) $N(R^{10})_2$,
5) $C(O)NR^{10}$,
6) $NR^{10}C(O)$,
7) $S(O)_m$,
8) $OS(O)_m$,
9) $NR^{10}C(O)NR^{10}$,
10) OC(O),
11) C(O)O
12) CH=CH,
13) C≡C,
14) $OC(O)NR^{10}$, or
15) $NR^{10}C(O)O$;

R^{1a}, R^{1b}, R^{1c} and R^{1d} are independently selected from

1) H,
2) unsubstituted or substituted C_1-C_6 alkyl,
3) unsubstituted or substituted C_2-C_8 alkenyl,
4) unsubstituted or substituted C_2-C_8 alkynyl,
5) unsubstituted or substituted aryl,
6) unsubstituted or substituted C_3-C_{10} cycloalkyl,
7) unsubstituted or substituted heterocycle,
8) $-OR^{10}$,
9) $R^{11}S(O)_m$-,
10) $-N(R^{10})_2$,
11) $R^{10}C(O)$-,
12) $-C(O)NR^6R^7$,
13) $R^{10}C(O)NR^{10}$-,

14) $(R^{10})_2NC(O)NR^{10}$-,
15) $R^{10}OC(O)$-,
16) $R^{10}C(O)O$-, or
17) $R^{11}OC(O)NR^{10}$-;

R^3 is independently selected from
1) H,
2) halo,
3) -CN,
4) $-NO_2$,
5) unsubstituted or substituted C_1-C_6 alkyl,
6) unsubstituted or substituted C_2-C_8 alkenyl,
7) unsubstituted or substituted C_2-C_8 alkynyl,
8) unsubstituted or substituted aryl,
9) unsubstituted or substituted heterocycle,
10) C_1-C_6 perfluoroalkyl,
11) $-OR^{10}$,
12) OCF_3,
13) $-CH_2CF_3$,
14) unsubstituted or substituted C_3-C_{10} cycloalkyl,
15) NR^6R^7,
16) $-C(O)R^{10}$,
17) $-OC(O)R^{10}$,
18) $-O(C_1$-C_6 alkyl$)OR^{10}$-,
19) $-S(O)_mR^{11}$,
20) $-C(O)NR^6R^7$,
21) $-NR^6C(O)R^{10}$,
22) $-(C_1$-C_6 alkyl$)OR^{10}$, or
23) $-(C_1$-C_6 alkyl$)C(O)R^{10}$;

R^4 is independently selected from
1) H,
2) unsubstituted or substituted C_1-C_6 alkyl,
3) unsubstituted or substituted C_2-C_8 alkenyl,

4) unsubstituted or substituted C_2-C_8 alkynyl,
5) unsubstituted or substituted aryl,
6) unsubstituted or substituted heterocycle,
7) unsubstituted or substituted C_3-C_{10} cycloalkyl,
8) unsubstituted or substituted C_1-C_6 perfluoroalkyl,
9) halo,
10) $-OR^{10}$,
11) CN,
12) $-S(O)_mR^{11}$,
13) $-C(O)NR^6R^7$,
14) $-NR^{10}C(O)R^{10}$,
15) NO_2,
16) $-N(R^{10})_2$,
17) $-C(O)R^{10}$,
18) $-OC(O)R^{10}$,
19) $R^{10})_2NC(O)NR^{10}-$, or
20) $R^{10}OC(O)NR^{10}-$;

R^5 is independently selected from
1) H,
2) unsubstituted or substituted C_1-C_6 alkyl,
3) unsubstituted or substituted C_2-C_8 alkenyl,
4) unsubstituted or substituted C_2-C_8 alkynyl,
5) unsubstituted or substituted C_3-C_{10} cycloalkyl,
6) unsubstituted or substituted aryl,
7) unsubstituted or substituted heterocycle,
8) $-S(O)_mR^{11}$,
9) $-C(O)NR^6R^7$,
10) $-S(O)_2NR^6R^7$,
11) $-C(O)R^6$,
12) $-C(O)OR^6$,
13) $-N(R^{10})_2$,
14) CN,
15) halo,

16) $-OR^{10}$,
17) NO_2, or
18) $R^{11}OC(O)NR^{10}$-;

R^6 and R^7 are independently selected from: H, C_1-C_6 alkyl, C_{3-6} cycloalkyl, heterocycle, aryl, aroyl, heteroaroyl, arylsulfonyl, heteroarylsulfonyl, unsubstituted or substituted with:

a) C_{1-6} alkoxy,
b) C_1-C_{20} alkyl
c) aryl or heterocycle,
d) halogen,
e) HO,
f) $-C(O)R^{11}$,
g) $-SO_2R^{11}$, or
h) $N(R^{10})_2$; or

R^6 and R^7 may be joined in a ring;

R^{10} is independently selected from

1) H,
2) unsubstituted or substituted C_1-C_6 alkyl,
3) unsubstituted or substituted perfluoroalkyl,
4) unsubstituted or substituted aralkyl,
5) unsubstituted or substituted aryl, or
6) unsubstituted or substituted heterocycle;

R^{11} is independently selected from

1) unsubstituted or substituted C_1-C_6 alkyl,
2) unsubstituted or substituted aralkyl,
3) unsubstituted or substituted aryl, or
4) unsubstituted or substituted heterocycle;

G is selected from H_2 or O;

V is selected from

1) heterocycle or
2) aryl;

W is a heterocycle;

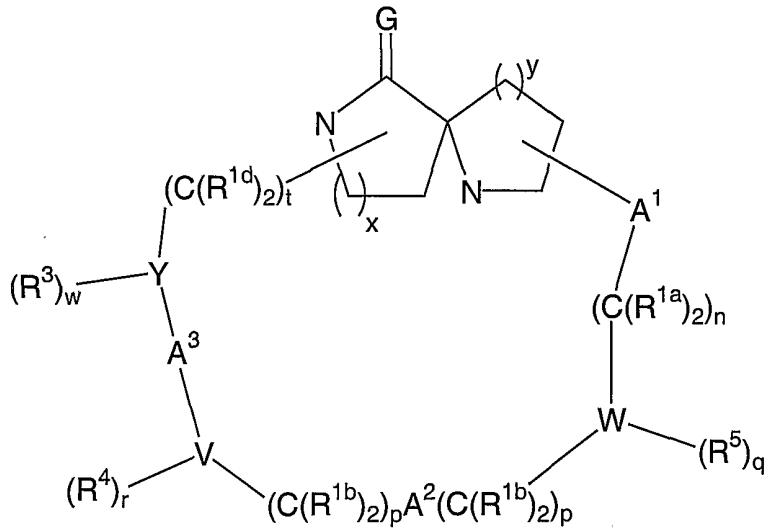
Y is selected from

1) a bond,
2) C_1-C_8 alkyl,
3) C_2-C_8 alkenyl,
4) C_2-C_8 alkynyl,
5) C_3-C_{10} cycloalkyl,
6) aryl, or
7) heterocycle;

m is 0, 1 or 2;
n is 0, 1, 2, 3, 4, 5 or 6;
p is 0, 1, 2, 3, 4, 5 or 6;
q is 0, 1, 2, 3 or 4;
r is 0, 1, 2, 3 or 4;
s is 0, 1, 2, 3, 4, 5 or 6;
t is 0, 1, 2, 3, or 4;
w is 0, 1, 2, 3 or 4, provided Y is not a bond;
x is 0, 1, 2 or 3; and
y is 0, 1, 2 or 3;

or a pharmaceutically acceptable salt or stereoisomer thereof.

In another embodiment, the compounds of the instant invention are illustrated by formula A:



A

wherein

A^1, A^2 and A^3 are independently selected from

1) a bond,
2) O,
3) C(O),
4) $N(R^{10})_2$,
5) $C(O)NR^{10}$,
6) $NR^{10}C(O)$,
7) $NR^{10}C(O)NR^{10}$,
8) OC(O),
9) C(O)O
10) $OC(O)NR^{10}$, or
11) $NR^{10}C(O)O$;

R^{1a}, R^{1b} and R^{1d} are independently selected from

1) H,
2) unsubstituted or substituted C_1-C_6 alkyl,
3) unsubstituted or substituted aryl,
4) unsubstituted or substituted C_3-C_{10} cycloalkyl,

5) $-OR^{10}$,
6) $-N(R^{10})_2$,
7) $R^{10}C(O)-$,
8) $-C(O)NR^6R^7$, or
9) $R^{10}C(O)NR^{10}-$;

R^3 is independently selected from
1) H,
2) halo,
3) -CN,
4) $-NO_2$,
5) unsubstituted or substituted C_1-C_6 alkyl,
6) unsubstituted or substituted aryl,
7) $-OR^{10}$,
8) OCF_3,
9) unsubstituted or substituted C_3-C_{10} cycloalkyl,
10) NR^6R^7,
11) $-C(O)R^{10}$,
12) $-C(O)NR^6R^7$,
13) $-NR^6C(O)R^{10}$,
14) $-(C_1$-C_6 alkyl$)OR^{10}$, or
15) $-(C_1$-C_6 alkyl$)C(O)R^{10}$;

R^4 is independently selected from
1) H,
2) unsubstituted or substituted C_1-C_6 alkyl,
3) unsubstituted or substituted C_2-C_8 alkenyl,
4) unsubstituted or substituted C_2-C_8 alkynyl,
5) unsubstituted or substituted aryl,
6) unsubstituted or substituted heterocycle,
7) unsubstituted or substituted C_3-C_{10} cycloalkyl,
8) halo,
9) $-OR^{10}$,
10) CN,

11) $-C(O)NR^6R^7$,
12) $-NR^{10}C(O)R^{10}$, or
13) $-N(R^{10})_2$;

R^5 is independently selected from
1) H,
2) unsubstituted or substituted C_1-C_6 alkyl,
3) unsubstituted or substituted C_3-C_{10} cycloalkyl,
4) unsubstituted or substituted aryl,
5) $-C(O)NR^6R^7$,
6) $-C(O)R^6$,
7) $-C(O)OR^6$,
8) halo, or
9) $-OR^{10}$;

R^6 and R^7 are independently selected from: H, C_1-C_6 alkyl, C_{3-6} cycloalkyl, heterocycle, aryl, aroyl, heteroaroyl, arylsulfonyl, heteroarylsulfonyl, unsubstituted or substituted with:
a) C_{1-6} alkoxy,
b) C_1-C_{20} alkyl
c) aryl or heterocycle,
d) halogen,
e) HO,
f) $-C(O)R^{11}$,
g) $-SO_2R^{11}$, or
h) $N(R^{10})_2$; or

R^6 and R^7 may be joined in a ring;

R^{10} is independently selected from
1) H,
2) unsubstituted or substituted C_1-C_6 alkyl,
3) unsubstituted or substituted perfluoroalkyl,
4) unsubstituted or substituted aralkyl,

5) unsubstituted or substituted aryl, or
6) unsubstituted or substituted heterocycle;

R^{11} is independently selected from
1) unsubstituted or substituted C_1-C_6 alkyl,
2) unsubstituted or substituted aralkyl,
3) unsubstituted or substituted aryl, or
4) unsubstituted or substituted heterocycle;

G is selected from H_2 or O;

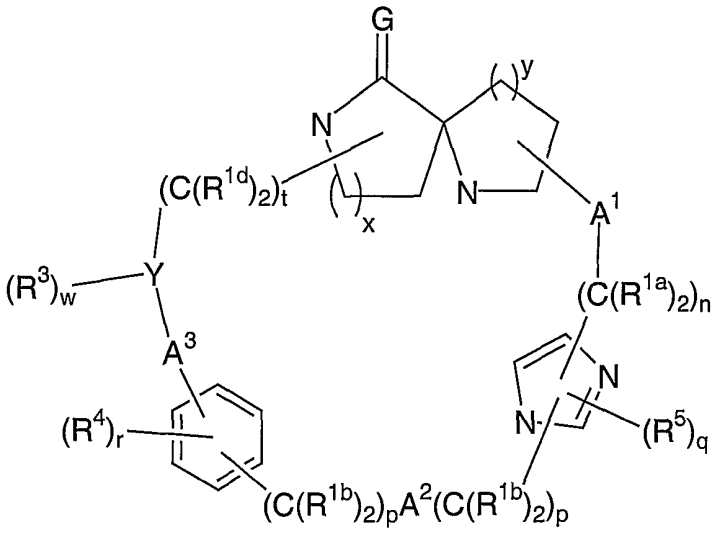
V is selected from
1) heterocycle or
2) aryl;

W is a heterocycle selected from imidazolyl, pyridyl;

Y is selected from
1) a bond,
2) C_3-C_{10} cycloalkyl,
3) aryl, or
4) heterocycle;

m is 0, 1 or 2;
n is 0, 1, 2, 3, 4, 5 or 6;
p is 0, 1, 2, 3, 4, 5 or 6;
q is 0, 1, 2, 3 or 4;
r is 0, 1, 2, 3 or 4;
t is 0, 1, 2, 3, or 4;
w is 0, 1, 2, 3 or 4, provided Y is not a bond;
x is 1 or 2; and
y is 1 or 2;
or a pharmaceutically acceptable salt or stereoisomer thereof.

In another embodiment, the compounds of the instant invention are illustrated by formula B:



B

wherein

A^1 and A^2 are independently selected from

1) a bond,
2) O,
3) C(O),
4) $C(O)NR^{10}$,
5) $NR^{10}C(O)$,
6) OC(O), or
7) C(O)O;

A^3 is selected from

1) a bond,
2) O,
3) C(O),
4) $C(O)NR^{10}$, or
5) $NR^{10}C(O)$;

R^{1a}, R^{1b} and R^{1d} are independently selected from

1) H,
2) unsubstituted or substituted C_1-C_6 alkyl,
3) unsubstituted or substituted aryl,
4) unsubstituted or substituted C_3-C_{10} cycloalkyl,
5) $-OR^{10}$,
6) $-N(R^{10})_2$, or
7) $R^{10}C(O)$-;

R^3 is independently selected from

1) H,
2) halo,
3) -CN,
4) $-NO_2$,
5) unsubstituted or substituted C_1-C_6 alkyl,
6) unsubstituted or substituted aryl,
7) unsubstituted or substituted heterocycle,
8) $-OR^{10}$,
9) OCF_3,
10) unsubstituted or substituted C_3-C_{10} cycloalkyl,
11) NR^6R^7,
12) $-C(O)R^{10}$,
13) $-C(O)NR^6R^7$, or
14) $-NR^6C(O)R^{10}$;

R^4 is independently selected from

1) H,
2) unsubstituted or substituted C_1-C_6 alkyl,
3) unsubstituted or substituted C_2-C_8 alkenyl,
4) unsubstituted or substituted C_2-C_8 alkynyl,
5) unsubstituted or substituted aryl,
6) unsubstituted or substituted heterocycle,
7) unsubstituted or substituted C_3-C_{10} cycloalkyl,
8) halo,

9) $-OR^{10}$,
10) CN, or
11) $-N(R^{10})_2$;

R^5 is independently selected from

1) H,
2) unsubstituted or substituted C_1-C_6 alkyl,
3) unsubstituted or substituted C_3-C_{10} cycloalkyl,
4) unsubstituted or substituted aryl,
5) $-C(O)R^6$,
6) $-C(O)OR^6$,
7) halo, or
8) $-OR^{10}$;

R^6 and R^7 are independently selected from: H, C_1-C_6 alkyl, C_{3-6} cycloalkyl, heterocycle, aryl, aroyl, heteroaroyl, arylsulfonyl, heteroarylsulfonyl, unsubstituted or substituted with:

a) C_{1-6} alkoxy,
b) C_1-C_{20} alkyl
c) aryl or heterocycle,
d) halogen,
e) HO,
f) $-C(O)R^{11}$,
g) $-SO_2R^{11}$, or
h) $N(R^{10})_2$; or

R^6 and R^7 may be joined in a ring;

R^{10} is independently selected from

1) H,
2) unsubstituted or substituted C_1-C_6 alkyl,
3) unsubstituted or substituted perfluoroalkyl,
4) unsubstituted or substituted aralkyl,
5) unsubstituted or substituted aryl, or

6) unsubstituted or substituted heterocycle;

R^{11} is independently selected from

1) unsubstituted or substituted C_1-C_6 alkyl,
2) unsubstituted or substituted aralkyl,
3) unsubstituted or substituted aryl, or
4) unsubstituted or substituted heterocycle;

G is selected from H_2 or O;

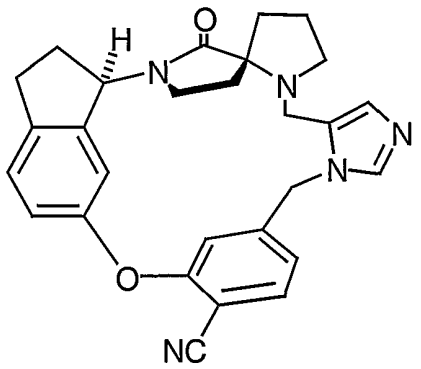
Y is selected from

1) a bond,
2) C_3-C_{10} cycloalkyl,
3) aryl, or
4) heterocycle;

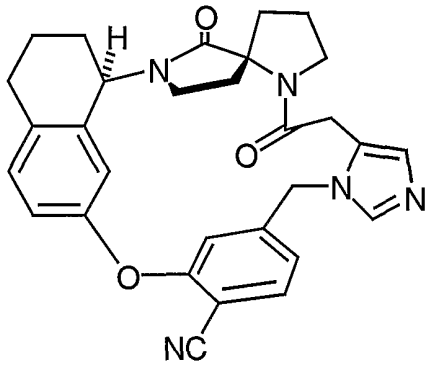
m is 0, 1 or 2;
n is 0, 1, 2, 3, 4, 5 or 6;
p is 0, 1, 2, 3, 4, 5 or 6;
q is 0, 1, 2, 3 or 4;
r is 0, 1, 2, 3 or 4;
t is 0, 1, 2, 3, or 4;
w is 0, 1, 2, 3 or 4, provided Y is not a bond;
x is 1 or 2; and
y is 1 or 2;

or a pharmaceutically acceptable salt or stereoisomer thereof.

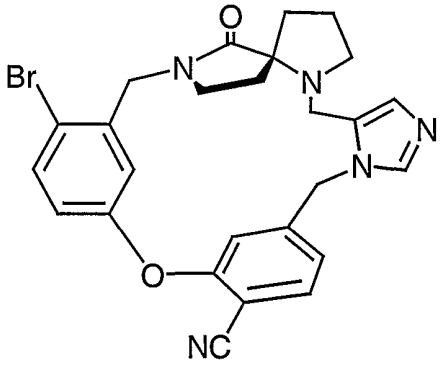
Examples of the compounds of the instant invention are:



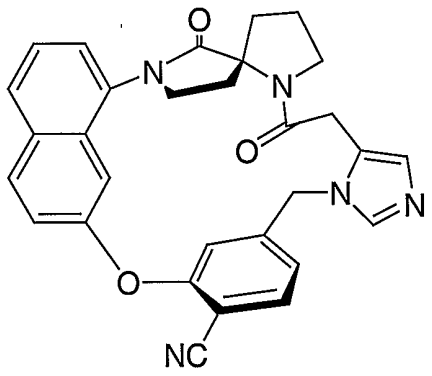
(17*R*, 20*R*)-19,20,21,22-tetrahydro-19-oxo-17*H*-15,17:18,20-diethano-6,10:12,16-dimetheno-20,21-propano-16*H*-imidazo[3,4-*h*][1,8,11,14]oxatriazacycloeicosine-9-carbonitrile;



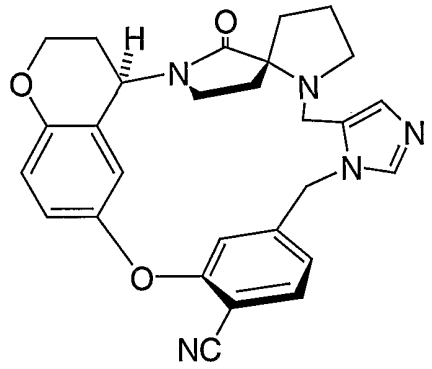
(*R*,*R*)-15,16,17,17a,19,20,22,23-octahydro-19,22-dioxo-5*H*,21*H*-18,20-ethano-12,14-etheno-6,10-metheno-20,21-propanobenz[*d*]imimidazo[4,4-*l*] [1,6,9,13] oxatriazacyclononadecosine-9-carbonitrile;



(*R*)-15-bromo-19,20,21,22-tetrahydro-19-oxo-17*H*-18,20-ethano-6,10:12,16-dimetheno-20,21-propano-16*H*-imidazo[3,4-*h*][1,8,11,14]oxatriazacycloeicosine-9-carbonitrile;



(*R*)-19,20,22,23-tetrahydro-19,22-dioxo-5*H*,21*H*-18,20-ethano-12,14-etheno-6,10-metheno-20,21-propanobenz[*d*]imidazo[4,3-*l*][1,6,9,13]oxatriazacyclononadecosine-9-carbonitrile;



(*R*,*R*)-15,16,17,17a,19,20,21,22-octahydro-15-oxa-19-oxo-5*H*-18,20-ethano-12,14-etheno-6,10-metheno-20,21-propano-18*H*-benz[*d*]imidazo[4,3-*k*][1,6,9,12] oxatriazacyclooctadecosine-9-carbonitrile;

or a pharmaceutically acceptable salt, optical isomer or stereoisomer thereof.

The compounds of the present invention may have asymmetric centers, chiral axes and chiral planes, and occur as racemates, racemic mixtures, and as individual diastereomers, with all possible isomers, including optical isomers, being included in the present invention. (See E.L. Eliel and S.H. Wilen *Stereochemistry*

of Carbon Compounds (John Wiley and Sons, New York 1994), in particular pages 1119-1190) When any variable (e.g. aryl, heterocycle, R^{1a}, R^3 etc.) occurs more than one time in any constituent, its definition on each occurrence is independent at every other occurrence. Also, combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

As used herein, "alkyl" is intended to include both branched and straight-chain saturated aliphatic hydrocarbon groups having 1 to 6 carbon atoms, unless otherwise indicated; "alkoxy" represents an alkyl group having 1 to 6 carbon atoms, unless otherwise indicated, attached through an oxygen bridge. "Halogen" or "halo" as used herein means fluoro, chloro, bromo and iodo.

As used herein, "cycloalkyl" is intended to include non-aromatic hydrocarbon groups having having from 3 to 10 carbon atoms, unless otherwise specified. Examples of such cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclohexyl, cycloheptyl, cyclooctyl, admantyl and the like. Optionally, a carbon atom in the cycloalkyl may be replaced with a heteroatom, such as O, N or S.

If no number of carbon atoms is specified, the term "alkenyl" refers to a non-aromatic hydrocarbon, straight, branched or cyclic, containing from 2 to 10 carbon atoms, unless otherwise indicated, and at least one carbon to carbon double bond. Preferably one carbon to carbon double bond is present, and up to four non-aromatic carbon-carbon double bonds may be present. Thus, "C_2-C_8 alkenyl" means an alkenyl radical having from 2 to 8 carbon atoms. Examples of such alkenyl groups include, but are not limited to, ethenyl, propenyl, butenyl and cyclohexenyl. As described above with respect to alkyl, the straight, branched or cyclic portion of the alkenyl group may contain double bonds and may be substituted if a substituted alkenyl group is indicated.

The term "alkynyl" refers to a hydrocarbon radical straight, branched or cyclic, containing from 2 to 10 carbon atoms, unless otherwise indicated, and at least one carbon to carbon triple bond. Up to three carbon-carbon triple bonds may be present. Thus, "C_2-C_8 alkynyl" means an alkynyl radical having from 2 to 8 carbon atoms. Examples of such alkynyl groups include, but are not limited to, ethynyl, propynyl and butynyl. As described above with respect to alkyl, the straight, branched or cyclic portion of the alkynyl group may contain triple bonds and may be substituted if a substituted alkynyl group is indicated.

As used herein, "aryl" is intended to mean any stable monocyclic or bicyclic carbon ring of up to 7 members in each ring, wherein at least one ring is aromatic. Examples of such aryl elements include, but are not limited to, phenyl, naphthyl, tetrahydronaphthyl, indanyl, biphenyl, phenanthryl, anthryl, acenaphthyl and the like.

As used herein, "aralkyl" is intended to mean an aryl moiety, as defined above, attached through a C_1-C_6 alkyl linker, where alkyl is defined above. Examples of aralkyls include, but are not limited to, benzyl, naphthylmethyl, phenylbutyl and the like.

The term heterocycle or heterocyclic, as used herein, represents a stable 5- to 7-membered monocyclic or stable 8- to 11-membered bicyclic heterocyclic ring which is either saturated or unsaturated, and which consists of carbon atoms and from one to four heteroatoms selected from the group consisting of N, O, and S, and including any bicyclic group in which any of the above-defined heterocyclic rings is fused to a benzene ring. The heterocyclic ring may be attached at any heteroatom or carbon atom which results in the creation of a stable structure. The term heterocycle or heterocyclic includes heteroaryl moieties. Examples of such heterocyclic elements include, but are not limited to, azepinyl, benzimidazolyl, benzisoxazolyl, benzofurazanyl, benzopyranyl, benzothiopyranyl, benzofuryl, benzothiazolyl, benzothienyl, benzoxazolyl, chromanyl, cinnolinyl, dihydrobenzofuryl, dihydrobenzothienyl, dihydrobenzothiopyranyl, dihydrobenzothiopyranyl sulfone, 1,3-dioxolanyl, furyl, imidazolidinyl, imidazolinyl, imidazolyl, indolinyl, indolyl, isochromanyl, isoindolinyl, isoquinolinyl, isothiazolidinyl, isothiazolyl, isothiazolidinyl, morpholinyl, naphthyridinyl, oxadiazolyl, 2-oxoazepinyl, oxazolyl, 2-oxopiperazinyl, 2-oxopiperdinyl, 2-oxopyrrolidinyl, piperidyl, piperazinyl, pyridyl, 2-pyridinonyl pyrazinyl, pyrazolidinyl, pyrazolyl, pyridazinyl, pyrimidinyl, pyrrolidinyl, pyrrolyl, quinazolinyl, quinolinyl, quinoxalinyl, tetrahydrofuryl, tetrahydroisoquinolinyl, tetrahydroquinolinyl, thiamorpholinyl, thiamorpholinyl sulfoxide, thiazolyl, thiazolinyl, thienofuryl, thienothienyl, thienyl and triazolyl.

As used herein, "heteroaryl" is intended to mean any stable monocyclic or bicyclic carbon ring of up to 7 members in each ring, wherein at least one ring is aromatic and wherein from one to four carbon atoms are replaced by heteroatoms selected from the group consisting of N, O, and S. Examples of such heterocyclic elements include, but are not limited to, benzimidazolyl, benzisoxazolyl, benzofurazanyl, benzopyranyl, benzothiopyranyl, benzofuryl, benzothiazolyl,

benzothienyl, benzoxazolyl, chromanyl, cinnolinyl, dihydrobenzofuryl, dihydrobenzothienyl, dihydrobenzothiopyranyl, dihydrobenzothiopyranyl sulfone, furyl, imidazolyl, indolinyl, indolyl, isochromanyl, isoindolinyl, isoquinolinyl, isothiazolyl, naphthyridinyl, oxadiazolyl, pyridyl, pyrazinyl, pyrazolyl, pyridazinyl, pyrimidinyl, pyrrolyl, quinazolinyl, quinolinyl, quinoxalinyl, tetrahydroisoquinolinyl, tetrahydroquinolinyl, thiazolyl, thienofuryl, thienothienyl, thienyl and triazolyl.

As used herein, "heterocyclylalkyl" is intended to mean a heterocyclic moiety, as defined above, attached through a C_1-C_6 alkyl linker, where alkyl is defined above. Examples of heterocyclylalkyls include, but are not limited to, 2-pyridylmethyl, 2-imidazolylethyl, 2-quinolinylmethyl, 2-imidazolylmethyl, 1-piperazineethyl, and the like.

As used herein, the terms "substituted alkyl", "substituted alkenyl", "substituted alkynyl" and "substituted alkoxy", unless otherwise defined, are intended to include the branch or straight-chain alkyl group of the specified number of carbon atoms, wherein the carbon atoms may be substituted with F, Cl, Br, I, CF_3, OCF_3, CN, N_3, NO_2, NH_2, $N(C_1$-C_6 alkyl$)_2$, oxo, OH, -O(C_1-C_6 alkyl), -C(O)H, $S(O)_{0-2}$, (C_1-C_6 alkyl)$S(O)_{0-2}$-, C_2-C_6 alkenyl, C_2-C_6 alkynyl, -(C_1-C_6 alkyl)$S(O)_{0-2}$ (C_1-C_6 alkyl), C_3-C_{20} cycloalkyl, -C(O)NH_2, HC(O)NH-, (C_1-C_6 alkyl)C(O)NH-, $H_2NC(O)NH$-, (C_1-C_6 alkyl)C(O)-, -O(C_1-C_6 alkyl)CF_3, (C_1-C_6 alkyl)OC(O)-, (C_1-C_6 alkyl)O(C_1-C_6 alkyl)-, (C_1-C_6 alkyl)C$(O)_2$(C_1-C_6 alkyl)-, (C_1-C_6 alkyl) OC(O)NH-, aryl, heterocycle, aralkyl, heterocyclylalkyl, halo-aryl, halo-aralkyl, halo-heterocycle, halo-heterocyclylalkyl, cyano-aryl, cyano-aralkyl, cyano-heterocycle and cyano-heterocyclylalkyl.

As used herein, the terms "substituted aryl", "substituted heterocycle", "substituted heteroaryl", "substituted cycloalkyl", "substituted benzyl", "substituted aralkyl" and "substituted heterocyclylalkyl", unless otherwise defined, are intended to include the cyclic group containing from 1 to 3 substitutents in addition to the point of attachment to the rest of the compound. Such substitutents are preferably selected from the group which includes but is not limited to F, Cl, Br, I, CF_3, OCF_3, NH_2, $N(C_1$-C_6 alkyl$)_2$, NO_2, CN, N_3, C_1-C_{20} alkyl, C_3-C_{20} cycloalkyl, -OH, -O(C_1-C_6 alkyl), $S(O)_{0-2}$, (C_1-C_6 alkyl)$S(O)_{0-2}$-, (C_1-C_6 alkyl)$S(O)_{0-2}$(C_1-C_6 alkyl)-, -C(O)NH_2, HC(O)NH-, (C_1-C_6 alkyl)C(O)NH-, $H_2NC(O)NH$-, (C_1-C_6 alkyl)C(O)-, (C_1-C_6 alkyl)OC(O)-, (C_1-C_6 alkyl)O(C_1-C_6 alkyl)-, (C_1-C_6)C$(O)_2$(C_1-C_6 alkyl)-, (C_1-C_6 alkyl)OC(O)NH-, aryl, aralkyl, heterocycle, heterocyclylalkyl, halo-aryl,

halo-aralkyl, haloheterocycle, haloheterocyclylalkyl, cyano-aryl, cyano-aralkyl, cyano-heterocycle and cyanoheterocyclylalkyl.

Lines drawn into the ring systems from substituents (such as from A^3, R^4, R^5 etc.) indicate that the indicated bond may be attached to any of the substitutable ring carbon or nitrogen atoms.

Preferably, R^{1a}, R^{1b} R^{1c} and R^{1d} are independently selected from: hydrogen or unsubstituted or substituted C_1-C_6 alkyl.

Preferably, A^1, A^2 and A^3 are independently selected from a bond, O or C(O). Most preferably, A^1 is a bond or C(O), A^2 is a bond and A^3 is a bond or O.

Preferably, R^3 is independently selected from H, halo, unsubstituted or substituted C_1-C_6 alkyl or OR^{10}. Most preferably, R^3 is independently selected from H or halo.

Preferably, R^4 is independently selected from H, halo, CN, unsubstituted or substituted aryl or unsubstituted or substituted C_1-C_6 alkyl. Most preferably, variable r is 1 to 3, and at least one R^4 is CN.

Preferably R^5 is independently selected from H, unsubstituted or substituted C_1-C_6 alkyl or unsubstituted or substituted aryl. Most preferably, R^5 is hydrogen.

Preferably, G is O.

Preferably, V is aryl. Most preferably, V is phenyl.

Most preferably, W is imidazolyl.

Preferably, n, p, s and t are independently selected from 0, 1 or 2.

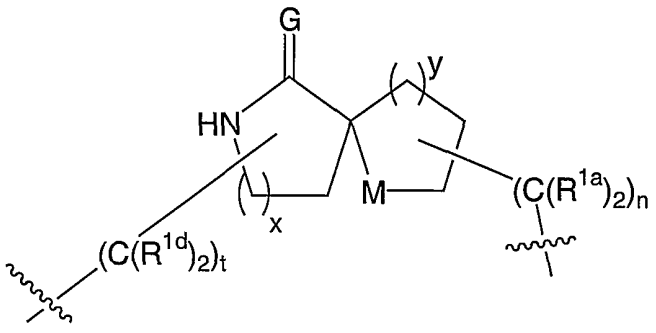
Preferably, q is 0 or 1.

Preferably, w is 0, 1 or 2.

Preferably, x is 1.

Preferably, y is 1.

Preferably, the moiety



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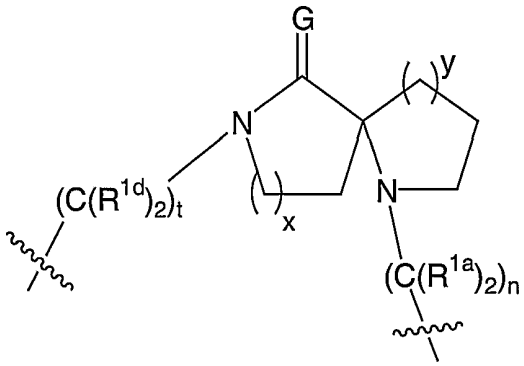
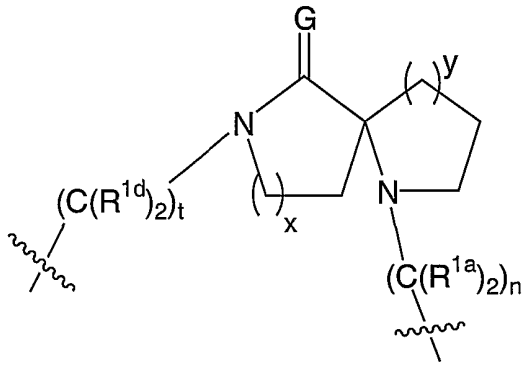


More preferably, the moiety



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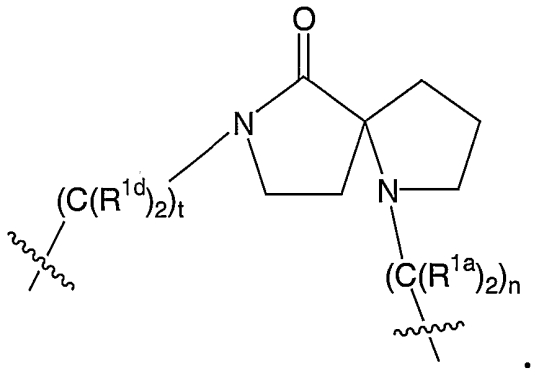
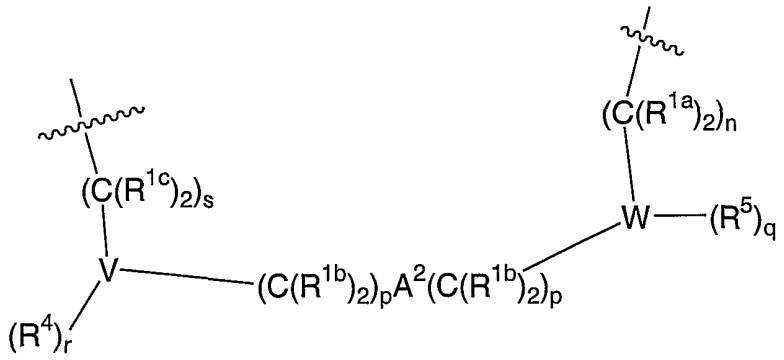
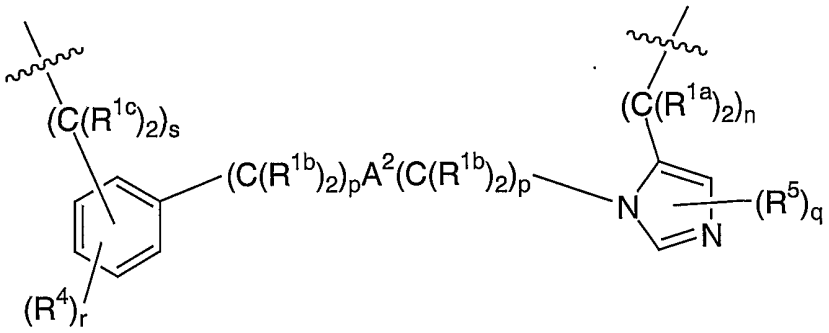


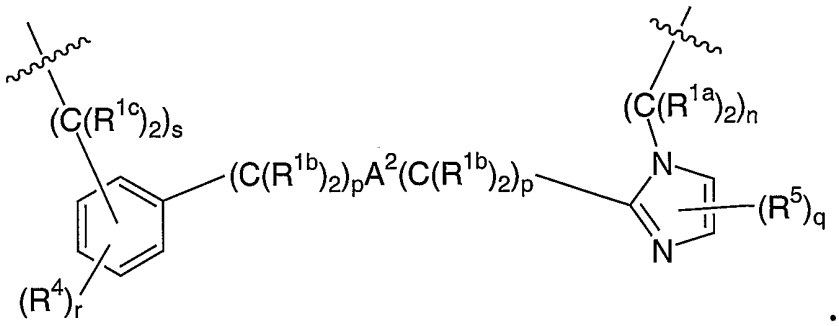
Preferably, the moiety



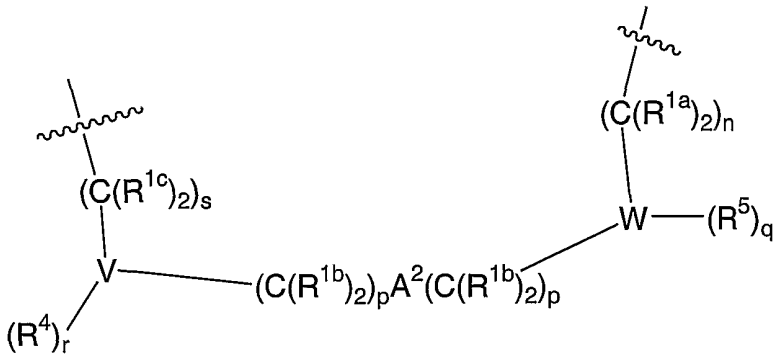
is selected from



5 or



Most preferably, the moiety



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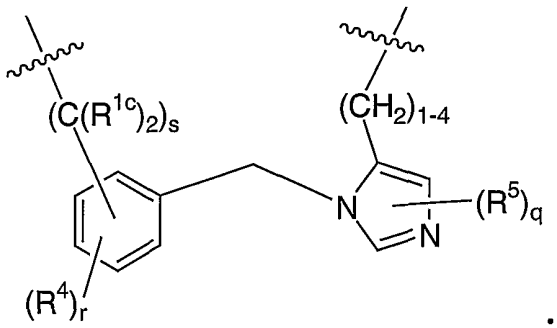


.

It is intended that the definition of any substituent or variable (e.g., R^{1a}, R^3, n, etc.) at a particular location in a molecule be independent of its definitions elsewhere in that molecule. Thus, $-N(R^{10})_2$ represents -NHH, $-NHCH_3$, $-NHC_2H_5$, etc. It is understood that substituents and substitution patterns on the compounds of the instant invention can be selected by one of ordinary skill in the art to provide compounds that are chemically stable and that can be readily synthesized by techniques known in the art, as well as those methods set forth below, from readily available starting materials.

The pharmaceutically acceptable salts of the compounds of this invention include the conventional non-toxic salts of the compounds of this invention as formed, e.g., from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like: and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxy-benzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, trifluoroacetic and the like.

The pharmaceutically acceptable salts of the compounds of this invention can be synthesized from the compounds of this invention which contain a basic moiety by conventional chemical methods. Generally, the salts are prepared either by ion exchange chromatography or by reacting the free base with stoichiometric amounts or with an excess of the desired salt-forming inorganic or organic acid in a suitable solvent or various combinations of solvents.

Abbreviations which may be used in the description of the chemistry and in the Examples that follow include:

Ac_2O	Acetic anhydride;
AcOH	Acetic acid;
AIBN	2,2'-Azobisisobutyronitrile;
BINAP	2,2'-Bis(diphenylphosphino)-1,1' binaphthyl;
Bn	Benzyl;
BOC/Boc	*tert*-Butoxycarbonyl;
CBz	Carbobenzyloxy;
DBAD	Di-*tert*-butyl azodicarboxylate;
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene;
DCE	1,2-Dichloroethane;
DIEA	*N,N*-Diisopropylethylamine;
DMAP	4-Dimethylaminopyridine;
DME	1,2-Dimethoxyethane;
DMF	*N,N*-Dimethylformamide;
DMSO	Methyl sulfoxide;
DPPA	Diphenylphosphoryl azide;
DTT	Dithiothreitol;
EDC	1-(3-Dimethylaminopropyl)-3-ethyl-carbodiimide-hydrochloride;
EDTA	Ethylenediaminetetraacetic acid;
Et_2	Diethyl ether;
Et_3N	Triethylamine;
EtOAc	Ethyl acetate;
EtOH	Ethanol;
FAB	Fast atom bombardment;
HEPES	4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid;
HOAc	Acetic acid;

HOBT	1-Hydroxybenzotriazole hydrate;
HOOBT	3-Hydroxy-1,2,2-benzotriazin-4(3*H*)-one;
HPLC	High-performance liquid chromatography;
KOtBu	Potassium *tert*-butoxide;
LAH	Lithium aluminum hydride;
LCMS	Liquid Chromatography Mass Spectroscopy;
MCPBA	*m*-Chloroperoxybenzoic acid;
Me	Methyl;
MeOH	Methanol;
Ms	Methanesulfonyl;
MsCl	Methanesulfonyl chloride;
n-Bu	*n*-butyl;
n-Bu_3P	Tri-*n*-butylphosphine;
NaHMDS	Sodium bis(trimethylsilyl)amide;
NBS	*N*-Bromosuccinimide;
$Pd(PPh_3)_4$	Palladium tetrakis(triphenylphosphine);
$Pd_2(dba)_2$	Tris(dibenzylideneacetone)dipalladium (0)
Ph	phenyl;
PMSF	α-Toluenesulfonyl chloride;
Py or pyr	Pyridine;
PYBOP (or PyBOP)	Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate;
t-Bu	*tert*-Butyl;
TBAF	Tetrabutylammonium fluoride;
RPLC	Reverse Phase Liquid Chromatography;
RT	Room Temperature;
TBSCl	*tert*-Butyldimethylsilyl chloride;
TFA	Trifluoroacetic acid;
THF	Tetrahydrofuran;
TIPS	Triisopropylsilyl;
TMS	Tetramethylsilane; and
Tr	Trityl.

These reactions may be employed in a linear sequence to provide the compounds of the invention or they may be used to synthesize fragments which are subsequently joined by the alkylation reactions described in the Schemes.

Synopsis of Schemes

Scheme 1 illustrates the synthesis of the key amine intermediate **5** in optically enriched form. The 6-hydroxy-1-indanone **1** is protected as the silyl ether **2**, which is reduced to the (*S*)-alcohol **3** using (*R*)-methyl oxazaborolidine as shown. Treatment of **3** with DPPA and DBU in toluene provides the azide **4** with clean inversion of stereochemistry, and this is reduced using LAH to give the (*S*)-amine **5**.

In Scheme 2, the synthesis of (*R*)-2-allyl-*N*-(*tert*-butoxycarbonyl) proline **9** (described in Khalil, Subasinghe & Johnson, *Tetrahedron Lett.*, **37**, 3441-3444 (1996)) is summarized. This acid can be coupled to amine **5** to afford the amide **10** under standard PyBOP conditions.

Scheme 2A illustrates how a pipecolinate analogue (**9A**) of the proline derivative **9** may be synthesized. This intermediate **9A** could be used in analogy to **9** in the other Schemes to produce alternative spiro products. The Cbz protecting group in **9A** may be removed as necessary under standard conditions that are known to one skilled in the art.

The imidazolecarboxaldehyde intermediate 14 can be synthesized as shown in Scheme 3. Palladium-catalyzed cyanation, followed by bromination, of toluene **11** affords the benzyl bromide **12**, which can be used to alkylate the trityl-protected imidazole as shown. The synthesis of such protected imidazoles has been previously described in Williams et al., *J. Med. Chem.*, **42**, 3779-3784 (1999). The initial imidazolium ion formed in the alkylation is treated with methanol to give the product **13**. Removal of the acetyl protecting group, followed by oxidation with pyridine-sulfur trioxide complex, provided the desired compound **14**.

Amide **10** can be converted to the alcohol **15** by the oxidative cleavage–reduction sequence shown in Scheme 4. Mitsunobu cyclodehydration conditions may be used to convert this alcohol to the spiropyrrolidine **16**, and the BOC group is removed by treatment with TFA. The resulting amine **17** is reductively alkylated with aldehyde **14** under standard $NaCNBH_3$ conditions to provide compound **18**, and this may be converted to the macrocycle **19** via one pot ether cleavage and cyclization, mediated by TBAF.

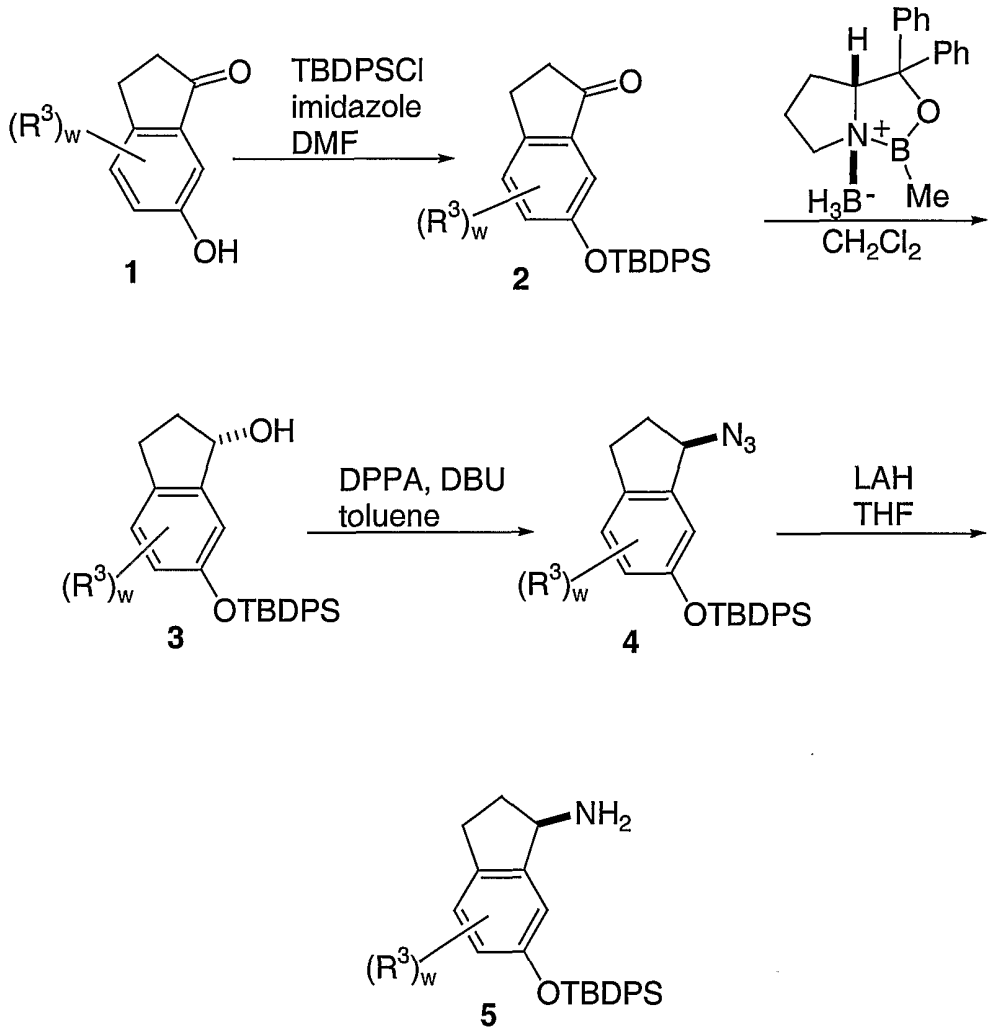
In Scheme 5, a simple deprotection-reprotection sequence is used to convert the anisole **20** to silyl ether **22**. This compound is then subjected to borane reduction, conversion of the resulting alcohol to the azide **24**, and finally reduction of this azide to the amine **25**. Coupling of this amine with the acid **9** yields the proline derivative **26**. Subjection of amide **26** to an analogous sequence to that used for amide **10** in Scheme 4 leads to the macrocyclic product **31**, as shown.

The indanone **1** (Scheme 1) may be replaced with similar compounds, such as α-tetralones or 4-chromanones, to afford analogous macrocyclic products via the same route used for **1**. A representative synthesis is shown in Scheme 6, using compound **32** as either an α-tetralone (X = CH_2) or 4-chromanone (X = O) starting material.

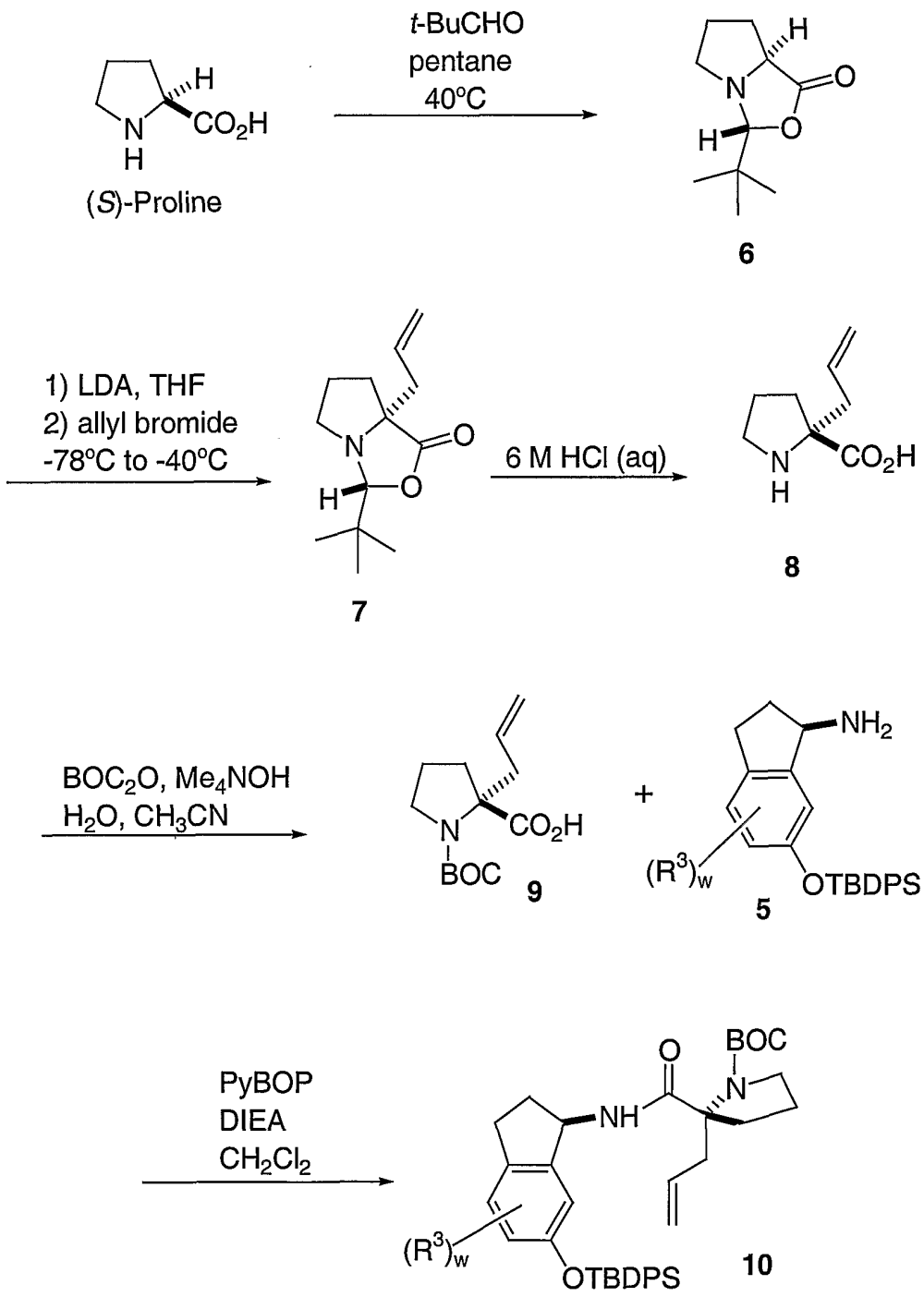
In Scheme 7, analogous chemistry is performed using the amino-naphthalene **44** as a substitute for the indanyl amine **5**. Amine **44** is realized via protection of 1-amino-7-hydroxynaphthalene **41** to give **43** followed by selective removal of the BOC protecting group with TFA, as shown. The standard protocols already describe lead to the spiro intermediate **48**.

Alkylation of trityl-protected imidazole derivatives with benzyl bromides like **A**, to yield 1,5-disubstituted imidazoles **B** (as detailed in Scheme 8) has been previously described by Anthony *et al.*, *J. Med. Chem.*, **42**, 3356-3368 (1999). Ester **B** may be saponified using lithium hydroxide to afford the desired carboxylate **50**, and this can be coupled with amines such as **49**, as shown in Scheme 9. Removal of the silyl ether followed by macrocyclization affords the desired final product **53**.

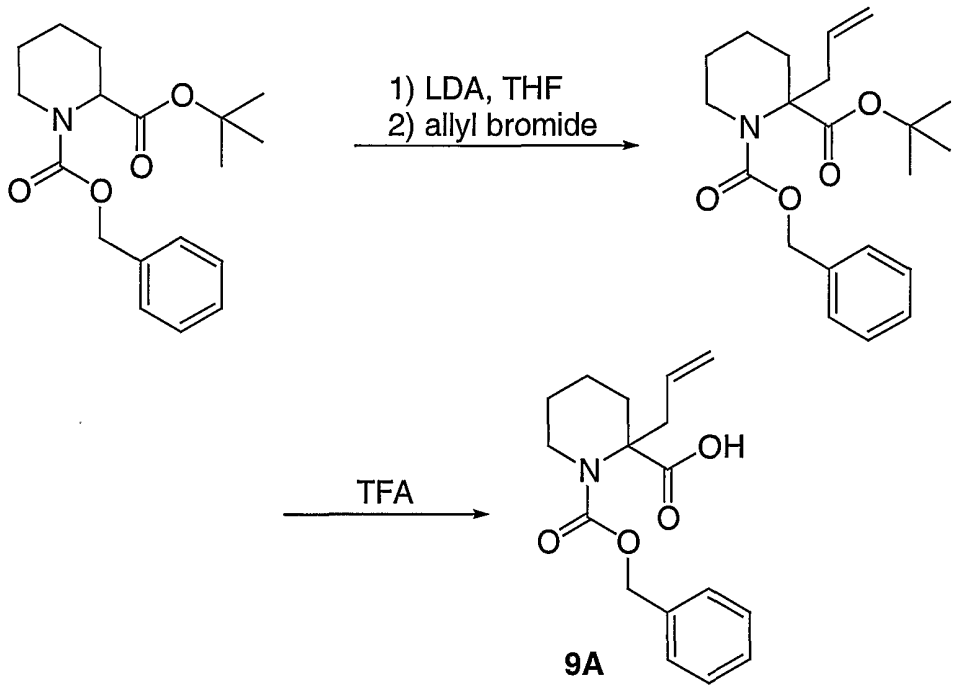
SCHEME 1


TBDPSCl
imidazole
DMF
$(R^3)_w$
OH
OTBDPS
Ph
H
Ph
O
N
B
Me
H_3B^-
CH_2Cl_2
N_3
DPPA, DBU
toluene
LAH
THF
NH_2
1
2
3
4
5

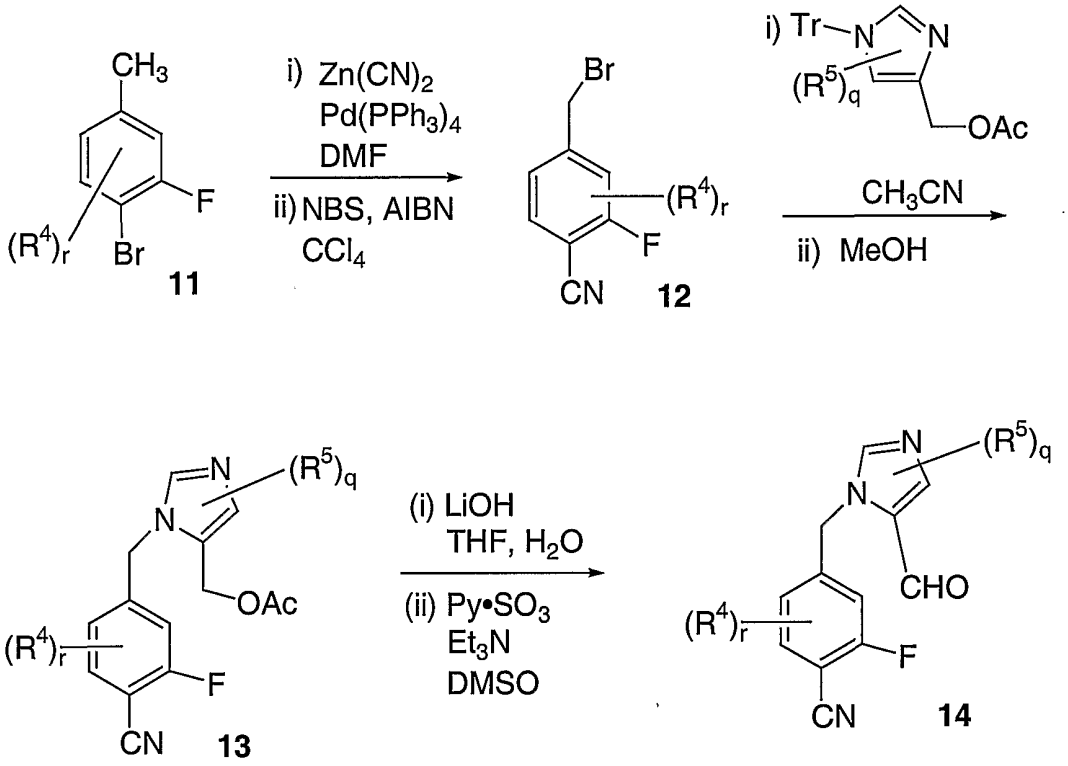
SCHEME 2


t-BuCHO
pentane
40°C
(S)-Proline
6
1) LDA, THF
2) allyl bromide
-78°C to -40°C
7
6 M HCl (aq)
8
BOC2O, Me4NOH
H2O, CH3CN
9
5
PyBOP
DIEA
CH2Cl2
10

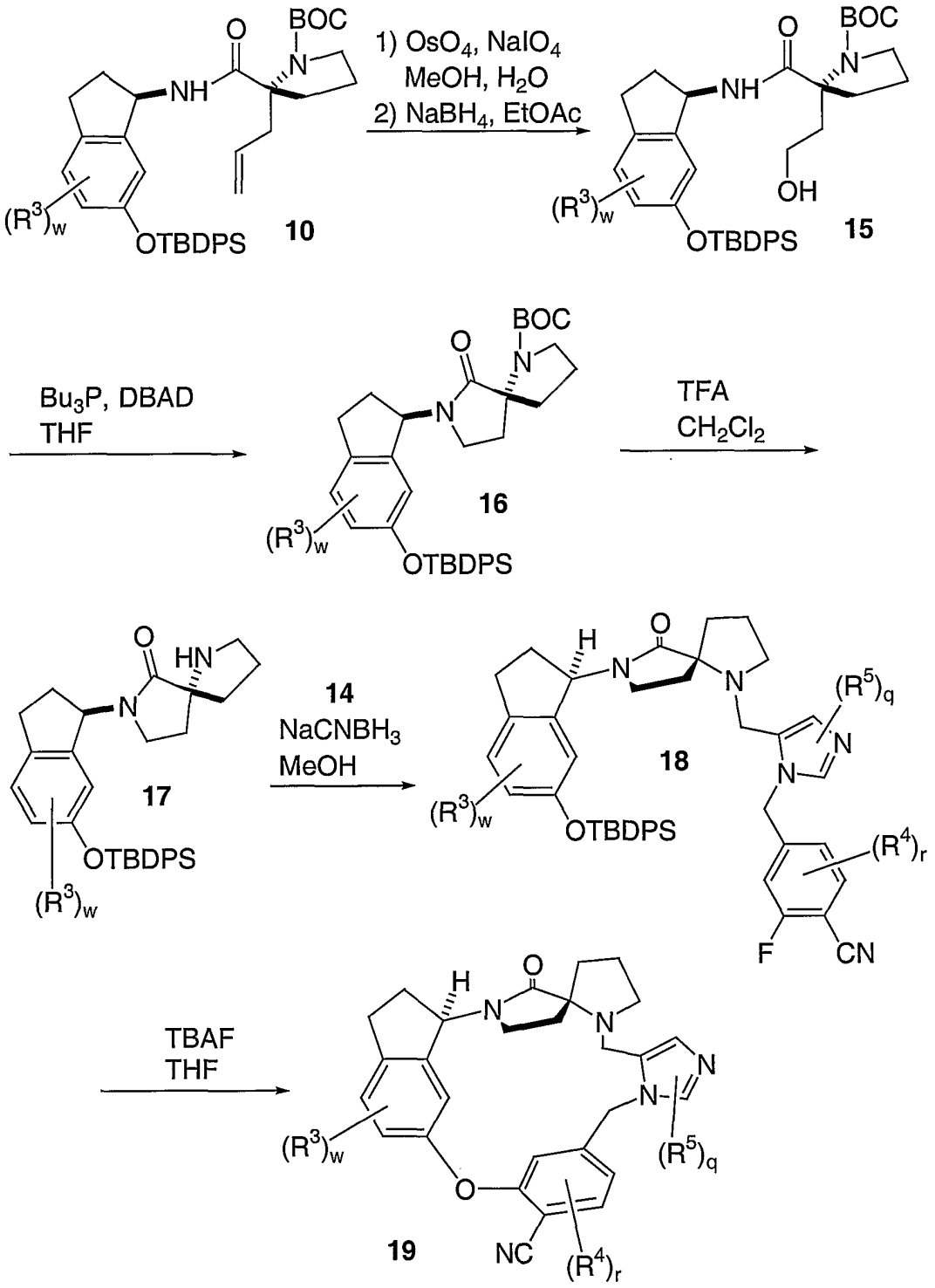
SCHEME 2A



SCHEME 3


CH3
F
(R4)r
Br
11
i) Zn(CN)2
Pd(PPh3)4
DMF
ii) NBS, AIBN
CCl4
Br
(R4)r
F
CN
12
i) Tr
N
N
(R5)q
OAc
CH3CN
ii) MeOH
N
(R5)q
N
OAc
(R4)r
F
CN
13
(i) LiOH
THF, H2O
(ii) Py•SO3
Et3N
DMSO
N
(R5)q
N
CHO
(R4)r
F
CN
14

SCHEME 4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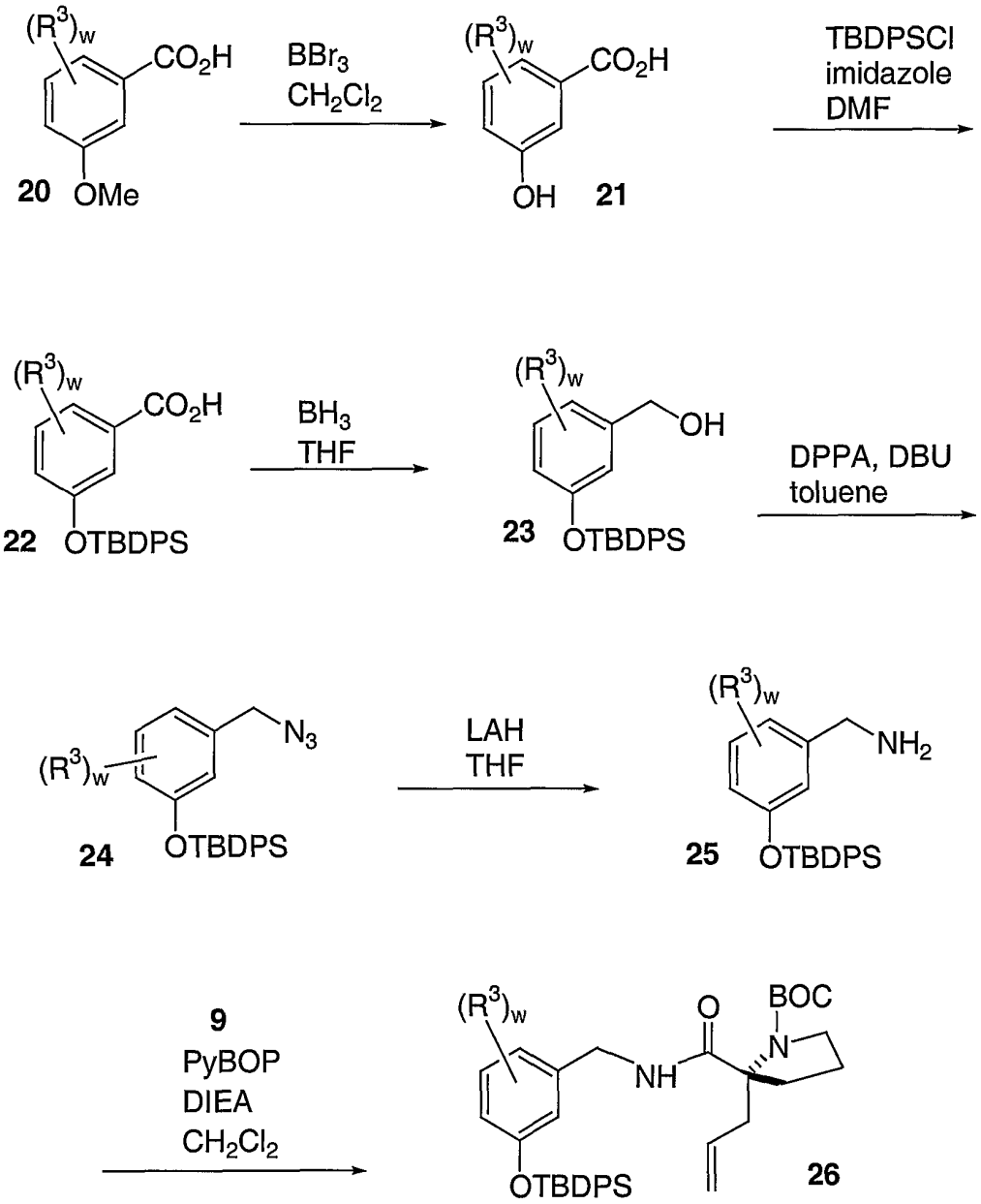
O
N
NH
$(R^3)_w$
OTBDPS
10
1) OsO_4, $NaIO_4$
MeOH, H_2O
2) $NaBH_4$, EtOAc
OH
15
Bu_3P, DBAD
THF
16
TFA
CH_2Cl_2
HN
17
14
$NaCNBH_3$
MeOH
H
18
$(R^5)_q$
$(R^4)_r$
F
CN
TBAF
THF
19
NC

SCHEME 5


(R3)w
CO2H
20 OMe
BBr3
CH2Cl2
(R3)w
CO2H
OH 21
TBDPSCl
imidazole
DMF
(R3)w
CO2H
22 OTBDPS
BH3
THF
(R3)w
OH
23 OTBDPS
DPPA, DBU
toluene
(R3)w
N3
24 OTBDPS
LAH
THF
(R3)w
NH2
25 OTBDPS
9
PyBOP
DIEA
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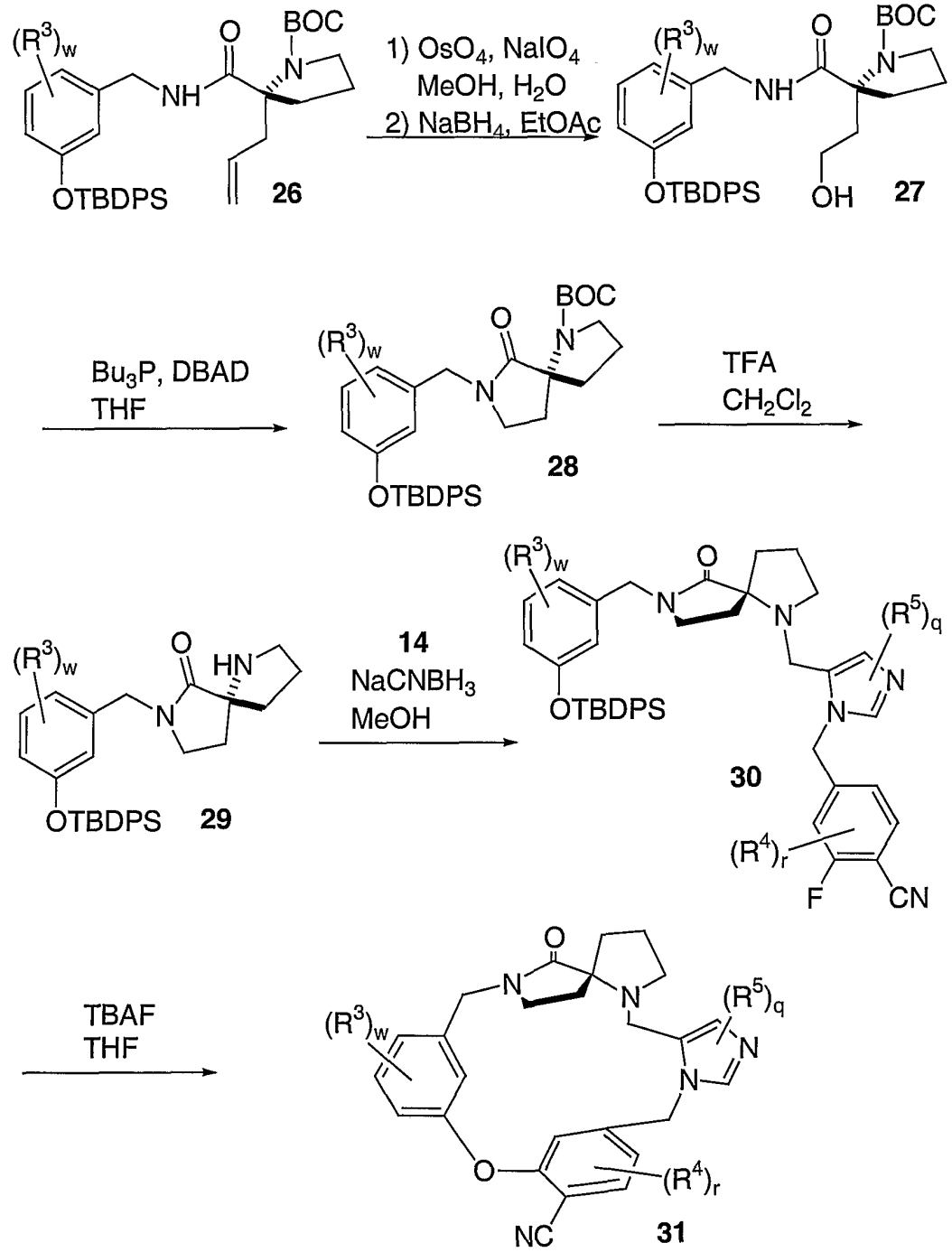
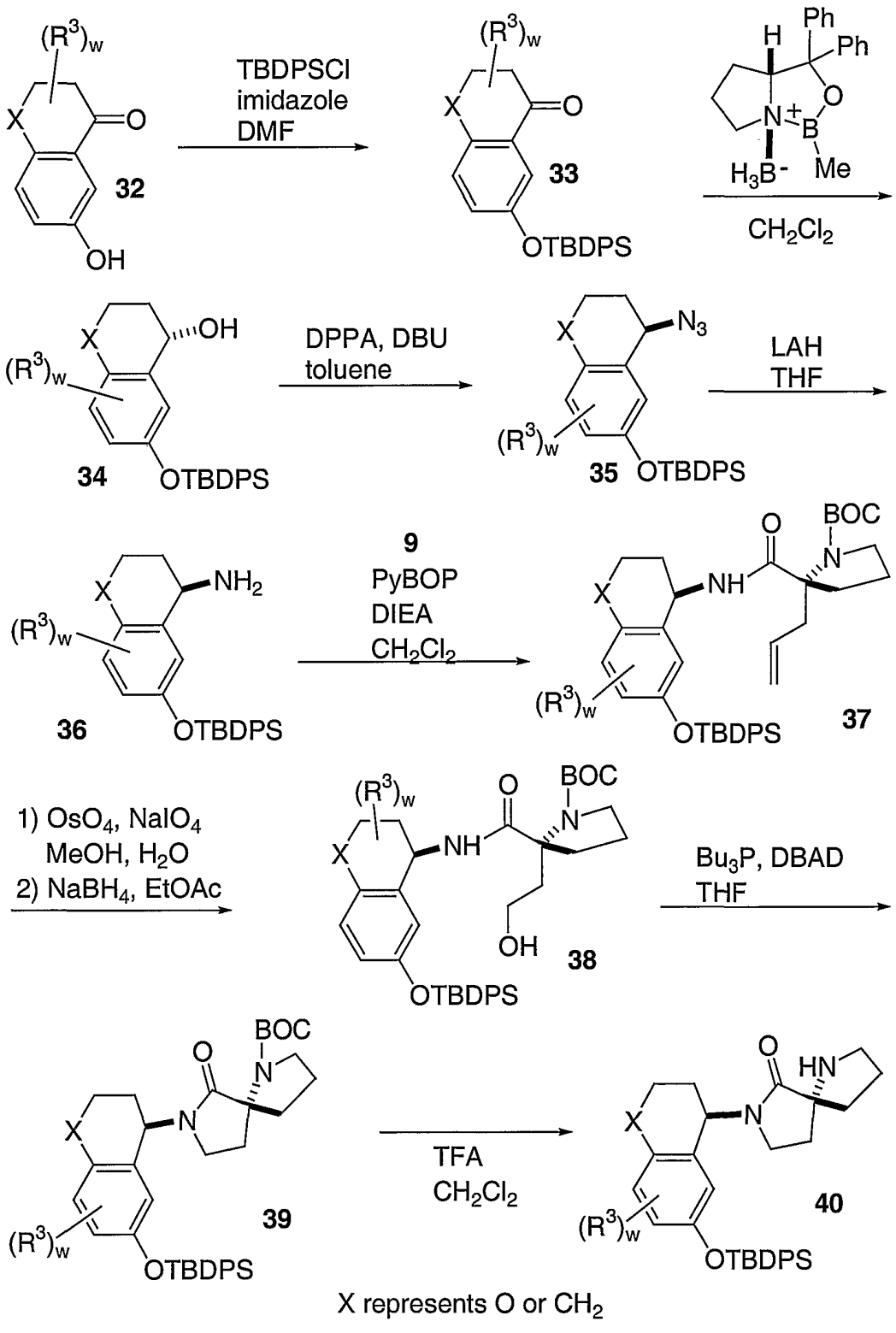
(R3)w
O
BOC
N
NH
OTBDPS
26

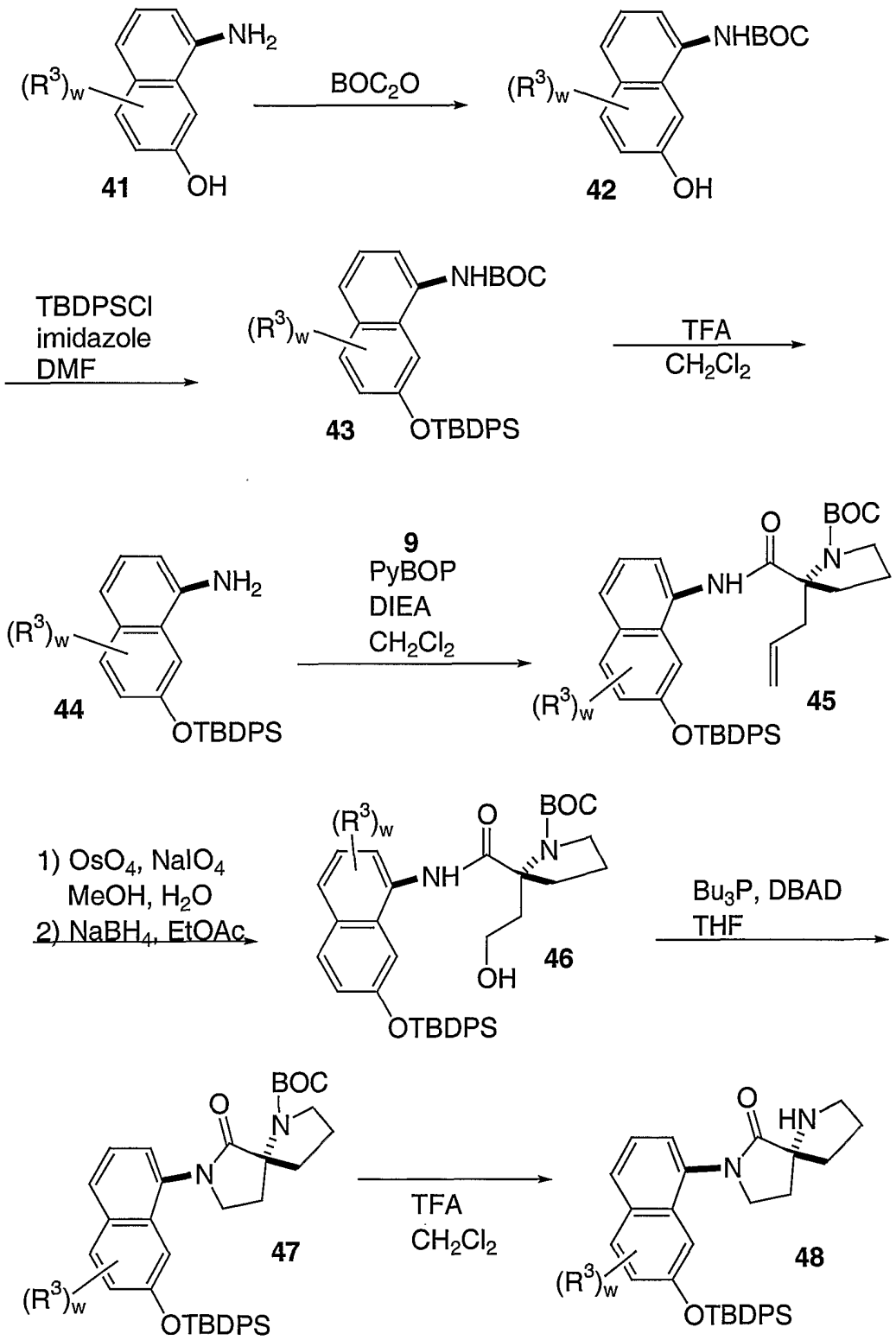
SCHEME 5 (CONTINUED)



SCHEME 6


TBDPSCl
imidazole
DMF
32
33
OTBDPS
OH
Ph
H
Ph
O
N+
B
H3B-
Me
CH2Cl2
DPPA, DBU
toluene
LAH
THF
34
35
36
37
9
PyBOP
DIEA
CH2Cl2
BOC
NH
NH2
N3
1) OsO4, NaIO4
MeOH, H2O
2) NaBH4, EtOAc
Bu3P, DBAD
THF
38
39
40
TFA
CH2Cl2
HN
X represents O or CH2

SCHEME 7


NH2
(R3)w
BOC2O
NHBOC
41
OH
42
TBDPSCl
imidazole
DMF
43
OTBDPS
TFA
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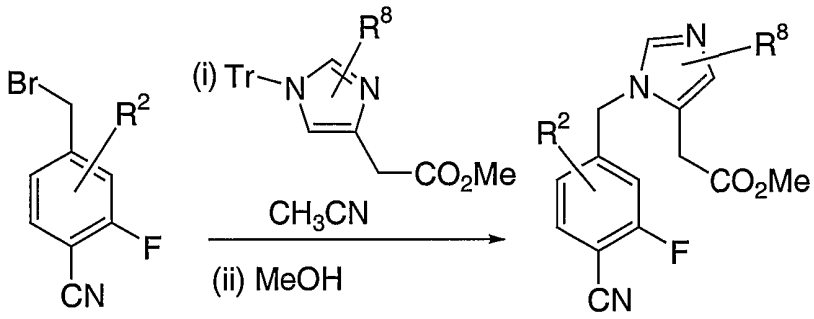
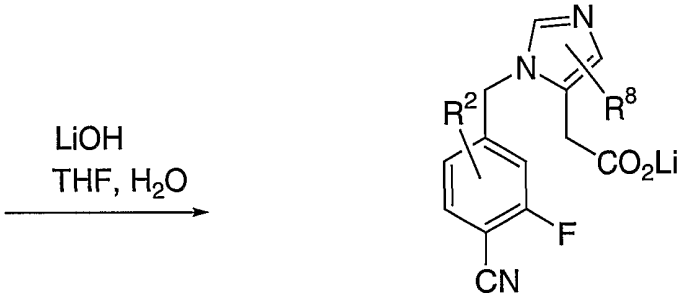
9
PyBOP
DIEA
CH2Cl2
44
45
BOC
O
N
NH
1) OsO4, NaIO4
MeOH, H2O
2) NaBH4, EtOAc
46
Bu3P, DBAD
THF
47
48
HN
TFA
CH2Cl2

SCHEME 8

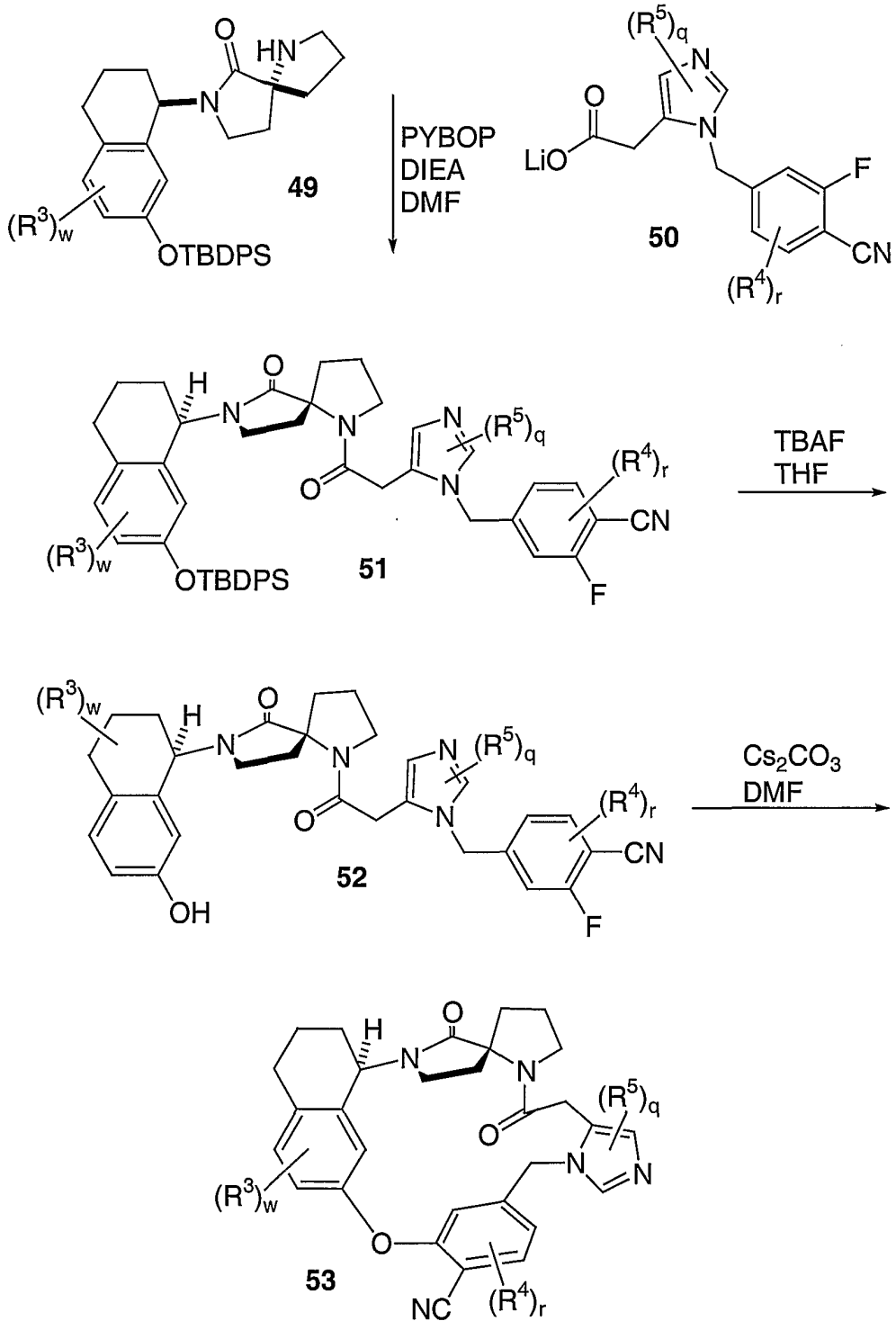


A B



50

SCHEME 9



In a preferred embodiment of the instant invention the compounds of the invention are selective inhibitors of farnesyl-protein transferase. A compound is considered a selective inhibitor of farnesyl-protein transferase, for example, when its *in vitro* farnesyl-protein transferase inhibitory activity, as assessed by the assay described in Example 4, is at least 100 times greater than the *in vitro* activity of the same compound against geranylgeranyl-protein transferase-type I in the assay described in Example 5. Preferably, a selective compound exhibits at least 1000 times greater activity against one of the enzymatic activities when comparing geranylgeranyl-protein transferase-type I inhibition and farnesyl-protein transferase inhibition.

It is also preferred that the selective inhibitor of farnesyl-protein transferase is further characterized by:

a) an IC_{50} (a measure of in vitro inhibitory activity) for inhibition of the prenylation of newly synthesized K-Ras protein more than about 100-fold higher than the EC_{50} for the inhibition of the farnesylation of hDJ protein.

When measuring such IC_{50}s and EC_{50}s the assays described in Example 9 may be utilized.

It is also preferred that the selective inhibitor of farnesyl-protein transferase is further characterized by:

b) an IC_{50} (a measurement of *in vitro* inhibitory activity) for inhibition of K4B-Ras dependent activation of MAP kinases in cells at least 100-fold greater than the EC_{50} for inhibition of the farnesylation of the protein hDJ in cells.

It is also preferred that the selective inhibitor of farnesyl-protein transferase is further characterized by:

c) an IC_{50} (a measurement of *in vitro* inhibitory activity) against H-Ras dependent activation of MAP kinases in cells at least 1000 fold lower than the inhibitory activity (IC_{50}) against H-*ras*-CVLL (SEQ.ID.NO.: 1) dependent activation of MAP kinases in cells.

When measuring Ras dependent activation of MAP kinases in cells the assays described in Example 8 may be utilized.

In another preferred embodiment of the instant invention the compounds of the invention are dual inhibitors of farnesyl-protein transferase and geranylgeranyl-protein transferase type I. Such a dual inhibitor may be termed a Class II prenyl-protein transferase inhibitor and will exhibit certain characteristics when assessed in *in vitro* assays, which are dependent on the type of assay employed.

In a SEAP assay, such as described in Example 8, it is preferred that the dual inhibitor compound has an *in vitro* inhibitory activity (IC_{50}) that is less than about 12μM against K4B-Ras dependent activation of MAP kinases in cells.

The Class II prenyl-protein transferase inhibitor may also be characterized by:

a) an IC_{50} (a measurement of *in vitro* inhibitory activity) for inhibiting K4B-Ras dependent activation of MAP kinases in cells between 0.1 and 100 times the IC_{50} for inhibiting the farnesylation of the protein hDJ in cells; and

b) an IC_{50} (a measurement of *in vitro* inhibitory activity) for inhibiting K4B-Ras dependent activation of MAP kinases in cells greater than 5-fold lower than the inhibitory activity (IC_{50}) against expression of the SEAP protein in cells transfected with the pCMV-SEAP plasmid that constitutively expresses the SEAP protein.

The Class II prenyl-protein transferase inhibitor may also be characterized by:

a) an IC_{50} (a measurement of *in vitro* inhibitory activity) against H-Ras dependent activation of MAP kinases in cells greater than 2 fold lower but less than 20,000 fold lower than the inhibitory activity (IC_{50}) against H-*ras*-CVLL (SEQ.ID.NO.: 1) dependent activation of MAP kinases in cells; and

b) an IC_{50} (a measurement of *in vitro* inhibitory activity) against H-*ras*-CVLL dependent activation of MAP kinases in cells greater than 5-fold lower than the inhibitory activity (IC_{50}) against expression of the SEAP protein in cells transfected with the pCMV-SEAP plasmid that constitutively expresses the SEAP protein.

The Class II prenyl-protein transferase inhibitor may also be characterized by:

a) an IC_{50} (a measurement of *in vitro* inhibitory activity) against H-Ras dependent activation of MAP kinases in cells greater than 10-fold lower but less than 2,500 fold lower than the inhibitory activity (IC_{50}) against H-*ras*-CVLL (SEQ.ID.NO.: 1) dependent activation of MAP kinases in cells; and

b) an IC_{50} (a measurement of *in vitro* inhibitory activity) against H-*ras*-CVLL dependent activation of MAP kinases in cells greater than 5 fold lower than the inhibitory activity (IC_{50}) against expression of the SEAP protein in cells transfected with the pCMV-SEAP plasmid that constitutively expresses the SEAP protein.

A method for measuring the activity of the inhibitors of prenyl-protein transferase, as well as the instant combination compositions, utilized in the instant methods against Ras dependent activation of MAP kinases in cells is described in Example 8.

In yet another embodiment, a compound of the instant invention may be a more potent inhibitor of geranylgeranyl-protein transferase-type I than it is an inhibitor of farnesyl-protein transferase.

The instant compounds are useful as pharmaceutical agents for mammals, especially for humans. These compounds may be administered to patients for use in the treatment of cancer. Examples of the type of cancer which may be treated with the compounds of this invention include, but are not limited to, colorectal carcinoma, exocrine pancreatic carcinoma, myeloid leukemias and neurological tumors. Such tumors may arise by mutations in the ras genes themselves, mutations in the proteins that can regulate Ras activity (i.e., neurofibromin (NF-1), neu, src, abl, lck, fyn) or by other mechanisms.

The compounds of the instant invention inhibit farnesyl-protein transferase and the farnesylation of the oncogene protein Ras. The instant compounds may also inhibit tumor angiogenesis, thereby affecting the growth of tumors (J. Rak et al. *Cancer Research*, 55:4575-4580 (1995)). Such anti-angiogenesis properties of the instant compounds may also be useful in the treatment of certain forms of vision deficit related to retinal vascularization.

The compounds of this invention are also useful for inhibiting other 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 compounds of the invention to a mammal in need of such treatment. For example, the composition is useful in the treatment of neurofibromatosis, which is a benign proliferative disorder.

The instant compounds may also be useful in the treatment of certain viral infections, in particular in the treatment of hepatitis delta and related viruses (J.S. Glenn et al. *Science*, 256:1331-1333 (1992).

The compounds of the instant invention are also useful in the prevention of restenosis after percutaneous transluminal coronary angioplasty by inhibiting neointimal formation (C. Indolfi et al. *Nature medicine*, 1:541-545(1995).

The instant compounds may also be useful in the treatment and prevention of polycystic kidney disease (D.L. Schaffner et al. *American Journal of Pathology*, 142:1051-1060 (1993) and B. Cowley, Jr. et al.*FASEB Journal*, 2:A3160 (1988)).

The instant compounds may also be useful for the treatment of fungal infections.

The instant compounds may also be useful as inhibitors of proliferation of vascular smooth muscle cells and therefore useful in the prevention and therapy of arteriosclerosis and diabetic vascular pathologies.

The compounds of the instant invention may also be useful in the prevention and treatment of endometriosis, uterine fibroids, dysfunctional uterine bleeding and endometrial hyperplasia.

In such methods of prevention and treatment as described herein, the prenyl-protein transferase inhibitors of the instant invention may also be co-administered with other well known therapeutic agents that are selected for their particular usefulness against the condition that is being treated. For example, the prenyl-protein transferase inhibitor may be useful in further combination with drugs known to supress the activity of the ovaries and slow the growth of the endometrial tissue. Such drugs include but are not limited to oral contraceptives, progestins, danazol and GnRH (gonadotropin-releasing hormone) agonists.

Administration of the prenyl-protein transferase inhibitor may also be combined with surgical treatment of endometriosis (such as surgical removal of misplaced endometrial tissue) where appropriate.

The instant compounds may also be useful as inhibitors of corneal inflammation. These compounds may improve the treatment of corneal opacity which results from cauterization-induced corneal inflammation. The instant compounds may also be useful in reducing corneal edema and neovascularization. (K. Sonoda et al., *Invest. Ophthalmol. Vis. Sci.*, 1998, vol. 39, p 2245-2251).

The compounds of this invention may be administered to mammals, preferably humans, either alone or, preferably, in combination with pharmaceutically acceptable carriers, excipients or diluents, in a pharmaceutical composition, according to standard pharmaceutical practice. The compounds can be administered orally or parenterally, including the intravenous, intramuscular, intraperitoneal, subcutaneous, rectal and topical routes of administration.

Additionally, the compounds of the instant invention may be administered to a mammal in need thereof using a gel extrusion mechanism (GEM) device, such as that described in U.S. Serial No. 60/144,643, filed on July 20, 1999, which is hereby incorporated by reference.

As used herein, the term "composition" is intended to encompass a product comprising the specified ingredients in the specific amounts, as well as any product which results, directly or indirectly, from combination of the specific ingredients in the specified amounts.

The pharmaceutical compositions containing the active ingredient may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, microcrystalline cellulose, sodium crosscarmellose, corn starch, or alginic acid; binding agents, for example starch, gelatin, polyvinyl-pyrrolidone or acacia, and lubricating agents, for example, magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to mask the unpleasant taste of the drug or delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a water soluble taste masking material such as hydroxypropyl-methylcellulose or hydroxypropyl-cellulose, or a time delay material such as ethyl cellulose, cellulose acetate buryrate may be employed.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water soluble carrier such as polyethyleneglycol or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

Aqueous suspensions contain the active material in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethyl-cellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally-occurring phosphatide, for example lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethylene-oxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose, saccharin or aspartame.

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as butylated hydroxyanisol or alpha-tocopherol.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

The pharmaceutical compositions of the invention may also be in the form of an oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring phosphatides, for example soy bean lecithin, and esters or partial esters derived

from fatty acids and hexitol anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening, flavoring agents, preservatives and antioxidants.

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, flavoring and coloring agents and antioxidant.

The pharmaceutical compositions may be in the form of a sterile injectable aqueous solutions. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution.

The sterile injectable preparation may also be a sterile injectable oil-in-water microemulsion where the active ingredient is dissolved in the oily phase. For example, the active ingredient may be first dissolved in a mixture of soybean oil and lecithin. The oil solution then introduced into a water and glycerol mixture and processed to form a microemulation.

The injectable solutions or microemulsions may be introduced into a patient's blood stream by local bolus injection. Alternatively, it may be advantageous to administer the solution or microemulsion in such a way as to maintain a constant circulating concentration of the instant compound. In order to maintain such a constant concentration, a continuous intravenous delivery device may be utilized. An example of such a device is the Deltec CADD-PLUS™ model 5400 intravenous pump.

The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleagenous suspension for intramuscular and subcutaneous administration. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butane diol. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Compounds of Formula I may also be administered in the form of a suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at

ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include cocoa butter, glycerinated gelatin, hydrogenated vegetable oils, mixtures of polyethylene glycols of various molecular weights and fatty acid esters of polyethylene glycol.

For topical use, creams, ointments, jellies, solutions or suspensions, etc., containing the compound of Formula I are employed. (For purposes of this application, topical application shall include mouthwashes and gargles.)

The compounds for the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles and delivery devices, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in the art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen. Compounds of the present invention may also be delivered as a suppository employing bases such as cocoa butter, glycerinated gelatin, hydrogenated vegetable oils, mixtures of polyethylene glycols of various molecular weights and fatty acid esters of polyethylene glycol.

When a compound according to this invention is administered into a human subject, the daily dosage will normally be determined by the prescribing physician with the dosage generally varying according to the age, weight, sex and response of the individual patient, as well as the severity of the patient's symptoms.

In one exemplary application, a suitable amount of compound is administered to a mammal undergoing treatment for cancer. Administration occurs in an amount between about 0.1 mg/kg of body weight to about 60 mg/kg of body weight per day, preferably of between 0.5 mg/kg of body weight to about 40 mg/kg of body weight per day.

The compounds of the instant invention may also be co-administered with other well known therapeutic agents that are selected for their particular usefulness against the condition that is being treated. For example, the compounds of the instant invention may also be co-administered with other well known cancer therapeutic agents that are selected for their particular usefulness against the condition that is being treated. Included in such combinations of therapeutic agents are combinations of the instant prenyl-protein transferase inhibitors and an antineoplastic agent. It is also understood that such a combination of antineoplastic agent and inhibitor of prenyl-protein transferase may be used in conjunction with other methods of treating cancer and/or tumors, including radiation therapy and surgery. It is further

understood that any of the therapeutic agents described herein may also be used in combination with a compound of the instant invention and an antineoplastic agent.

Examples of an antineoplastic agent include, in general, microtubule-stabilizing agents such as paclitaxel (also known as Taxol®), docetaxel (also known as Taxotere®), epothilone A, epothilone B, desoxyepothilone A, desoxyepothilone B or their derivatives); microtubule-disruptor agents; alkylating agents, for example, nitrogen mustards, ethyleneimine compounds, alkyl sulfonates and other compounds with an alkylating action such as nitrosoureas, cisplatin, and dacarbazine; anti-metabolites, for example, folic acid, purine or pyrimidine antagonists; epidophyllotoxin; an antineoplastic enzyme; a topoisomerase inhibitor; procarbazine; mitoxantrone; platinum coordination complexes; biological response modifiers and growth inhibitors; mitotic inhibitors, for example, vinca alkaloids and derivatives of podophyllotoxin; cytotoxic antibiotics; hormonal/anti-hormonal therapeutic agents, haematopoietic growth factors and antibodies (such as trastuzumab, also known as Herceptin™).

Example classes of antineoplastic agents include, for example, the anthracycline family of drugs, the vinca drugs, the mitomycins, the bleomycins, the cytotoxic nucleosides, the taxanes, the epothilones, discodermolide, the pteridine family of drugs, diynenes and the podophyllotoxins. Particularly useful members of those classes include, for example, doxorubicin, carminomycin, daunorubicin, aminopterin, methotrexate, methopterin, dichloro-methotrexate, mitomycin C, porfiromycin, 5-fluorouracil, 6-mercaptopurine, gemcitabine, cytosine arabinoside, podophyllotoxin or podo-phyllotoxin derivatives such as etoposide, etoposide phosphate or teniposide, melphalan, vinblastine, vincristine, leurosidine, vindesine, leurosine, paclitaxel and the like. Other useful antineoplastic agents include estramustine, cisplatin, carboplatin, cyclophosphamide, bleomycin, tamoxifen, ifosamide, melphalan, hexamethyl melamine, thiotepa, cytarabin, idatrexate, trimetrexate, dacarbazine, L-asparaginase, dactinomycin, mechlorethamine (nitrogen mustard), streptozocin, cyclophosphamide, carmustine (BCNU), lomustine (CCNU), procarbazine, mitomycin, cytarabine, etoposide, methotrexate, bleomycin, chlorambucil, camptothecin, CPT-11, topotecan, ara-C, bicalutamide, flutamide, leuprolide, pyridobenzoindole derivatives, interferons and interleukins. Particular examples of antineoplastic, or chemotherapeutic, agents are described, for example, by D. J. Stewart in "Nausea and Vomiting: Recent Research and Clinical Advances", Eds. J. Kucharczyk, et al., CRC Press Inc., Boca Raton, Florida, USA (1991), pages

177-203, especially page 188. See also, R. J. Gralla, et al., Cancer Treatment Reports, 68(1), 163-172 (1984).

The preferred class of antineoplastic agents is the taxanes and the preferred antineoplastic agent is paclitaxel.

The compounds of the instant invention may also be co-administered with antisense oligonucleotides which are specifically hybridizable with RNA or DNA deriving from human *ras* gene. Such antisense oligonucleotides are described in U.S. Patent No. 5,576,208 and PCT Publication No. WO 99/22772. The instant compounds are particularly useful when co-administered with the antisense oligonucleotide comprising the amino acid sequence of SEQ.ID.NO: 2 of U.S. Patent No. 5,576,208.

Certain compounds of the instant invention may exhibit very low plasma concentrations and significant inter-individual variation in the plasma levels of the compound. It is believed that very low plasma concentrations and high inter-subject variability achieved following administration of certain prenyl-protein transferase inhibitors to mammals may be due to extensive metabolism by cytochrome P450 enzymes prior to entry of drug into the systemic circulation. Prenyl-protein transferase inhibitors may be metabolized by cytochrome P450 enzyme systems, such as CYP3A4, CYP2D6, CYP2C9, CYP2C19 or other cytochrome P450 isoform. If a compound of the instant invention demonstrates an affinity for one or more of the cytochrome P450 enzyme systems, another compound with a higher affinity for the P450 enzyme(s) involved in metabolism should be administered concomitantly. Examples of compounds that have a comparatively very high affinity for CYP3A4, CYP2D6, CYP2C9, CYP2C19 or other P450 isoform include, but are not limited to, piperonyl butoxide, troleandomycin, erythromycin, proadifen, isoniazid, allylisopropylacetamide, ethinylestradiol, chloramphenicol, 2-ethynyl-naphthalene and the like. Such a high affinity compound, when employed in combination with a compound of formula I, may reduce the inter-individual variation and increase the plasma concentration of a compound of formula I to a level having substantial therapeutic activity by inhibiting the metabolism of the compound of formula I. Additionally, inhibiting the metabolism of a compound of the instant invention prolongs the pharmacokinetic half-life, and thus the pharmacodynamic effect, of the compound.

A compound of the present invention may be employed in

conjunction with antiemetic agents to treat nausea or emesis, including acute, delayed, late-phase, and anticipatory emesis, which may result from the use of a compound of the present invention, alone or with radiation therapy. For the prevention or treatment of emesis a compound of the present invention may be used in conjunction with other anti-emetic agents, especially neurokinin-1 receptor antagonists, 5HT3 receptor antagonists, such as ondansetron, granisetron, tropisetron, and zatisetron, GABAB receptor agonists, such as baclofen, or a corticosteroid such as Decadron (dexamethasone), Kenalog, Aristocort, Nasalide, Preferid, Benecorten or others such as disclosed in U.S.Patent Nos. 2,789,118, 2,990,401, 3,048,581, 3,126,375, 3,929,768, 3,996,359, 3,928,326 and 3,749,712. For the treatment or prevention of emesis, conjunctive therapy with a neurokinin-1 receptor antagonist, a 5HT3 receptor antagonist and a corticosteroid is preferred.

Neurokinin-1 receptor antagonists of use in conjunction with the compounds of the present invention are fully described, for example, in U.S. Patent Nos. 5,162,339, 5,232,929, 5,242,930, 5,373,003, 5,387,595, 5,459,270, 5,494,926, 5,496,833, 5,637,699, 5,719,147; European Patent Publication Nos. EP 0 360 390, 0 394 989, 0 428 434, 0 429 366, 0 430 771, 0 436 334, 0 443 132, 0 482 539, 0 498 069, 0 499 313, 0 512 901, 0 512 902, 0 514 273, 0 514 274, 0 514 275, 0 514 276, 0 515 681, 0 517 589, 0 520 555, 0 522 808, 0 528 495, 0 532 456, 0 533 280, 0 536 817, 0 545 478, 0 558 156, 0 577 394, 0 585 913,0 590 152, 0 599 538, 0 610 793, 0 634 402, 0 686 629, 0 693 489, 0 694 535, 0 699 655, 0 699 674, 0 707 006, 0 708 101, 0 709 375, 0 709 376, 0 714 891, 0 723 959, 0 733 632 and 0 776 893; PCT International Patent Publication Nos. WO 90/05525, 90/05729, 91/09844, 91/18899, 92/01688, 92/06079, 92/12151, 92/15585, 92/17449, 92/20661, 92/20676, 92/21677, 92/22569, 93/00330, 93/00331, 93/01159, 93/01165, 93/01169, 93/01170, 93/06099, 93/09116, 93/10073, 93/14084, 93/14113, 93/18023, 93/19064, 93/21155, 93/21181, 93/23380, 93/24465, 94/00440, 94/01402, 94/02461, 94/02595, 94/03429, 94/03445, 94/04494, 94/04496, 94/05625, 94/07843, 94/08997, 94/10165, 94/10167, 94/10168, 94/10170, 94/11368, 94/13639, 94/13663, 94/14767, 94/15903, 94/19320, 94/19323, 94/20500, 94/26735, 94/26740, 94/29309, 95/02595, 95/04040, 95/04042, 95/06645, 95/07886, 95/07908, 95/08549, 95/11880, 95/14017, 95/15311, 95/16679, 95/17382, 95/18124, 95/18129, 95/19344, 95/20575, 95/21819, 95/22525, 95/23798, 95/26338, 95/28418, 95/30674, 95/30687, 95/33744, 96/05181, 96/05193, 96/05203, 96/06094, 96/07649, 96/10562, 96/16939, 96/18643, 96/20197, 96/21661, 96/29304, 96/29317, 96/29326, 96/29328, 96/31214, 96/32385, 96/37489,

97/01553, 97/01554, 97/03066, 97/08144, 97/14671, 97/17362, 97/18206, 97/19084, 97/19942 and 97/21702; and in British Patent Publication Nos. 2 266 529, 2 268 931, 2 269 170, 2 269 590, 2 271 774, 2 292 144, 2 293 168, 2 293 169, and 2 302 689. The preparation of such compounds is fully described in the aforementioned patents and publications.

A particularly preferred neurokinin-1 receptor antagonist for use in conjunction with the compounds of the present invention is 2-(R)-(1-(R)-(3,5-bis (trifluoromethyl)phenyl)ethoxy)-3-(S)-(4-fluorophenyl)-4-(3-(5-oxo-1H,4H-1,2,4-triazolo)methyl)morpholine, or a pharmaceutically acceptable salt thereof, which is described in U.S. Patent No. 5,719,147.

For the treatment of cancer, it may be desirable to employ a compound of the present invention in conjunction with another pharmacologically active agent(s). A compound of the present invention and the other pharmacologically active agent(s) may be administered to a patient simultaneously, sequentially or in combination. For example, the present compound may be employed directly in combination with the other active agent(s), or it may be administered prior, concurrent or subsequent to the administration of the other active agent(s). In general, the currently available dosage forms of the known therapeutic agents for use in such combinations will be suitable.

For example, a compound of the present invention may be presented together with another therapeutic agent in a combined preparation, such as with an antiemetic agent for simultaneous, separate, or sequential use in the relief of emesis associated with employing a compound of the present invention and radiation therapy. Such combined preparations may be, for example, in the form of a twin pack. A preferred combination comprises a compound of the present invention with antiemetic agents, as described above.

Radiation therapy, including x-rays or gamma rays which are delivered from either an externally applied beam or by implantation of tiny radioactive sources, may also be used in combination with the instant inhibitor of prenyl-protein transferase alone to treat cancer.

Additionally, compounds of the instant invention may also be useful as radiation sensitizers, as described in WO 97/38697, published on October 23, 1997, and herein incorporated by reference.

The instant compounds may also be useful in combination with other inhibitors of parts of the signaling pathway that links cell surface growth

factor receptors to nuclear signals initiating cellular proliferation. Thus, the instant compounds may be utilized in combination with farnesyl pyrophosphate competitive inhibitors of the activity of farnesyl-protein transferase or in combination with a compound which has Raf antagonist activity. The instant compounds may also be co-administered with compounds that are selective inhibitors of geranylgeranyl protein transferase.

In particular, if the compound of the instant invention is a selective inhibitor of farnesyl-protein transferase, co-administration with a compound(s) that is a selective inhibitor of geranylgeranyl protein transferase may provide an improved therapeutic effect.

In particular, the compounds disclosed in the following patents and publications may be useful as farnesyl pyrophosphate-competitive inhibitor component of the instant composition: U.S. Serial Nos. 08/254,228 and 08/435,047. Those patents and publications are incorporated herein by reference.

In practicing methods of this invention, which comprise administering, simultaneously or sequentially or in any order, two or more of a protein substrate-competitive inhibitor and a farnesyl pyrophosphate-competitive inhibitor, such administration can be oral or parenteral, including intravenous, intramuscular, intraperitoneal, subcutaneous, rectal and topical routes of administration. It is preferred that such administration be oral. It is more preferred that such administration be oral and simultaneous. When the protein substrate-competitive inhibitor and farnesyl pyrophosphate-competitive inhibitor are administered sequentially, the administration of each can be by the same method or by different methods.

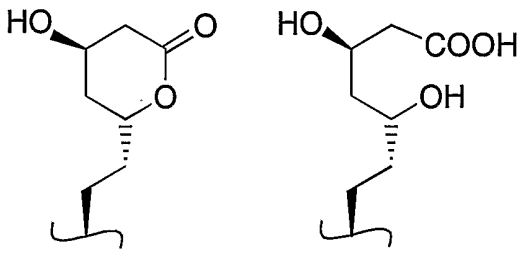
The instant compounds may also be useful in combination with an integrin antagonist for the treatment of cancer, as described in U.S. Serial No. 09/055,487, filed April 6, 1998, and WO 98/44797, published on October 15, 1998, which are incorporated herein by reference.

As used herein the term "integrin antagonist" refers to a compound which selectively antagonize, inhibit or counteract binding of a physiological ligand to an integrin(s) that is involved in the regulation of angiogenisis, or in the growth and invasiveness of tumor cells. In particular, the term refers to compounds which selectively antagonize, inhibit or counteract binding of a physiological ligand to the $\alpha v\beta 3$ integrin, which selectively antagonize, inhibit or counteract binding of a physiological ligand to the $\alpha v\beta 5$ integrin, which antagonize, inhibit or counteract binding of a physiological ligand to both the $\alpha v\beta 3$ integrin and the $\alpha v\beta 5$ integrin,

or which antagonize, inhibit or counteract the activity of the particular integrin(s) expressed on capillary endothelial cells. The term also refers to antagonists of the $\alpha 1\beta 1$, $\alpha 2\beta 1$, $\alpha 5\beta 1$, $\alpha 6\beta 1$ and $\alpha 6\beta 4$ integrins. The term also refers to antagonists of any combination of $\alpha v\beta 3$ integrin, $\alpha v\beta 5$ integrin, $\alpha 1\beta 1$, $\alpha 2\beta 1$, $\alpha 5\beta 1$, $\alpha 6\beta 1$ and $\alpha 6\beta 4$ integrins. The instant compounds may also be useful with other agents that inhibit angiogenisis and thereby inhibit the growth and invasiveness of tumor cells, including, but not limited to angiostatin and endostatin.

The instant compounds may also be useful in combination with an inhibitor of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) for the treatment of cancer. Compounds which have inhibitory activity for HMG-CoA reductase can be readily identified by using assays well-known in the art. For example, see the assays described or cited in U.S. Patent No. 4,231,938 at col. 6, and WO 84/02131 at pages 30-33. The terms "HMG-CoA reductase inhibitor" and "inhibitor of HMG-CoA reductase" have the same meaning when used herein.

Examples of HMG-CoA reductase inhibitors that may be used include but are not limited to lovastatin (MEVACOR®; see US Patent No. 4,231,938, 4,294,926 and 4,319,039), simvastatin (ZOCOR®; see US Patent No. 4,444,784, 4,820,850 and 4,916,239), pravastatin (PRAVACHOL®; see US Patent Nos. 4,346,227, 4,537,859, 4,410,629; 5,030,447 and 5,180,589), fluvastatin (LESCOL®; see US Patent Nos. 5,354,772, 4,911,165, 4,929,437, 5,189,164, 5,118,853, 5,290,946 and 5,356,896), atorvastatin (LIPITOR®; see US Patent Nos. 5,273,995, 4,681,893, 5,489,691 and 5,342,952) and cerivastatin (also known as rivastatin and BAYCHOL®; see US Patent No. 5,177,080). The structural formulas of these and additional HMG-CoA reductase inhibitors that may be used in the instant methods are described at page 87 of M. Yalpani, "Cholesterol Lowering Drugs", *Chemistry & Industry*, pp. 85-89 (5 February 1996) and US Patent Nos. 4,782,084 and 4,885,314. The term HMG-CoA reductase inhibitor as used herein includes all pharmaceutically acceptable lactone and open-acid forms (i.e., where the lactone ring is opened to form the free acid) as well as salt and ester forms of compounds which have HMG-CoA reductase inhibitory activity, and therefor the use of such salts, esters, open-acid and lactone forms is included within the scope of this invention. An illustration of the lactone portion and its corresponding open-acid form is shown below as structures I and II.



Lactone
I

Open-Acid
II

In HMG-CoA reductase inhibitors where an open-acid form can exist, salt and ester forms may preferably be formed from the open-acid, and all such forms are included within the meaning of the term "HMG-CoA reductase inhibitor" as used herein. Preferably, the HMG-CoA reductase inhibitor is selected from lovastatin and simvastatin, and most preferably simvastatin. Herein, the term "pharmaceutically acceptable salts" with respect to the HMG-CoA reductase inhibitor shall mean non-toxic salts of the compounds employed in this invention which are generally prepared by reacting the free acid with a suitable organic or inorganic base, particularly those formed from cations such as sodium, potassium, aluminum, calcium, lithium, magnesium, zinc and tetramethylammonium, as well as those salts formed from amines such as ammonia, ethylenediamine, N-methylglucamine, lysine, arginine, ornithine, choline, N,N'-dibenzylethylenediamine, chloroprocaine, diethanolamine, procaine, N-benzylphenethylamine, 1-p-chlorobenzyl-2-pyrrolidine-1'-yl-methyl-benzimidazole, diethylamine, piperazine, and tris(hydroxymethyl) aminomethane. Further examples of salt forms of HMG-CoA reductase inhibitors may include, but are not limited to, acetate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, bromide, calcium edetate, camsylate, carbonate, chloride, clavulanate, citrate, dihydrochloride, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycollylarsanilate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroxynapthoate, iodide, isothionate, lactate, lactobionate, laurate, malate, maleate, mandelate, mesylate, methylsulfate, mucate, napsylate, nitrate, oleate, oxalate, pamaote, palmitate, panthothenate, phosphate/ diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, tannate, tartrate, teoclate, tosylate, triethiodide, and valerate.

Ester derivatives of the described HMG-CoA reductase inhibitor compounds may act as prodrugs which, when absorbed into the bloodstream of a

warm-blooded animal, may cleave in such a manner as to release the drug form and permit the drug to afford improved therapeutic efficacy.

Similarly, the instant compounds may be useful in combination with agents that are effective in the treatment and prevention of NF-1, restenosis, polycystic kidney disease, infections of hepatitis delta and related viruses and fungal infections.

If formulated as a fixed dose, such combination products employ the combinations of this invention within the dosage range described above and the other pharmaceutically active agent(s) within its approved dosage range. Combinations of the instant invention may alternatively be used sequentially with known pharmaceutically acceptable agent(s) when a multiple combination formulation is inappropriate.

The instant compounds may also be useful in combination with prodrugs of antineoplastic agents. In particular, the instant compounds may be co-administered either concurrently or sequentially with a conjugate (termed a "PSA conjugate") which comprises an oligopeptide, that is selectively cleaved by enzymatically active prostate specific antigen (PSA), and an antineoplastic agent. Such co-administration will be particularly useful in the treatment of prostate cancer or other cancers which are characterized by the presence of enzymatically active PSA in the immediate surrounding cancer cells, which is secreted by the cancer cells.

Compounds which are PSA conjugates and are therefore useful in such a co-administration, and methods of synthesis thereof, can be found in the following patents, pending patent applications and publications which are herein incorporated by references:

U.S. Patent No. 5,599,686, granted on February 4, 1997;

WO 96/00503 (January 11, 1996); U.S. Serial No. 08/404,833, filed on March 15, 1995; U.S. Serial No. 08/468,161, filed on June 6, 1995;

U.S. Patent No. 5,866,679, granted on February 2, 1999;

U.S. Patent No. 5,998,362, granted on December 7, 1999;

U.S. Patent No. 5,948,750, granted on September 7, 1999;

WO 99/02175 (January 21, 1999); U.S. Serial No. 09/112,656, filed on July 9, 1998; and

WO 99/28345 (June 10, 1999); U.S. Serial No. 09/193,365, filed on November 17, 1998.

Compounds which are described as prodrugs wherein the active therapeutic agent is released by the action of enzymatically active PSA and therefore may be useful in such a co-administration, and methods of synthesis thereof, can be found in the following patents, pending patent applications and publications, which are herein incorporated by reference: WO 98/52966 (November 26, 1998).

All patents, publications and pending patent applications identified are herein incorporated by reference.

The compounds of the instant invention are also useful as a component in an assay to rapidly determine the presence and quantity of farnesyl-protein transferase (FPTase) in a composition. Thus the composition to be tested may be divided and the two portions contacted with mixtures which comprise a known substrate of FPTase (for example a tetrapeptide having a cysteine at the amine terminus) and farnesyl pyrophosphate and, in one of the mixtures, a compound of the instant invention. After the assay mixtures are incubated for a sufficient period of time, well known in the art, to allow the FPTase to farnesylate the substrate, the chemical content of the assay mixtures may be determined by well known immuno-logical, radiochemical or chromatographic techniques. Because the compounds of the instant invention are selective inhibitors of FPTase, absence or quantitative reduction of the amount of substrate in the assay mixture without the compound of the instant invention relative to the presence of the unchanged substrate in the assay containing the instant compound is indicative of the presence of FPTase in the composition to be tested.

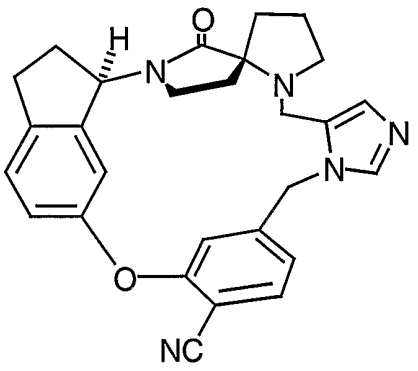
It would be readily apparent to one of ordinary skill in the art that such an assay as described above would be useful in identifying tissue samples which contain farnesyl-protein transferase and quantitating the enzyme. Thus, potent inhibitor compounds of the instant invention may be used in an active site titration assay to determine the quantity of enzyme in the sample. A series of samples composed of aliquots of a tissue extract containing an unknown amount of farnesyl-

protein transferase, an excess amount of a known substrate of FPTase (for example a tetrapeptide having a cysteine at the amine terminus) and farnesyl pyrophosphate are incubated for an appropriate period of time in the presence of varying concentrations of a compound of the instant invention. The concentration of a sufficiently potent inhibitor (i.e., one that has a Ki substantially smaller than the concentration of enzyme in the assay vessel) required to inhibit the enzymatic activity of the sample by 50% is approximately equal to half of the concentration of the enzyme in that particular sample.

EXAMPLES

Examples provided are intended to assist in a further understanding of the invention. Particular materials employed, species and conditions are intended to be further illustrative of the invention and are not intended to limit the reasonable scope thereof.

EXAMPLE 1



Preparation of (17*R*, 20*R*)-19,20,21,22-tetrahydro-19-oxo-17*H*-15,17:18,20-diethano-6,10:12,16-dimetheno-20,21-propano-16*H*-imidazo[3,4-*h*][1,8,11,14] oxatriazacycloeicosine-9-carbonitrile

Step A: 4-(Hydroxymethyl)-1-(triphenylmethyl)imidazole

To a solution of 4-(hydroxymethyl)imidazole hydrochloride (35.0 g, 260 mmol) in dry DMF (250 mL) at room temperature was added triethylamine (90.6 mL, 650 mmol). A white solid precipitated from the solution. Chlorotriphenylmethane (76.1 g, 273 mmol) in DMF (500 mL) was added dropwise. The reaction mixture was stirred for 20 hours, poured over ice, filtered, and washed with ice water. The resulting product was slurried with cold dioxane, filtered, and dried *in vacuo* to provide the titled product.

Step B: 4-(Acetoxymethyl)-1-(triphenylmethyl)imidazole

4-(Hydroxymethyl)-1-(triphenylmethyl)imidazole, as described above in Step A, (88.5 g, 260 mmol) was suspended in pyridine (500 mL). Acetic anhydride (74 mL, 780 mmol) was added dropwise, and the reaction was stirred for 48 hours during which it became homogeneous. The solution was poured into EtOAc, and washed sequentially with water, 5% aqueous HCl solution, saturated aqueous $NaHCO_3$ solution, and brine. The organic extracts were dried (Na_2SO_4), and concentrated *in vacuo* to provide the titled ester.

Step C: 4-Cyano-3-fluorotoluene

To a deoxygenated solution of 4-bromo-3-fluorotoluene (25.0 g, 132 mmol) in DMF (500 mL) was added $Zn(CN)_2$ (10.1 g, 86 mmol) and $Pd(PPh_3)_4$ (15 g, 13 mmol). The reaction was stirred at 100°C for 18 hours, then cooled to room temperature. The solution was poured into toluene (1 L), washed with 30% aqueous NH_4OH (2 × 1 L), then brine (800 mL), then dried (Na_2SO_4), filtered, and concentrated *in vacuo* to provide the crude product. Purification by silica gel chromatography, eluting with a gradient of hexane - 0% to 7% EtOAc, yielded the titled product.

Step D: 4-Cyano-3-fluorobenzyl bromide

To a solution of 4-cyano-3-fluorotoluene, as described above in Step C, (5.0 g, 37.0 mmol) in carbon tetrachloride (300 mL) was added *N*-bromosuccinimide (7.57 g, 42.6 mmol) and 2,2'-azobisisobutyronitrile (610 mg, 3.7 mmol). The reaction mixture was heated to reflux under argon for 24 hours, then cooled to room temperature, filtered, and concentrated under reduced pressure. The

residue was purified by silica gel chromatography, eluting with a gradient of hexane - 4% to 7% EtOAc, to yield the titled product.

Step E: 5-(Acetoxymethyl)-1-(4-cyano-3-fluorobenzyl)imidazole hydrobromide

A mixture of 4-(acetoxymethyl)-1-(triphenylmethyl)imidazole, as described above in Step B, (19.7 g, 51.4 mmol) and 4-cyano-3-fluorobenzyl bromide, as described above in Step D, (11.0 g, 51.4 mmol) in dry CH_3CN (140 mL) was stirred at 50°C for 3 hours, during which a white precipitate formed. The reaction was cooled to room temperature and filtered to provide the solid imidazolium bromide salt. The filtrate was concentrated *in vacuo* to a volume of 70 mL, reheated at 50°C for 2 hours, cooled to room temperature, and filtered again. The solid material was combined and dissolved in MeOH (500 mL), and the solution was heated to reflux for 2 hours. The solution was concentrated *in vacuo* to a volume of 20 mL, then cold hexane - EtOAc (1:1, 500 mL) was added and the precipitate was collected and dried *in vacuo*.

Step F: 1-(4-Cyano-3-fluorobenzyl)-5-(hydroxymethyl)imidazole

To a solution of 5-(acetoxymethyl)-1-(4-cyano-3-fluorobenzyl) imidazole, as described above in Step E, (19.8 g, 72.5 mmol) in 5:1 THF/water (430 mL) at ambient temperature was added lithium hydroxide monohydrate (3.33 g, 79.4 mmol). After 4 hours, the solution was adjusted to pH 7 with 1.0 N hydrochloric acid and concentrated *in vacuo*. The residue was concentrated from toluene *in vacuo* (3 × 100 mL) to give the titled product.

Step G: 1-(4-Cyano-3-fluorobenzyl)-5-imidazolecarboxaldehyde

To a solution of 1-(4-cyano-3-fluorobenzyl)-5-(hydroxymethyl) imidazole, as described above in Step F, (2.31 g, 10.0 mmol) in 20 mL of DMSO at 0°C was added triethylamine (5.6 mL, 40 mmol), then SO_3-pyridine complex (3.89 g, 25 mmol). After 30 minutes, the reaction was poured into EtOAc, washed with water and brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to provide the titled aldehyde.

Step H: 6-*tert*-Butyldiphenylsilyloxy-1-indanone

6-Hydroxy-1-indanone was prepared according to the procedure described by Nayak & Chakraborti, *Tetrahedron Lett.*, **38**, 8749-8752 (1997). A mixture of 6-hydroxy-1-indanone (5.00 g, 33.8 mmol), *tert*-butyldiphenylsilyl chloride (23.1 g, 84.2 mmol), and imidazole (6.90 g, 101.4 mmol) in degassed DMF (100 mL) was heated at 60°C for 18 h. The solvent was removed *in vacuo* and the residue purified by silica gel chromatography, eluting with a gradient of hexane - 10% to 15% EtOAc, to yield the titled product.

Step I: (*S*)-6-*tert*-Butyldiphenylsilyloxy-1-indanol

To a stirred solution of borane-methyl sulfide (0.155 mL, 1.55 mmol) in dry CH_2Cl_2 (4 mL), under argon, was added (*R*)-methyl oxazaborolidine (1 M in toluene, 1.55 mL, 1.55 mmol). To the resulting solution was added a solution of 6-*tert*-butyldiphenylsilyloxy-1-indanone, as described above in Step H, (500 mg, 1.29 mmol) in CH_2Cl_2 (5.5 mL), dropwise. The reaction mixture was stirred at ambient temperature for 2 h, then added carefully to MeOH (20 mL). The solvent was removed by distillation at 1 atm. This MeOH addition-distillation procedure was repeated twice more, then the residue was purified by silica gel chromatography, eluting with CH_2Cl_2, to yield the titled product.

Step J: (*R*)-6-*tert*-Butyldiphenylsilyloxy-1-indanyl azide

To a solution of (*S*)-6-*tert*-butyldiphenylsilyloxy-1-indanol, as described above in Step I, (283 mg, 0.728 mmol) and diphenylphosphoryl azide (240 mg, 0.874 mmol) in toluene (1.3 mL) at 0°C was added 1,8-diazabicyclo[5.4.0]undec-7-ene (0.12 mL, 0.80 mmol), dropwise. The mixture was allowed to slowly warm to ambient temperature and was stirred for 18 h then diluted with CH_2Cl_2 (15 mL). The resulting mixture was washed with 0.5 N HCl (5 mL), then H_2O (5 mL), then brine (5 mL) then dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel chromatography, eluting with a gradient of hexane – 0% to 4% EtOAc, to yield the titled product.

Step K: (*R*)-6-*tert*-Butyldiphenylsilyloxy-1-indanylamine

To a stirred solution of (*R*)-6-*tert*-butyldiphenylsilyloxy-1-indanyl azide, as described above in Step J, (273 mg, 0.66 mmol) in THF (8 mL) at 0°C was added $LiAlH_4$ (1.0 M in THF, 0.79 mL, 0.79 mmol), dropwise. The reaction mixture

was allowed to slowly warm to ambient temperature and was stirred for 18 h. EtOAc (0.035 mL) was added, followed by H_2O (0.035 mL), then 15% aqueous NaOH (0.035 mL), then H_2O (0.10 mL). The quenched mixture was stirred at ambient temperature for 30 min, then filtered, and concentrated *in vacuo*. The residue was purified by silica gel chromatography, eluting with a gradient of CH_2Cl_2 - 0% to 6% MeOH – 0% to 0.6% NH_4OH, to yield the titled product.

Step L: (*R*,*R*)-2-Allyl-1-(*tert*-butoxycarbonyl)-*N*-[6-(*tert*-butyldiphenylsilyloxy)indan-1-yl]pyrrolidine-2-carboxamide

(*R*)-2-Allyl-*N*-(*tert*-butoxycarbonyl)proline was prepared in analogy with the procedure described by Khalil, Subasinghe & Johnson, *Tetrahedron Lett.*, **37**, 3441-3444 (1996). To (*R*)-2-allyl-*N*-(*tert*-butoxycarbonyl)proline (587 mg, 2.30 mmol) in dry CH_2Cl_2 (5 mL), at 0°C, under argon were added PYBOP (1.32 g, 2.53 mmol), (*R*)-6-*tert*-butyldiphenylsilyloxy-1-indanylamine, as described above in Step K, (980 mg, 2.53 mmol), and *N*,*N*-diisopropylethylamine (0.44 mL, 2.53 mmol). The reaction mixture was stirred for 1 h, then poured into CH_2Cl_2 (50 mL) and 10% aqueous citric acid (20 mL), and the organic layer was extracted, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica, eluting with a gradient of CH_2Cl_2 - 0% to 5% EtOAc to yield the desired product.

Step M: (*R*,*R*)-1-(*tert*-Butoxycarbonyl)-*N*-[6-(*tert*-butyldiphenylsilyloxy)indan-1-yl]-2-(2-hydroxyethyl)pyrrolidine-2-carboxamide

To a stirred solution of (*R*,*R*)-2-allyl-1-(*tert*-butoxycarbonyl)-*N*-[6-(*tert*-butyldiphenylsilyloxy)indan-1-yl]pyrrolidine-2-carboxamide, as described above in Step L, (410 mg, 0.656 mmol), in MeOH (20 mL) and H_2O (5 mL) at ambient temperature was added osmium tetroxide (2.5% solution in *tert*-BuOH, 0.6 mL, 0.048 mol) followed by sodium periodate (421 mg, 1.97 mmol). The resulting mixture was stirred for 90 min, then poured into H_2O and EtOAc and extracted with EtOAc (2 × 75 mL). The combined organic extracts were washed with H_2O, then brine, then dried over Na_2SO_4, filtered, and concentrated *in vacuo*. This crude product was dissolved in EtOAc (20 mL), the solution was cooled to –78°C and $NaBH_4$ (0.1 M in *i*-PrOH, 2.5 mL, 0.26 mmol) was added dropwise. The mixture was allowed to warm slowly for 2 h, then was poured into brine (10 mL) and the organic layer was extracted. The aqueous layer was extracted further with EtOAc

(10 mL), then the combined organic extracts were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica, eluting with a gradient of CH_2Cl_2 - 0% to 50% EtOAc to yield the desired product.

Step N: (*R*,*R*)-*tert*-Butyl 7-[6-(*tert*-butyldiphenylsilyloxy)indan-1-yl]-6-oxo-1,7-diazaspiro[4.4]nonane-1-carboxylate

To a solution of di-*tert*-butyl azodicarboxylate (40 mg, 0.176 mmol) in THF (0.5 mL) was added tri-*n*-butylphosphine (36 mg, 0.176 mmol) and the mixture was stood for 5 min, then added dropwise to a solution of (*R*,*R*)-1-(*tert*-butoxycarbonyl)-*N*-[6-(*tert*-butyldiphenylsilyloxy)indan-1-yl]-2-(2-hydroxyethyl) pyrrolidine-2-carboxamide, as described above in Step M, (85 mg, 0.135 mmol), in THF (1 mL) at 0°C. The reaction mixture was allowed to warm slowly to ambient temperature and was stirred for 18 h, then poured into saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (2 × 10 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica, eluting with a gradient of CH_2Cl_2 - 0% to 35% EtOAc to yield the desired product.

Step O: (*R*,*R*)-7-[6-(*tert*-Butyldiphenylsilyloxy)indan-1-yl]-6-oxo-1,7-diazaspiro[4.4]nonane trifluoroacetate

A solution of *tert*-butyl 7-[6-(*tert*-butyldiphenylsilyloxy)indan-1-yl]-6-oxo-1,7-diazaspiro[4.4]nonane-1-carboxylate, as described above in Step N, (62 mg, 0.101 mmol) in CH_2Cl_2 (1 mL) and trifluoroacetic acid (0.5 mL) was stood at ambient temperature for 20 min, then concentrated *in vacuo* to yield the titled salt.

Step P: (*R*,*R*)-4-[5-({7-[6-(*tert*-Butyldiphenylsilyloxy)indan-1-yl]-6-oxo-1,7-diazaspiro[4.4]non-1-yl}methyl)-1*H*-imidazol-1-ylmethyl]-2-fluorobenzonitrile

(*R*,*R*)-7-[6-(*tert*-Butyldiphenylsilyloxy)indan-1-yl]-6-oxo-1,7-diazaspiro[4.4]nonane trifluoroacetate, as described above in Step O, (62 mg, 0.099 mmol) and 1-(4-cyano-3-fluorobenzyl)-5-imidazolecarboxaldehyde, as described above in Step G, (23 mg, 0.099 mmol), were stirred in MeOH (1 mL) and *N*,*N*-diisopropylethylamine was added dropwise to adjust the mixture to ca. pH 5, as

judged by wetted pH paper. The mixture was stirred for 1 h at ambient temperature, then $NaCNBH_3$ (9 mg, 0.143 mmol) was added, the pH was adjusted to pH 5 with AcOH, and stirring was continued for 18 h. The reaction was quenched with saturated aqueous $NaHCO_3$ (6 mL) and extracted with CH_2Cl_2 (2 × 15 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica, eluting with a gradient of $CHCl_3$ - 0% to 3% MeOH – 0% to 0.3% NH_4OH, to yield the titled product.

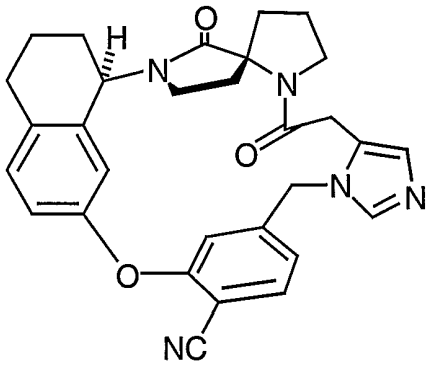
Step Q: (17*R*, 20*R*)-19,20,21,22-Tetrahydro-19-oxo-17*H*-15,17:18,20-diethano-6,10:12,16-dimetheno-20,21-propano-16*H*-imidazo [3,4-*h*][1,8,11,14]oxatriazacycloeicosine-9-carbonitrile

To a stirred solution of (*R,R*)-4-[5-({7-[6-(*tert*-butyldiphenylsilyloxy) indan-1-yl]-6-oxo-1,7-diazaspiro[4.4]non-1-yl}methyl)-1*H*-imidazol-1-ylmethyl]-2-fluorobenzonitrile, as described above in Step P, (74 mg, 0.10 mmol) in THF (2 mL) was added TBAF (1.0 M in THF, 0.11 mL, 0.11 mmol), dropwise, and the resulting mixture was stirred at ambient temperature for 18 h. The reaction was quenched with saturated aqueous $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (2 × 20 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica, eluting with a gradient of CH_2Cl_2 - 0% to 5% MeOH – 0% to 0.5% NH_4OH, to yield the titled product.

^{1}H NMR ($CDCl_3$): δ 7.62 (1H, d, *J* = 8.3 Hz), 7.61 (1H, s), 7.28 (1H, d, *J* = 8.1 Hz), 7.17 (1H, dd, *J* = 8.3, 2.4 Hz), 7.06 (1H, d, *J* = 1.0 Hz), 6.99 (1H, s), 6.83 (1H, dd, *J* = 8.1, 1.2 Hz), 6.69 (1H, d, *J* = 2.2 Hz), 5.67 (1H, dd, *J* = 8.8, 4.9 Hz), 5.23 (1H, d, *J* = 16.4 Hz), 5.01 (1H, d, *J* = 16.1 Hz), 3.70 (1H, d, *J* = 14.7 Hz), 3.33 (1H, d, *J* = 14.9 Hz), 3.23 (1H, m), 3.09 (1H, td, *J* = 8.1, 2.9 Hz), 3.05-2.90 (3H, m), 2.80 (1H, td, *J* = 9.5. 2.9 Hz), 2.45 (1H, m), 2.20 (1H, dt, *J* = 12.2, 8.3 Hz), 2.09-1.78 (6H, m).

ES MS: 466 (MH^+).

EXAMPLE 2



Preparation of (*R*,*R*)-15,16,17,17a,19,20,22,23-octahydro-19,22-dioxo-5*H*,21*H*-18,20-ethano-12,14-etheno-6,10-metheno-20,21-propanobenz[*d*]imidazo[4,3-*l*][1,6,9,13]oxatriazacyclononadecosine-9-carbonitrile

Step A: Methyl (imidazol-4-yl)acetate hydrochloride

A solution of 4-imidazoleacetic acid hydrochloride (25 mmol) in MeOH (100 mL) is saturated with HCl (g). Trimethyl orthoformate (100 mmol) is added and the mixture stands at ambient temperature for 18 hours, then is concentrated to dryness *in vacuo* to afford the titled ester.

Step B: Methyl [1-(triphenylmethyl)-1*H*-imidazol-4-yl]acetate

To a solution of methyl 4-imidazoleacetate hydrochloride, as described above in Step A, (24.3 mmol) in dry DMF (50 mL) are added triethylamine (53.5 mmol), then triphenylmethyl bromide (26.7 mmol). The mixture is stirred at ambient temperature, then partitioned between H_2O and EtOAc. The organic layer is dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue is purified by flash column chromatography on silica to yield the titled product.

Step C: Methyl [1-(4-cyano-3-fluorobenzyl)-1*H*-imidazol-5-yl]acetate

A mixture of methyl [1-(triphenylmethyl)-1*H*-imidazol-4-yl]acetate, as described above in Step B, (1.40 mmol) and 4-cyano-3-fluorobenzyl bromide, as described in Example 1, Step D, (1.40 mmol) in acetonitrile (3 mL) is heated to 50°C for 2 h. The mixture is allowed to cool, and the solid is collected by filtration.

The acetonitrile filtrate is concentrated *in vacuo* to a volume of approximately 1 mL and then reheated to 50°C for 2 h, cooled, and the solid is removed by filtration. The two crops of precipitated imidazolium salts are combined in MeOH (30 mL) and the solution is heated to reflux for 2 h, then concentrated *in vacuo*. The residue is partitioned between saturated aqueous $NaHCO_3$ and $CHCl_3$. The aqueous layer is extracted further with $CHCl_3$ (2 ×). The combined organic extracts are dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product is purified by flash column chromatography on silica to yield the titled product.

Step D: Lithium [1-(4-cyano-3-fluorobenzyl)-1*H*-imidazol-5-yl]acetate

Methyl [1-(4-cyanobenzyl)-1*H*-imidazol-5-yl]acetate, as described above in Step C, (free base) (0.95 mmol) is dissolved in THF (5 mL) and H_2O (1 mL). Lithium hydroxide (0.95 mmol) is added and the resulting mixture is stirred at ambient temperature until the reaction is complete, as judged by HPLC, then is adjusted to pH 7 with 1.0 N aqueous HCl and is concentrated to dryness *in vacuo* to give the titled lithium salt.

Step E: (*R*,*R*)-7-[7-(*tert*-Butyldiphenylsilyloxy)-1,2,3,4-tetrahydronaphthalen-1-yl]-6-oxo-1,7-diazaspiro[4.4]nonane trifluoroacetate

Following the procedures described in Example 1, Steps H to O, but using 7-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one, in place of 6-hydroxy-1-indanone in Step H, the above-titled compound is obtained.

Step F: (*R*,*R*)-4-[5-(2-{7-[7-(*tert*-Butyldiphenylsilyloxy)-1,2,3,4-tetrahydronaphthalen-1-yl]-6-oxo-1,7-diazaspiro[4.4]non-1-yl}-2-oxoethyl)-1*H*-imidazol-1-ylmethyl]-2-fluorobenzonitrile

To lithium [1-(4-cyano-3-fluorobenzyl)-1*H*-imidazol-5-yl]acetate, as described above in Step D, (2.0 mmol) in dry DMF (5 mL) are added PYBOP (2.2 mmol), (*R*,*R*)-7-[7-(*tert*-butyldiphenylsilyloxy)-1,2,3,4-tetrahydronaphthalen-1-yl]-6-oxo-1,7-diazaspiro[4.4]nonane trifluoroacetate, as described above in Step E, (2.2 mmol), and *N*,*N*-diisopropylethylamine (2.2 mmol). The reaction mixture is stirred until the reaction is complete as judged by HPLC, then poured into EtOAc and 10% aqueous citric acid, and the organic layer is extracted, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product is purified by flash column chromatography on silica to yield the desired product.

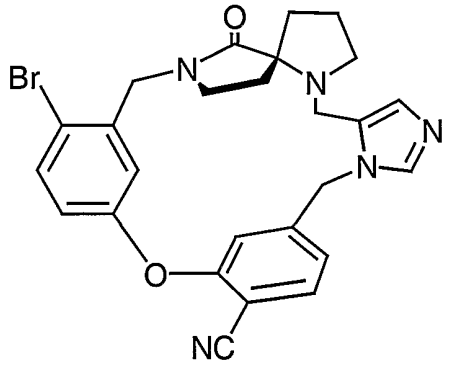
Step G: (*R*,*R*)-2-Fluoro-4-(5-{2-[7-(7-hydroxy-1,2,3,4-tetrahydronaphthalen-1-yl)-6-oxo-1,7-diazaspiro[4.4]non-1-yl]-2-oxoethyl}-1*H*-imidazol-1-ylmethyl)benzonitrile

To a stirred solution of (*R*,*R*)-4-[5-(2-{7-[7-(*tert*-butyldiphenylsilyl-oxy)-1,2,3,4-tetrahydronaphthalen-1-yl]-6-oxo-1,7-diazaspiro[4.4]non-1-yl}-2-oxoethyl)-1*H*-imidazol-1-ylmethyl]-2-fluorobenzonitrile, as described above in Step F, (1.0 mmol) in THF (10 mL) is added TBAF (1.0 M in THF, 1.1 mmol), dropwise, and the resulting mixture is stirred at ambient temperature until the reaction is complete as judged by HPLC. The reaction is quenched with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (2 ×). The combined organic extracts are dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product is purified by flash column chromatography on silica to yield the titled product.

Step H: (*R*,*R*)-15,16,17,17a,19,20,22,23-Octahydro-19,22-dioxo-5*H*,21*H*-18,20-ethano-12,14-etheno-6,10-metheno-20,21-propanobenz[*d*] imidazo[4,3-*l*] [1,6,9,13]oxatriazacyclononadecosine-9-carbonitrile

A mixture of (*R*,*R*)-2-fluoro-4-(5-{2-[7-(7-hydroxy-1,2,3,4-tetrahydronaphthalen-1-yl)-6-oxo-1,7-diazaspiro[4.4]non-1-yl]-2-oxoethyl}-1*H*-imidazol-1-ylmethyl)benzonitrile, as described above in Step G, (0.31 mmol) and Cs_2CO_3 (0.62 mmol) in dry, degassed DMF (40 mL) is stirred at 50°C under argon until the reaction is complete as judged by LCMS. The solvent is removed under reduced pressure, and the residue is partitioned between saturated aqueous $NaHCO_3$ and $CHCl_3$. The aqueous layer is extracted further with $CHCl_3$ (2 ×). The combined organic extracts are dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product is purified by flash column chromatography on silica to yield the titled product.

EXAMPLE 3



Preparation of (*R*)-15-bromo-19,20,21,22-tetrahydro-19-oxo-17*H*-18,20-ethano-6,10:12,16-dimetheno-20,21-propano-16*H*-imidazo[3,4-*h*][1,8,11,14] oxatriazacycloeicosine-9-carbonitrile

Step A: 2-Bromo-5-hydroxybenzoic acid

To a solution of 2-bromo-5-methoxybenzoic acid (60 mmol) in CH_2Cl_2 (500 mL) at –78°C is added BBr_3 (1 M solution in CH_2Cl_2, 132 mmol) dropwise. The reaction mixture is allowed to warm slowly to ambient temperature and stirred for 18 h. The reaction is quenched with 10% aqueous citric acid and extracted with EtOAc (3×). The combined organic extracts are dried over Na_2SO_4, filtered, and concentrated *in vacuo* to yield the titled product.

Step B: 2-Bromo-5-(*tert*-butyldiphenylsilyloxy)benzoic acid

A solution of 2-bromo-5-hydroxybenzoic acid, as described above in Step A, (50 mmol), *tert*-butyldiphenylsilyl chloride (55 mmol), and imidazole (100 mmol) in dry DMF (50 mL) is heated to 65°C, under argon, until the reaction is complete, as judged by HPLC. The reaction is poured into H_2O and extracted with EtOAc (3×). The combined organic extracts are dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product is purified by flash column chromatography on silica to yield the titled product.

Step C: 2-Bromo-5-(*tert*-butyldiphenylsilyloxy)benzyl alcohol

To a stirred solution of 2-bromo-5-(*tert*-butyldiphenylsilyloxy)benzoic acid, as described above in Step B, (25 mmol) in dry THF (50 mL) at 0°C is added borane-THF (1 M solution in THF, 37.5 mmol) dropwise. The mixture is allowed to warm to ambient temperature slowly and stirring is continued until the reaction is complete, as judged by HPLC. The reaction is quenched carefully with H_2O and extracted with EtOAc (3×). The combined organic extracts are dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product is purified by flash column chromatography on silica to yield the titled product.

Step D: (*R*)-15-Bromo-19,20,21,22-tetrahydro-19-oxo-17*H*-18,20-ethano-6,10:12,16-dimetheno-20,21-propano-16*H*-imidazo[3,4-*h*] [1,8,11,14]oxatriazacycloeicosine-9-carbonitrile

Following the procedures described in Example 1, Steps J to Q, but using 2-bromo-5-(*tert*-butyldiphenylsilyloxy)benzyl alcohol, in place of (*S*)-6-*tert*-butyldiphenylsilyloxy-1-indanol in Step J, the above-titled compound is obtained.

EXAMPLE 4

In vitro inhibition of ras farnesyl transferase

Transferase Assays. Isoprenyl-protein transferase activity assays are carried out at 30°C unless noted otherwise. A typical reaction contains (in a final volume of 50 µL): [^{3}H]farnesyl diphosphate, Ras protein, 50 mM HEPES, pH 7.5, 5 mM $MgCl_2$, 5 mM dithiothreitol, 10 µM $ZnCl_2$, 0.1% polyethyleneglycol (PEG) (15,000-20,000 mw) and isoprenyl-protein transferase. The FPTase employed in the assay is prepared by recombinant expression as described in Omer, C.A., Kral, A.M., Diehl, R.E., Prendergast, G.C., Powers, S., Allen, C.M., Gibbs, J.B. and Kohl, N.E. (1993) Biochemistry 32:5167-5176. After thermally pre-equilibrating the assay mixture in the absence of enzyme, reactions are initiated by the addition of isoprenyl-protein transferase and stopped at timed intervals (typically 15 min) by the addition of 1 M HCl in ethanol (1 mL). The quenched reactions are allowed to stand for 15 m (to complete the precipitation process). After adding 2 mL of 100% ethanol, the reactions are vacuum-filtered through Whatman GF/C filters. Filters are washed four times with 2 mL aliquots of 100% ethanol, mixed with scintillation fluid (10 mL) and then counted in a Beckman LS3801 scintillation counter.

For inhibition studies, assays are run as described above, except inhibitors are prepared as concentrated solutions in 100% dimethyl sulfoxide and then diluted 20-fold into the enzyme assay mixture. Substrate concentrations for inhibitor IC_{50} determinations are as follows: FTase, 650 nM Ras-CVLS (SEQ.ID.NO.: 1), 100 nM farnesyl diphosphate.

The compounds of the instant invention are tested for inhibitory activity against human FPTase by the assay described above.

EXAMPLE 5

Modified *In vitro* GGTase inhibition assay

The modified geranylgeranyl-protein transferase inhibition assay is carried out at room temperature. A typical reaction contains (in a final volume of 50 μL): [^{3}H]geranylgeranyl diphosphate, biotinylated Ras peptide, 50 mM HEPES, pH 7.5, a modulating anion (for example 10 mM glycerophosphate or 5mM ATP), 5 mM $MgCl_2$, 10 μM $ZnCl_2$, 0.1% PEG (15,000-20,000 mw), 2 mM dithiothreitol, and geranylgeranyl-protein transferase type I(GGTase). The GGTase-type I enzyme employed in the assay is prepared as described in U.S. Patent No. 5,470,832, incorporated by reference. The Ras peptide is derived from the K4B-Ras protein and has the following sequence: biotinyl-GKKKKKKSKTKCVIM (single amino acid code) (SEQ.ID.NO.: 2). Reactions are initiated by the addition of GGTase and stopped at timed intervals (typically 15 min) by the addition of 200 μL of a 3 mg/mL suspension of streptavidin SPA beads (Scintillation Proximity Assay beads, Amersham) in 0.2 M sodium phosphate, pH 4, containing 50 mM EDTA, and 0.5% BSA. The quenched reactions are allowed to stand for 2 hours before analysis on a Packard TopCount scintillation counter.

For inhibition studies, assays are run as described above, except inhibitors are prepared as concentrated solutions in 100% dimethyl sulfoxide and then diluted 25 fold into the enzyme assay mixture. IC_{50} values are determined with Ras peptide near K_M concentrations. Enzyme and substrate concentrations for inhibitor IC_{50} determinations are as follows: 75 pM GGTase-I, 1.6 μM Ras peptide, 100 nM geranylgeranyl diphosphate.

The compounds of the instant invention are tested for inhibitory activity against human GGTase-type I by the assay described above.

EXAMPLE 6

Cell-based *in vitro* ras farnesylation assay

The cell line used in this assay is a v-ras line derived from either Ratl or NIH3T3 cells, which expressed viral Ha-ras p21. The assay is performed essentially as described in DeClue, J.E. et al., Cancer Research 51:712-717, (1991). Cells in 10 cm dishes at 50-75% confluency are treated with the test compound (final concentration of solvent, methanol or dimethyl sulfoxide, is 0.1%). After 4 hours at 37°C, the cells are labeled in 3 ml methionine-free DMEM supple-mented with 10% regular DMEM, 2% fetal bovine serum and 400 μCi[^{35}S]methionine (1000 Ci/mmol). After an additional 20 hours, the cells are lysed in 1 ml lysis buffer (1% NP40/20 mM HEPES, pH 7.5/5 mM $MgCl_2$/1mM DTT/10 mg/ml aprotinen/2 mg/ml leupeptin/2 mg/ml antipain/0.5 mM PMSF) and the lysates cleared by centrifugation at 100,000 x g for 45 min. Aliquots of lysates containing equal numbers of acid-precipitable counts are bought to 1 ml with IP buffer (lysis buffer lacking DTT) and immuno-precipitated with the ras-specific monoclonal antibody Y13-259 (Furth, M.E. et al., J. Virol. 43:294-304, (1982)). Following a 2 hour antibody incubation at 4°C, 200 μl of a 25% suspension of protein A-Sepharose coated with rabbit anti rat IgG is added for 45 min. The immuno-precipitates are washed four times with IP buffer (20 nM HEPES, pH 7.5/1 mM EDTA/1% Triton X-100.0.5% deoxycholate/0.1%/ SDS/0.1 M NaCl) boiled in SDS-PAGE sample buffer and loaded on 13% acrylamide gels. When the dye front reached the bottom, the gel is fixed, soaked in Enlightening, dried and autoradiographed. The intensities of the bands corresponding to farnesyl-ated and nonfarnesylated ras proteins are compared to determine the percent inhibition of farnesyl transfer to protein.

EXAMPLE 7

Cell-based *in vitro* growth inhibition assay

To determine the biological consequences of FPTase inhibition, the effect of the compounds of the instant invention on the anchorage-independent growth of Ratl cells transformed with either a v-ras, v-raf, or v-mos oncogene is tested. Cells transformed by v-Raf and v-Mos maybe included in the analysis to evaluate the specificity of instant compounds for Ras-induced cell transformation.

Rat 1 cells transformed with either v-ras, v-raf, or v-mos are seeded

at a density of 1 x 10^4 cells per plate (35 mm in diameter) in a 0.3% top agarose layer in medium A (Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum) over a bottom agarose layer (0.6%). Both layers contain 0.1% methanol or an appropriate concentration of the instant compound (dissolved in methanol at 1000 times the final concentration used in the assay). The cells are fed twice weekly with 0.5 ml of medium A containing 0.1% methanol or the concentration of the instant compound. Photomicrographs are taken 16 days after the cultures are seeded and comparisons are made.

EXAMPLE 8

Construction of SEAP reporter plasmid pDSE100

The SEAP reporter plasmid, pDSE100 was constructed by ligating a restriction fragment containing the SEAP coding sequence into the plasmid pCMV-RE-AKI. The SEAP gene is derived from the plasmid pSEAP2-Basic (Clontech, Palo Alto, CA). The plasmid pCMV-RE-AKI was constructed by Deborah Jones (Merck) and contains 5 sequential copies of the 'dyad symmetry response element' cloned upstream of a 'CAT-TATA' sequence derived from the cytomegalovirus immediate early promoter. The plasmid also contains a bovine growth hormone poly-A sequence.

The plasmid, pDSE100 was constructed as follows. A restriction fragment encoding the SEAP coding sequence was cut out of the plasmid pSEAP2-Basic using the restriction enzymes EcoR1 and HpaI. The ends of the linear DNA fragments were filled in with the Klenow fragment of *E. coli* DNA Polymerase I. The 'blunt ended' DNA containing the SEAP gene was isolated by electrophoresing the digest in an agarose gel and cutting out the 1694 base pair fragment. The vector plasmid pCMV-RE-AKI was linearized with the restriction enzyme Bgl-II and the ends filled in with Klenow DNA Polymerase I. The SEAP DNA fragment was blunt end ligated into the pCMV-RE-AKI vector and the ligation products were transformed into DH5-alpha *E. coli* cells (Gibco-BRL). Transformants were screened for the proper insert and then mapped for restriction fragment orientation. Properly oriented recombinant constructs were sequenced across the cloning junctions to verify the correct sequence. The resulting plasmid contains the SEAP coding sequence

downstream of the DSE and CAT-TATA promoter elements and upstream of the BGH poly-A sequence.

Alternative Construction of SEAP reporter plasmid, pDSE101

The SEAP repotrer plasmid, pDSE101 is also constructed by ligating a restriction fragment containing the SEAP coding sequence into the plasmid pCMV-RE-AKI. The SEAP gene is derived from plasmid pGEM7zf(-)/SEAP.

The plasmid pDSE101 was constructed as follows: A restriction fragment containing part of the SEAP gene coding sequence was cut out of the plasmid pGEM7zf(-)/SEAP using the restriction enzymes Apa I and KpnI. The ends of the linear DNA fragments were chewed back with the Klenow fragment of *E. coli* DNA Polymerase I. The "blunt ended" DNA containing the truncated SEAP gene was isolated by electrophoresing the digest in an agarose gel and cutting out the 1910 base pair fragment. This 1910 base pair fragment was ligated into the plasmid pCMV-RE-AKI which had been cut with Bgl-II and filled in with *E. coli* Klenow fragment DNA polymerase. Recombinant plasmids were screened for insert orientation and sequenced through the ligated junctions. The plasmid pCMV-RE-AKI is derived from plasmid pCMVIE-AKI-DHFR (Whang , Y., Silberklang, M., Morgan, A., Munshi, S., Lenny, A.B., Ellis, R.W., and Kieff, E. (1987) J. Virol., 61, 1796-1807) by removing an EcoRI fragment containing the DHFR and Neomycin markers. Five copies of the fos promoter serum response element were inserted as described previously (Jones, R.E., Defeo-Jones, D., McAvoy, E.M., Vuocolo, G.A., Wegrzyn, R.J., Haskell, K.M. and Oliff, A. (1991) Oncogene, 6, 745-751) to create plasmid pCMV-RE-AKI.

The plasmid pGEM7zf(-)/SEAP was constructed as follows. The SEAP gene was PCRed, in two segments from a human placenta cDNA library (Clontech) using the following oligos.

Sense strand N-terminal SEAP : 5' GAGAGGGAATTCGGGCCCTTCCTGCAT GCTGCTGCTGCTGCTGCTGCTGGGC 3' (SEQ.ID.NO.:4)

Antisense strand N-terminal SEAP: 5' GAGAGAGCTCGAGGTTAACCCGGGT GCGCGGCGTCGGTGGT 3' (SEQ.ID.NO.:5)

Sense strand C-terminal SEAP: 5' GAGAGAGTCTAGAGTTAACCCGTGGTCC

CCGCGTTGCTTCCT 3' (SEQ.ID.NO.:6)

Antisense strand C-terminal SEAP: 5' GAAGAGGAAGCTTGGTACCGCCACTG GGCTGTAGGTGGTGGCT 3' (SEQ.ID.NO.:7)

The N-terminal oligos (SEQ.ID.NO.: 4 and SEQ.ID.NO.: 5) were used to generate a 1560 bp N-terminal PCR product that contained EcoRI and HpaI restriction sites at the ends. The Antisense N-terminal oligo (SEQ.ID.NO.: 5) introduces an internal translation STOP codon within the SEAP gene along with the HpaI site. The C-terminal oligos (SEQ.ID.NO.: 6 and SEQ.ID.NO.: 7) were used to amplify a 412 bp C-terminal PCR product containing HpaI and HindIII restriction sites. The sense strand C-terminal oligo (SEQ.ID.NO.: 6) introduces the internal STOP codon as well as the HpaI site. Next, the N-terminal amplicon was digested with EcoRI and HpaI while the C-terminal amplicon was digested with HpaI and HindIII. The two fragments comprising each end of the SEAP gene were isolated by electro-phoresing the digest in an agarose gel and isolating the 1560 and 412 base pair fragments. These two fragments were then co-ligated into the vector pGEM7zf (-) (Promega) which had been restriction digested with EcoRI and HindIII and isolated on an agarose gel. The resulting clone, pGEM7zf(-)/SEAP contains the coding sequence for the SEAP gene from amino acids.

Construction of a constitutively expressing SEAP plasmid pCMV-SEAP-A

An expression plasmid constitutively expressing the SEAP protein was created by placing the sequence encoding a truncated SEAP gene downstream of the cytomegalovirus (CMV) IE-1 promoter. The expression plasmid also includes the CMV intron A region 5' to the SEAP gene as well as the 3' untranslated region of the bovine growth hormone gene 3' to the SEAP gene.

The plasmid pCMVIE-AKI-DHFR (Whang , Y., Silberklang, M., Morgan, A., Munshi, S., Lenny, A.B., Ellis, R.W., and Kieff, E. (1987) J. Virol., 61:1796-1807) containing the CMV immediate early promoter was cut with EcoRI generating two fragments. The vector fragment was isolated by agarose electro-phoresis and religated. The resulting plasmid is named pCMV-AKI. Next, the cytomegalovirus intron A nucleotide sequence was inserted downstream of the CMV IE1 promter in pCMV-AKI. The intron A sequence was isolated from a genomic clone bank and subcloned into pBR322 to generate plasmid p16T-286.

The intron A sequence was mutated at nucleotide 1856 (nucleotide numbering as in Chapman, B.S., Thayer, R.M., Vincent, K.A. and Haigwood, N.L., Nuc.Acids Res. 19, 3979-3986) to remove a SacI restriction site using site directed mutagenesis. The mutated intron A sequence was PCRed from the plasmid p16T-287 using the following oligos.

Sense strand: 5' GGCAGAGCTCGTTTAGTGAACCGTCAG 3' (SEQ.ID.NO.: 8)

Antisense strand: 5' GAGAGATCTCAAGGACGGTGACTGCAG 3' (SEQ.ID.NO.: 9)

These two oligos generate a 991 base pair fragment with a SacI site incorporated by the sense oligo and a Bgl-II fragment incorporated by the antisense oligo. The PCR fragment is trimmed with SacI and Bgl-II and isolated on an agarose gel. The vector pCMV-AKI is cut with SacI and Bgl-II and the larger vector fragment isolated by agarose gel electrophoresis. The two gel isolated fragments are ligated at their respective SacI and Bgl-II sites to create plasmid pCMV-AKI-InA.

The DNA sequence encoding the truncated SEAP gene is inserted into the pCMV-AKI-InA plasmid at the Bgl-II site of the vector. The SEAP gene is cut out of plasmid pGEM7zf(-)/SEAP (described above) using EcoRI and HindIII. The fragment is filled in with Klenow DNA polymerase and the 1970 base pair fragment isolated from the vector fragment by agarose gel electrophoresis. The pCMV-AKI-InA vector is prepared by digesting with Bgl-II and filling in the ends with Klenow DNA polymerase. The final construct is generated by blunt end ligating the SEAP fragment into the pCMV-AKI-InA vector. Transformants were screened for the proper insert and then mapped for restriction fragment orientation. Properly oriented recombinant constructs were sequenced across the cloning junctions to verify the correct sequence. The resulting plasmid, named pCMV-SEAP-A (deposited in the ATCC under Budapest Treaty on August 27, 1998, and designated ATCC), contains a modified SEAP sequence downstream of the cytomegalovirus immediately early promoter IE-1 and intron A sequence and upstream of the bovine growth hormone poly-A sequence. The plasmid expresses SEAP in a constitutive manner when transfected into mammalian cells.

Alternative construction of a constitutively expressing SEAP plasmid pCMV-SEAP-B

An expression plasmid constitutively expressing the SEAP protein can be created by placing the sequence encoding a truncated SEAP gene downstream of the cytomegalovirus (CMV) IE-1 promoter and upstream of the 3' unstranslated region of the bovine growth hormone gene.

The plasmid pCMVIE-AKI-DHFR (Whang, Y., Silberklang, M., Morgan, A., Munshi, S., Lenny, A.B., Ellis, R.W., and Kieff, E. (1987) J. Virol., 61:1796-1807) containing the CMV immediate early promoter and bovine growth hormone poly-A sequence can be cut with EcoRI generating two fragments. The vector fragment can be isolated by agarose electrophoresis and religated. The resulting plasmid is named pCMV-AKI. The DNA sequence encoding the truncated SEAP gene can be inserted into the pCMV-AKI plasmid at a unique Bgl-II in the vector. The SEAP gene is cut out of plasmid pGEMzf(-)/SEAP (described above) using EcoRI and HindIII. The fragments are filled in with Klenow DNA polymerase and the 1970 base pair fragment is isolated from the vector fragment by agarose gel electrophoresis. The pCMV-AKI vector is prepared by digesting with Bgl-II and filling in the ends with Klenow DNA polymerase. The final construct is generated by blunt end ligating the SEAP fragment into the vector and transforming the ligation reaction into *E. coli* DH5α cells. Transformants can then be screened for the proper insert and mapped for restriction fragment orientation. Properly oriented recombinant constructs would be sequenced across the cloning junctions to verify the correct sequence. The resulting plasmid, named pCMV-SEAP-B contains a modified SEAP sequence downstream of the cytomegalovirus immediate early promoter, IE1, and upstream of a bovine growth hormone poly-A sequence. The plasmid would express SEAP in a constitutive nammer when transfected into mammalian cells.

Cloning of a Myristylated viral-H-*ras* expression plasmid pSMS600

A DNA fragment containing viral-H-*ras* can be PCRed from plasmid "HB-11 (deposited in the ATCC under Budapest Treaty on August 27, 1997, and designated ATCC 209,218) using the following oligos.

Sense strand:

5'TCTCCTCGAGGCCACCATGGGGAGTAGCAAGAGCAAGCCTAAGGACCC CAGCCAGCGCCGGATGACAGAATACAAGCTTGTGGTGG 3'. (SEQ.ID.NO.: 10)

Antisense:
5'CACATCTAGATCAGGACAGCACAGACTTGCAGC 3'.
(SEQ.ID.NO.: 11)

A sequence encoding the first 15 aminoacids of the v-src gene, containing a myristylation site, is incorporated into the sense strand oligo. The sense strand oligo also optimizes the 'Kozak' translation initiation sequence immediately 5' to the ATG start site. To prevent prenylation at the viral-*ras* C-terminus, cysteine 186 would be mutated to a serine by substituting a G residue for a C residue in the C-terminal antisense oligo. The PCR primer oligos introduce an XhoI site at the 5' end and a XbaI site at the 3'end. The XhoI-XbaI fragment can be ligated into the mammalian expression plasmid pCI (Promega) cut with XhoI and XbaI. This results in a plasmid, pSMS600, in which the recombinant myr-viral-H-ras gene is constitutively transcribed from the CMV promoter of the pCI vector.

Cloning of a viral-H-*ras*-CVLL expression plasmid pSMS601

A viral-H-*ras* clone with a C-terminal sequence encoding the amino acids CVLL can be cloned from the plasmid "HB-11" by PCR using the following oligos.

Sense strand:
5'TCTCCTCGAGGCCACCATGACAGAATACAAGCTTGTGGTGG-3'
(SEQ.ID.NO.: 12)

Antisense strand:
5'CACTCTAGACTGGTGTCAGAGCAGCACACACTTGCAGC-3' (SEQ.ID.NO.: 13)

The sense strand oligo optimizes the 'Kozak' sequence and adds an XhoI site. The antisense strand mutates serine 189 to leucine and adds an XbaI site. The PCR fragment can be trimmed with XhoI and XbaI and ligated into the XhoI-XbaI cut vector pCI (Promega). This results in a plasmid, pSMS601, in which the mutated viral-H-*ras*-CVLL gene is constitutively transcribed from the CMV promoter of the pCI vector.

Cloning of cellular-H-*ras*-Leu61 expression plasmid pSMS620

The human cellular-H-*ras* gene can be PCRed from a human cerebral cortex cDNA library (Clontech) using the following oligonucleotide primers.

Sense strand:
5'-GAGAGAATTCGCCACCATGACGGAATATAAGCTGGTGG-3'
(SEQ.ID.NO.: 14)

Antisense strand:
5'-GAGAGTCGACGCGTCAGGAGAGCACACACTTGC-3' (SEQ.ID.NO.: 15)

The primers will amplify a c-H-Ras encoding DNA fragment with the primers contributing an optimized 'Kozak' translation start sequence, an EcoRI site at the N-terminus and a Sal I site at the C-terminal end. After trimming the ends of the PCR product with EcoRI and Sal I, the c-H-ras fragment can be ligated ligated into an EcoRI -Sal I cut mutagenesis vector pAlter-1 (Promega). Mutation of glutamine-61 to a leucine can be accomplished using the manufacturer's protocols and the following oligonucleotide:

5'-CCGCCGGCCTGGAGGAGTACAG-3' (SEQ.ID.NO.: 16)

After selection and sequencing for the correct nucleotide substitution, the mutated c-H-*ras*-Leu61 can be excised from the pAlter-1 vector, using EcoRI and Sal I, and be directly ligated into the vector pCI (Promega) which has been digested with EcoRI and Sal I. The new recombinant plasmid, pSMS620, will constitutively transcribe c-H-*ras*-Leu61 from the CMV promoter of the pCI vector.

Cloning of a c-N-*ras*-Val-12 expression plasmid pSMS630

The human c-N-*ras* gene can be PCRed from a human cerebral cortex cDNA library (Clontech) using the following oligonucleotide primers.

Sense strand:
5'-GAGAGAATTCGCCACCATGACTGAGTACAAACTGGTGG-3'
(SEQ.ID.NO.: 17)

Antisense strand:
5'-GAGAGTCGACTTGTTACATCACCACACATGGC-3' (SEQ.ID.NO.: 18)

The primers will amplify a c-N-Ras encoding DNA fragment with the primers contributing an optimized 'Kozak' translation start sequence, an EcoRI site at the N-terminus and a Sal I site at the C-terminal end. After trimming the ends of the PCR product with EcoRI and Sal I, the c-N-ras fragment can be ligated into an EcoRI -Sal I cut mutagenesis vector pAlter-1 (Promega). Mutation of glycine-12 to a valine can be accomplished using the manufacturer's protocols and the following oligonucleotide:

5'-GTTGGAGCAGTTGGTGTTGGG-3' (SEQ.ID.NO.: 19)

After selection and sequencing for the correct nucleotide substitution, the mutated c-N-*ras*-Val-12 can be excised from the pAlter-1 vector, using EcoRI and Sal I, and be directly ligated into the vector pCI (Promega) which has been digested with EcoRI and Sal I. The new recombinant plasmid, pSMS630, will constitutively transcribe c-N-*ras*-Val-12 from the CMV promoter of the pCI vector.

Cloning of a c-K4B-*ras*-Val-12 expression plasmid pSMS640

The human c-K4B-*ras* gene can be PCRed from a human cerebral cortex cDNA library (Clontech) using the following oligo-nucleotide primers.

Sense strand:
5'-GAGAGGTACCGCCACCATGACTGAATATAAACTTGTGG-3'
(SEQ.ID.NO.: 20)

Antisense strand:
5'-CTCTGTCGACGTATTTACATAATTACACACTTTGTC-3' (SEQ.ID.NO.: 21)

The primers will amplify a c-K4B-Ras encoding DNA fragment with the primers contributing an optimized 'Kozak' translation start sequence, a KpnI site at the N-terminus and a Sal I site at the C-terminal end. After trimming the ends of

the PCR product with Kpn I and Sal I, the c-K4B-*ras* fragment can be ligated into a KpnI -Sal I cut mutagenesis vector pAlter-1 (Promega). Mutation of cysteine-12 to a valine can be accomplished using the manufacturer's protocols and the following oligonucleotide:

5'-GTAGTTGGAGCT**GT**TGGCGTAGGC-3' (SEQ.ID.NO.: 22)

After selection and sequencing for the correct nucleotide substitution, the mutated c-K4B-*ras*-Val-12 can be excised from the pAlter-1 vector, using KpnI and Sal I, and be directly ligated into the vector pCI (Promega) which has been digested with KpnI and Sal I. The new recombinant plasmid will constitutively transcribe c-K4B-*ras*-Val-12 from the CMV promoter of the pCI vector.

Cloning of c-K-*ras*4A-Val-12 expression plasmid pSMS650

The human c-K4A-*ras* gene can be PCRed from a human cerebral cortex cDNA library (Clontech) using the following oligo-nucleotide primers.

Sense strand:
5'-GAGAGGTACCGCCACCATGACTGAATATAAACTTGTGG-3'
(SEQ.ID.NO.: 23)

Antisense strand:
5'-CTCTGTCGACAGATTACATTATAATGCATTTTTTAATTTTCACAC-3'
(SEQ.ID.NO.: 24)

The primers will amplify a c-K4A-Ras encoding DNA fragment with the primers contributing an optimized 'Kozak' translation start sequence, a KpnI site at the N-terminus and a Sal I stite at the C-terminal end. After trimming the ends of the PCR product with Kpn I and Sal I, the c-K-ras4A fragment can be ligated into a KpnI -Sal I cut mutagenesis vector pAlter-1 (Promega). Mutation of cysteine-12 to a valine can be accomplished using the manufacturer's protocols and the following oligonucleotide:

5'-GTAGTTGGAGCT**GT**TGGCGTAGGC-3' (SEQ.ID.NO.: 25)

After selection and sequencing for the correct nucleotide substitution, the mutated c-K4A-*ras*-Val-12 can be excised from the pAlter-1 vector, using KpnI and Sal I, and be directly ligated into the vector pCI (Promega) which has been digested with KpnI and Sal I. The new recombinant plasmid, pSMS650, will constitutively transcribe c-K4A-*ras*-Val-12 from the CMV promoter of the pCI vector.

SEAP assay

Human C33A cells (human epitheial carcenoma - ATTC collection) are seeded in 10cm tissue culture plates in DMEM + 10% fetal calf serum + 1X Pen/Strep + 1X glutamine + 1X NEAA. Cells are grown at 37°C in a 5% CO_2 atmosphere until they reach 50-80% of confluency.

The transient transfection is performed by the $CaPO_4$ method (Sambrook et al., 1989). Thus, expression plasmids for H-*ras*, N-*ras*, K-*ras*, Myr-*ras* or H-*ras*-CVLL are co-precipitated with the DSE-SEAP reporter construct. (A *ras* expression plasmid is not included when the cell is transfected with the pCMV-SEAP plasmid.) For 10 cm plates 600 µl of $CaCl_2$-DNA solution is added dropwise while vortexing to 600 µl of 2X HBS buffer to give 1.2 ml of precipitate solution (see recipes below). This is allowed to sit at room temperature for 20 to 30 minutes. While the precipitate is forming, the media on the C33A cells is replaced with DMEM (minus phenol red; Gibco cat. No. 31053-028)+ 0.5% charcoal stripped calf serum + 1X (Pen/Strep, Glutamine and nonessential aminoacids). The $CaPO_4$-DNA precipitate is added dropwise to the cells and the plate rocked gently to distribute. DNA uptake is allowed to proceed for 5-6 hrs at 37°C under a 5% CO_2 atmosphere.

Following the DNA incubation period, the cells are washed with PBS and trypsinized with 1ml of 0.05% trypsin. The 1 ml of trypsinized cells is diluted into 10 ml of phenol red free DMEM + 0.2% charcoal stripped calf serum + 1X (Pen/Strep, Glutamine and NEAA). Transfected cells are plated in a 96 well micro-titer plate (100 µl/well) to which drug, diluted in media, has already been added in a volume of 100 µl. The final volume per well is 200 µl with each drug concentration repeated in triplicate over a range of half-log steps.

Incubation of cells and drugs is for 36 hours at 37°C under CO_2. At the end of the incubation period, cells are examined micro-scopically for evidence of cell distress. Next, 100 µl of media containing the secreted alkaline phosphatase is removed from each well and transferred to a microtube array for heat treatment at

65°C for 1 hour to inactivate endogenous alkaline phosphatases (but not the heat stable secreted phosphatase).

The heat treated media is assayed for alkaline phosphatase by a luminescence assay using the luminescence reagent CSPD® (Tropix, Bedford, Mass.). A volume of 50 µl media is combined with 200 µl of CSPD cocktail and incubated for 60 minutes at room temperature. Luminesence is monitored using an ML2200 microplate luminometer (Dynatech). Luminescence reflects the level of activation of the fos reporter construct stimulated by the transiently expressed protein.

DNA-$CaPO_4$ precipitate for 10cm. plate of cells

Ras expression plasmid (1 µg/µl)	10 µl
DSE-SEAP Plasmid (1 µg/µl)	2 µl
Sheared Calf Thymus DNA (1 µg/µl)	8 µl
2M $CaCl_2$	74 µl
dH_2O	506 µl

2X HBS Buffer

280mM NaCl

10mM KCl

1.5mM Na_2HPO_4 $2H_2O$

12mM dextrose

50mM HEPES

Final pH = 7.05

Luminesence Buffer (26ml)

Assay Buffer	20ml
Emerald Reagent™ (Tropix)	2.5ml
100mM homoarginine	2.5ml
CSPD Reagent® (Tropix)	1.0ml

Assay Buffer

Add 0.05M Na_2CO_3 to 0.05M $NaHCO_3$ to obtain pH 9.5.

Make 1mM in $MgCl_2$

EXAMPLE 9

The processing assays employed are modifications of that described by DeClue et al [Cancer Research 51, 712-717, 1991].

K4B-Ras processing inhibition assay

PSN-1 (human pancreatic carcinoma) or viral-K4B-ras-transformed Rat1 cells are used for analysis of protein processing. Subconfluent cells in 100 mm dishes are fed with 3.5 ml of media (methionine-free RPMI supplemented with 2% fetal bovine serum or cysteine-free/methionine-free DMEM supplemented with 0.035 ml of 200 mM glutamine (Gibco), 2% fetal bovine serum, respectively) containing the desired concentration of test compound, lovastatin or solvent alone. Cells treated with lovastatin (5-10 μM), a compound that blocks Ras processing in cells by inhibiting a rate-limiting step in the isoprenoid biosynthetic pathway, serve as a positive control. Test compounds are prepared as 1000x concentrated solutions in DMSO to yield a final solvent concentration of 0.1%. Following incubation at 37°C for 2 hours 204 μCi/ml [^{35}S]Pro-Mix (Amersham, cell labeling grade) is added.

After introducing the label amino acid mixture, the cells are incubated at 37°C for an additional period of time (typically 6 to 24 hours). The media is then removed and the cells are washed once with cold PBS. The cells are scraped into 1 ml of cold PBS, collected by centrifugation (10,000 x g for 10 sec at room temperature), and lysed by vortexing in 1 ml of lysis buffer (1% Nonidet P-40, 20 mM HEPES, pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.5% deoxycholate, 0.1% SDS, 1 mM DTT, 10 μg/ml AEBSF, 10 μg/ml aprotinin, 2 μg/ml leupeptin and 2 μg/ml antipain). The lysate is then centrifuged at 15,000 x g for 10 min at 4°C and the supernatant saved.

For immunoprecipitation of Ki4B-Ras, samples of lysate supernatant containing equal amounts of protein are utilized. Protein concentration is determined by the bradford method utilizing bovine serum albumin as a standard. The appropriate volume of lysate is brought to 1 ml with lysis buffer lacking DTT and 8 μg of the pan Ras monoclonal antibody, Y13-259, added. The protein/antibody mixture is incubated on ice at 4°C for 24 hours. The immune complex is collected on pansorbin (Calbiochem) coated with rabbit antiserum to rat IgG (Cappel) by tumbling at 4°C for 45 minutes. The pellet is washed 3 times with 1 ml of lysis buffer lacking DTT and protease inhibitors and resuspended in 100 μl elution buffer (10 mM Tris pH 7.4, 1%

SDS). The Ras is eluted from the beads by heating at 95°C for 5 minutes, after which the beads are pelleted by brief centrifugation (15,000 x g for 30 sec. at room temperature).

The supernatant is added to 1 ml of Dilution Buffer 0.1% Triton X-100, 5 mM EDTA, 50 mM NaCl, 10 mM Tris pH 7.4) with 2 μg Kirsten-ras specific monoclonal antibody, c-K-ras Ab-1 (Calbiochem). The second protein/antibody mixture is incubated on ice at 4°C for 1-2 hours. The immune complex is collected on pansorbin (Calbiochem) coated with rabbit antiserum to rat IgG (Cappel) by tumbling at 4°C for 45 minutes. The pellet is washed 3 times with 1 ml of lysis buffer lacking DTT and protease inhibitors and resuspended in Laemmli sample buffer. The Ras is eluted from the beads by heating at 95°C for 5 minutes, after which the beads are pelleted by brief centrifugation. The supernatant is subjected to SDS-PAGE on a 12% acrylamide gel (bis-acrylamide:acrylamide, 1:100), and the Ras visualized by fluorography.

hDJ processing inhibition assay

PSN-1 cells are seeded in 24-well assay plates. For each compound to be tested, the cells are treated with a minimum of seven concentrations in half-log steps. The final solvent (DMSO) concentration is 0.1%. A vehicle-only control is included on each assay plate. The cells are treated for 24 hours at 37°C / 5% CO_2.

The growth media is then aspirated and the samples are washed with PBS. The cells are lysed with SDS-PAGE sample buffer containing 5% 2-mercaptoethanol and heated to 95°C for 5 minutes. After cooling on ice for 10 minutes, a mixture of nucleases is added to reduce viscosity of the samples.

The plates are incubated on ice for another 10 minutes. The samples are loaded onto pre-cast 8% acrylamide gels and electrophoresed at 15 mA/gel for 3-4 hours. The samples are then transferred from the gels to PVDF membranes by Western blotting.

The membranes are blocked for at least 1 hour in buffer containing 2% nonfat dry milk. The membranes are then treated with a monoclonal antibody to hDJ-2 (Neomarkers Cat. # MS-225), washed, and treated with an alkaline phosphatase-conjugated secondary antibody. The membranes are then treated with a fluorescent detection reagent and scanned on a phosphorimager.

For each sample, the percent of total signal corresponding to the unprenylated species of hDJ (the slower-migrating species) is calculated by

densitometry. Dose-response curves and EC_{50} values are generated using 4-parameter curve fits in SigmaPlot software.

EXAMPLE 10

Rap1 processing inhibition assay

Protocol A:

Cells are labeled, incubated and lysed as described in Example 9. For immunoprecipitation of Rap1, samples of lysate supernatant containing equal amounts of protein are utilized. Protein concentration is determined by the bradford method utilizing bovine serum albumin as a standard. The appropriate volume of lysate is brought to 1 ml with lysis buffer lacking DTT and 2 μg of the Rap1 antibody, Rap1/Krev1 (121) (Santa Cruz Biotech), is added. The protein/antibody mixture is incubated on ice at 4°C for 1 hour. The immune complex is collected on pansorbin (Calbiochem) by tumbling at 4°C for 45 minutes. The pellet is washed 3 times with 1 ml of lysis buffer lacking DTT and protease inhibitors and resuspended in 100 μl elution buffer (10 mM Tris pH 7.4, 1% SDS). The Rap1 is eluted from the beads by heating at 95°C for 5 minutes, after which the beads are pelleted by brief centrifugation (15,000 x g for 30 sec. at room temperature).

The supernatant is added to 1 ml of Dilution Buffer (0.1% Triton X-100, 5 mM EDTA, 50 mM NaCl, 10 mM Tris pH 7.4) with 2 μg Rap1 antibody, Rap1/Krev1 (121) (Santa Cruz Biotech). The second protein/antibody mixture is incubated on ice at 4°C for 1-2 hours. The immune complex is collected on pansorbin (Calbiochem) by tumbling at 4°C for 45 minutes. The pellet is washed 3 times with 1 ml of lysis buffer lacking DTT and protease inhibitors and resuspended in Laemmli sample buffer. The Rap1 is eluted from the beads by heating at 95°C for 5 minutes, after which the beads are pelleted by brief centrifugation. The supernatant is subjected to SDS-PAGE on a 12% acrylamide gel (bis-acrylamide:acrylamide, 1:100), and the Rap1 visualized by fluorography.

Protocol B:

PSN-1 cells are passaged every 3-4 days in 10cm plates, splitting

near-confluent plates 1:20 and 1:40. The day before the assay is set up, 5x 10^6 cells are plated on 15cm plates to ensure the same stage of confluency in each assay. The media for these cells is RPM1 1640 (Gibco), with 15% fetal bovine serum and 1x Pen/Strep antibiotic mix. The day of the assay, cells are collected from the 15 cm plates by trypsinization and diluted to 400,000 cells/ml in media. 0.5ml of these diluted cells are added to each well of 24-well plates, for a final cell number of 200,000 per well. The cells are then grown at 37°C overnight.

The compounds to be assayed are diluted in DMSO in 1/2-log dilutions. The range of final concentrations to be assayed is generally 0.1-100 μM. Four concentrations per compound is typical. The compounds are diluted so that each concentration is 1000x of the final concentration (i.e., for a 10 μM data point, a 10 mM stock of the compound is needed).

2 μL of each 1000x compound stock is diluted into 1 ml media to produce a 2X stock of compound. A vehicle control solution (2 μL DMSO to 1ml media), is utilized. 0.5 ml of the 2X stocks of compound are added to the cells.

After 24 hours, the media is aspirated from the assay plates. Each well is rinsed with 1ml PBS, and the PBS is aspirated. 180 μL SDS-PAGE sample buffer (Novex) containing 5% 2-mercapto-ethanol is added to each well. The plates are heated to 100°C for 5 minutes using a heat block containing an adapter for assay plates. The plates are placed on ice. After 10 minutes, 20 μL of an RNAse/DNase mix is added per well. This mix is 1mg/ml DNaseI (Worthington Enzymes), 0.25 mg/ml Rnase A (Worthington Enzymes), 0.5 M Tris-HCl pH 8.0 and 50 mM $MgCl_2$. The plate is left on ice for 10 minutes. Samples are then either loaded on the gel, or stored at -70°C until use.

Each assay plate (usually 3 compounds, each in 4-point titrations, plus controls) requires one 15-well 14% Novex gel. 25 μl of each sample is loaded onto the gel. The gel is run at 15 mA for about 3.5 hours. It is important to run the gel far enough so that there will be adequate separation between 21 kd (Rap1) and 29kd (Rab6).

The gels are then transferred to Novex pre-cut PVDF membranes for 1.5 hours at 30V (constant voltage). Immediately after transferring, the membranes are blocked overnight in 20ml Western blocking buffer (2% nonfat dry milk in Western wash buffer (PBS + 0.1% Tween-20). If blocked over the weekend, 0.02% sodium azide is added. The membranes are blocked at 4°C with slow rocking.

The blocking solution is discarded and 20ml fresh blocking solution containing the anti Rap1a antibody (Santa Cruz Biochemical SC1482) at 1:1000 (diluted in Western blocking buffer) and the anti Rab6 antibody (Santa Cruz Biochemical SC310) at 1:5000 (diluted in Western blocking buffer) are added. The membranes are incubated at room temperature for 1 hour with mild rocking. The blocking solution is then discarded and the membrane is washed 3 times with Western wash buffer for 15 minutes per wash. 20ml blocking solution containing 1:1000 (diluted in Western blocking buffer) each of two alkaline phosphatase conjugated antibodies (Alkaline phosphatase conjugated Anti-goat IgG and Alkaline phosphatase conjugated anti-rabbit IgG [Santa Cruz Biochemical]) is then added. The membrane is incubated for one hour and washed 3x as above.

About 2 ml per gel of the Amersham ECF detection reagent is placed on an overhead transparency (ECF) and the PVDF membranes are placed face down onto the detection reagent. This is incubated for one minute, then the membrane is placed onto a fresh transparency sheet.

The developed transparency sheet is scanned on a phosphorimager and the Rap1a Minimum Inhibitory Concentration is determined from the lowest concentration of compound that produces a detectable Rap1a Western signal. The Rap1a antibody used recognizes only unprenylated/unprocessed Rap1a, so that the precence of a detectable Rap1a Western signal is indicative of inhibition of Rap1a prenylation.

Protocol C:

This protocol allows the determination of an EC_{50} for inhibition of processing of Rap1a. The assay is run as described in Protocol B with the following modifications. 20 μl of sample is run on pre-cast 10-20% gradient acrylamide mini gels (Novex Inc.) at 15 mA/gel for 2.5-3 hours. Prenylated and unprenylated forms of Rap1a are detected by blotting with a polyclonal antibody (Rap1/Krev-1 Ab#121; Santa Cruz Research Products #sc-65), followed by an alkaline phosphatase-conjugated anti-rabbit IgG antibody. The percentage of unprenylated Rap1a relative to the total amount of Rap1a is determined by peak integration using Imagequant™ software (Molecular Dynamics). Unprenylated Rap1a is distinguished from prenylated protein by virtue of the greater apparent molecular weight of the prenylated protein. Dose-response curves and EC_{50} values are generated using 4-parameter curve fits in SigmaPlot software.

EXAMPLE 11

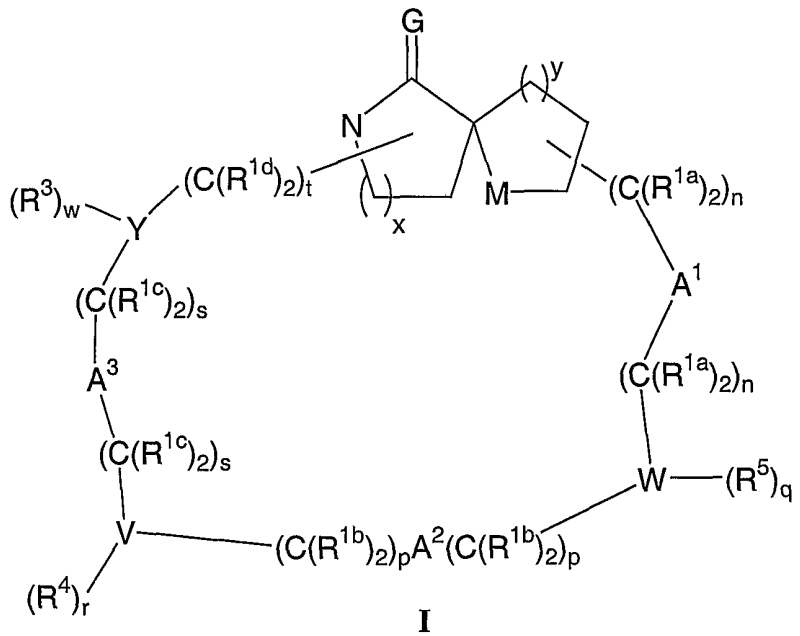
In vivo tumor growth inhibition assay (nude mouse)

In vivo efficacy as an inhibitor of the growth of cancer cells may be confirmed by several protocols well known in the art. Examples of such *in vivo* efficacy studies are described by N. E. Kohl et al. (Nature Medicine, 1:792-797 (1995)) and N. E. Kohl et al. (Proc. Nat. Acad. Sci. U.S.A., 91:9141-9145 (1994)).

Rodent fibroblasts transformed with oncogenically mutated human Ha-ras or Ki-ras (10^6 cells/animal in 1 ml of DMEM salts) are injected subcutaneously into the left flank of 8-12 week old female nude mice (Harlan) on day 0. The mice in each oncogene group are randomly assigned to a vehicle or compound treatment group. Animals are dosed subcutaneously starting on day 1 and daily for the duration of the experiment. Alternatively, the farnesyl-protein transferase inhibitor may be administered by a continuous infusion pump. Compound or vehicle is delivered in a total volume of 0.1 ml. Tumors are excised and weighed when all of the vehicle-treated animals exhibited lesions of 0.5-1.0 cm in diameter, typically 11-15 days after the cells were injected. The average weight of the tumors in each treatment group for each cell line is calculated.

WHAT IS CLAIMED IS:

1. A compound of the formula I:



I

wherein

M is selected from N, O or S;

A^1, A^2 and A^3 are independently selected from

1) a bond,
2) O,
3) C(O),
4) $N(R^{10})_2$,
5) $C(O)NR^{10}$,
6) $NR^{10}C(O)$,
7) $S(O)_m$,
8) $OS(O)_m$,
9) $NR^{10}C(O)NR^{10}$,
10) OC(O),
11) C(O)O

12) CH=CH,
13) C≡C,
14) $OC(O)NR^{10}$, or
15) $NR^{10}C(O)O$;

R^{1a}, R^{1b}, R^{1c} and R^{1d} are independently selected from

1) H,
2) unsubstituted or substituted C_1-C_6 alkyl,
3) unsubstituted or substituted C_2-C_8 alkenyl,
4) unsubstituted or substituted C_2-C_8 alkynyl,
5) unsubstituted or substituted aryl,
6) unsubstituted or substituted C_3-C_{10} cycloalkyl,
7) unsubstituted or substituted heterocycle,
8) $-OR^{10}$,
9) $R^{11}S(O)_m$-,
10) $-N(R^{10})_2$,
11) $R^{10}C(O)$-,
12) $-C(O)NR^6R^7$,
13) $R^{10}C(O)NR^{10}$-,
14) $(R^{10})_2NC(O)NR^{10}$-,
15) $R^{10}OC(O)$-,
16) $R^{10}C(O)O$-, or
17) $R^{11}OC(O)NR^{10}$-;

R^3 is independently selected from

1) H,
2) halo,
3) -CN,
4) $-NO_2$,
5) unsubstituted or substituted C_1-C_6 alkyl,
6) unsubstituted or substituted C_2-C_8 alkenyl,
7) unsubstituted or substituted C_2-C_8 alkynyl,
8) unsubstituted or substituted aryl,
9) unsubstituted or substituted heterocycle,
10) C_1-C_6 perfluoroalkyl,

11) -OR^{10},
12) OCF_3,
13) -CH_2CF_3,
14) unsubstituted or substituted C_3-C_{10} cycloalkyl,
15) NR^6R^7,
16) -$C(O)R^{10}$,
17) -$OC(O)R^{10}$,
18) -$O(C_1$-C_6 alkyl)OR^{10}-,
19) -$S(O)_mR^{11}$,
20) -$C(O)NR^6R^7$,
21) -$NR^6C(O)R^{10}$,
22) -(C_1-C_6 alkyl)OR^{10}, or
23) -(C_1-C_6 alkyl)$C(O)R^{10}$;

R^4 is independently selected from

1) H,
2) unsubstituted or substituted C_1-C_6 alkyl,
3) unsubstituted or substituted C_2-C_8 alkenyl,
4) unsubstituted or substituted C_2-C_8 alkynyl,
5) unsubstituted or substituted aryl,
6) unsubstituted or substituted heterocycle,
7) unsubstituted or substituted C_3-C_{10} cycloalkyl,
8) unsubstituted or substituted C_1-C_6 perfluoroalkyl,
9) halo,
10) -OR^{10},
11) CN,
12) -$S(O)_mR^{11}$,
13) -$C(O)NR^6R^7$,
14) -$NR^{10}C(O)R^{10}$,
15) NO_2,
16) -$N(R^{10})_2$,
17) -$C(O)R^{10}$,
18) -$OC(O)R^{10}$,
19) $R^{10})_2NC(O)NR^{10}$-, or

20) $R^{10}OC(O)NR^{10}$-;

R^5 is independently selected from

1) H,
2) unsubstituted or substituted C_1-C_6 alkyl,
3) unsubstituted or substituted C_2-C_8 alkenyl,
4) unsubstituted or substituted C_2-C_8 alkynyl,
5) unsubstituted or substituted C_3-C_{10} cycloalkyl,
6) unsubstituted or substituted aryl,
7) unsubstituted or substituted heterocycle,
8) $-S(O)_mR^{11}$,
9) $-C(O)NR^6R^7$,
10) $-S(O)_2NR^6R^7$,
11) $-C(O)R^6$,
12) $-C(O)OR^6$,
13) $-N(R^{10})_2$,
14) CN,
15) halo,
16) $-OR^{10}$,
17) NO_2, or
18) $R^{11}OC(O)NR^{10}$-;

R^6 and R^7 are independently selected from: H, C_1-C_6 alkyl, C_{3-6} cycloalkyl, heterocycle, aryl, aroyl, heteroaroyl, arylsulfonyl, heteroarylsulfonyl, unsubstituted or substituted with:

a) C_{1-6} alkoxy,
b) C_1-C_{20} alkyl
c) aryl or heterocycle,
d) halogen,
e) HO,
f) $-C(O)R^{11}$,
g) $-SO_2R^{11}$, or
h) $N(R^{10})_2$; or

R^6 and R^7 may be joined in a ring;

R^{10} is independently selected from

1) H,
2) unsubstituted or substituted C_1-C_6 alkyl,
3) unsubstituted or substituted perfluoroalkyl,
4) unsubstituted or substituted aralkyl,
5) unsubstituted or substituted aryl, or
6) unsubstituted or substituted heterocycle;

R^{11} is independently selected from

1) unsubstituted or substituted C_1-C_6 alkyl,
2) unsubstituted or substituted aralkyl,
3) unsubstituted or substituted aryl, or
4) unsubstituted or substituted heterocycle;

G is selected from H_2 or O;

V is selected from

1) heterocycle or
2) aryl;

W is a heterocycle;

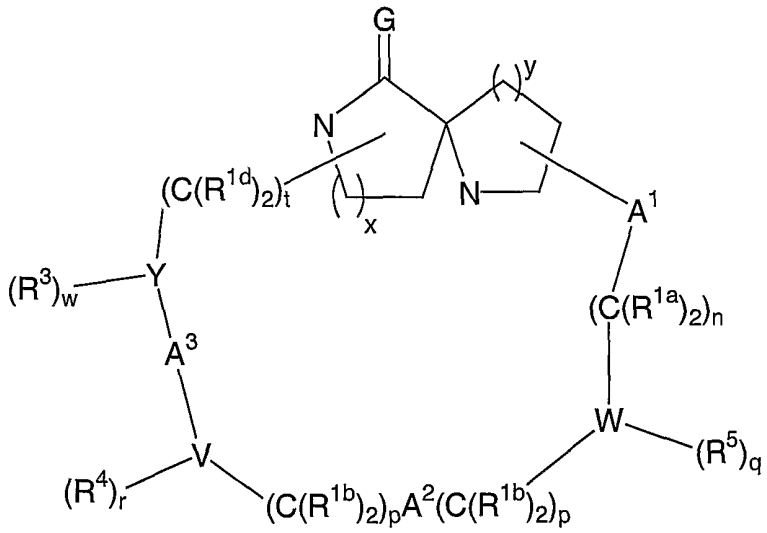
Y is selected from

1) a bond,
2) C_1-C_8 alkyl,
3) C_2-C_8 alkenyl,
4) C_2-C_8 alkynyl,
5) C_3-C_{10} cycloalkyl,
6) aryl, or
7) heterocycle;

m is 0, 1 or 2;

n is 0, 1, 2, 3, 4, 5 or 6;
p is 0, 1, 2, 3, 4, 5 or 6;
q is 0, 1, 2, 3 or 4;
r is 0, 1, 2, 3 or 4;
s is 0, 1, 2, 3, 4, 5 or 6;
t is 0, 1, 2, 3, or 4;
w is 0, 1, 2, 3 or 4, provided Y is not a bond;
x is 0, 1, 2 or 3; and
y is 0, 1, 2 or 3;
or a pharmaceutically acceptable salt or stereoisomer thereof.

2. The compound according to Claim 1 of formula A:



A

wherein

A^1, A^2 and A^3 are independently selected from

1) a bond,
2) O,
3) C(O),
4) $N(R^{10})_2$,
5) $C(O)NR^{10}$,

6) $NR^{10}C(O)$,
7) $NR^{10}C(O)NR^{10}$,
8) $OC(O)$,
9) $C(O)O$
10) $OC(O)NR^{10}$, or
11) $NR^{10}C(O)O$;

R^{1a}, R^{1b} and R^{1d} are independently selected from

1) H,
2) unsubstituted or substituted C_1-C_6 alkyl,
3) unsubstituted or substituted aryl,
4) unsubstituted or substituted C_3-C_{10} cycloalkyl,
5) $-OR^{10}$,
6) $-N(R^{10})_2$,
7) $R^{10}C(O)-$,
8) $-C(O)NR^6R^7$, or
9) $R^{10}C(O)NR^{10}-$;

R^3 is independently selected from

1) H,
2) halo,
3) -CN,
4) $-NO_2$,
5) unsubstituted or substituted C_1-C_6 alkyl,
6) unsubstituted or substituted aryl,
7) $-OR^{10}$,
8) OCF_3,
9) unsubstituted or substituted C_3-C_{10} cycloalkyl,
10) NR^6R^7,
11) $-C(O)R^{10}$,
12) $-C(O)NR^6R^7$,
13) $-NR^6C(O)R^{10}$,
14) $-(C_1$-C_6 alkyl$)OR^{10}$, or
15) $-(C_1$-C_6 alkyl$)C(O)R^{10}$;

R^4 is independently selected from

1) H,
2) unsubstituted or substituted C_1-C_6 alkyl,
3) unsubstituted or substituted C_2-C_8 alkenyl,
4) unsubstituted or substituted C_2-C_8 alkynyl,
5) unsubstituted or substituted aryl,
6) unsubstituted or substituted heterocycle,
7) unsubstituted or substituted C_3-C_{10} cycloalkyl,
8) halo,
9) $-OR^{10}$,
10) CN,
11) $-C(O)NR^6R^7$,
12) $-NR^{10}C(O)R^{10}$, or
13) $-N(R^{10})_2$;

R^5 is independently selected from

1) H,
2) unsubstituted or substituted C_1-C_6 alkyl,
3) unsubstituted or substituted C_3-C_{10} cycloalkyl,
4) unsubstituted or substituted aryl,
5) $-C(O)NR^6R^7$,
6) $-C(O)R^6$,
7) $-C(O)OR^6$,
8) halo, or
9) $-OR^{10}$;

R^6 and R^7 are independently selected from: H, C_1-C_6 alkyl, C_{3-6} cycloalkyl, heterocycle, aryl, aroyl, heteroaroyl, arylsulfonyl, heteroarylsulfonyl, unsubstituted or substituted with:

a) C_{1-6} alkoxy,
b) C_1-C_{20} alkyl
c) aryl or heterocycle,
d) halogen,
e) HO,

f) $-C(O)R^{11}$,
g) $-SO_2R^{11}$, or
h) $N(R^{10})_2$; or

R^6 and R^7 may be joined in a ring;

R^{10} is independently selected from
1) H,
2) unsubstituted or substituted C_1-C_6 alkyl,
3) unsubstituted or substituted perfluoroalkyl,
4) unsubstituted or substituted aralkyl,
5) unsubstituted or substituted aryl, or
6) unsubstituted or substituted heterocycle;

R^{11} is independently selected from
1) unsubstituted or substituted C_1-C_6 alkyl,
2) unsubstituted or substituted aralkyl,
3) unsubstituted or substituted aryl, or
4) unsubstituted or substituted heterocycle;

G is selected from H_2 or O;

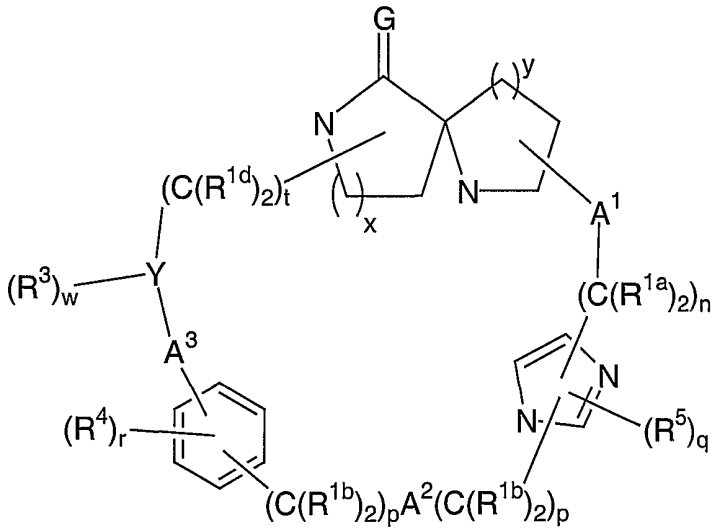
V is selected from
1) heterocycle or
2) aryl;

W is a heterocycle selected from imidazolyl, pyridyl;

Y is selected from
1) a bond,
2) C_3-C_{10} cycloalkyl,
3) aryl, or
4) heterocycle;

m is 0, 1 or 2;
n is 0, 1, 2, 3, 4, 5 or 6;
p is 0, 1, 2, 3, 4, 5 or 6;
q is 0, 1, 2, 3 or 4;
r is 0, 1, 2, 3 or 4;
t is 0, 1, 2, 3, or 4;
w is 0, 1, 2, 3 or 4, provided Y is not a bond;
x is 1 or 2; and
y is 1 or 2;
or a pharmaceutically acceptable salt or stereoisomer thereof.

3. The compound according to Claim 1 of formula B:



B

wherein

A^1 and A^2 are independently selected from

1) a bond,
2) O,
3) C(O),
4) $C(O)NR^{10}$,
5) $NR^{10}C(O)$,

6) OC(O), or
7) C(O)O;

A^3 is selected from
1) a bond,
2) O,
3) C(O),
4) $C(O)NR^{10}$, or
5) $NR^{10}C(O)$;

R^{1a}, R^{1b} and R^{1d} are independently selected from
1) H,
2) unsubstituted or substituted C_1-C_6 alkyl,
3) unsubstituted or substituted aryl,
4) unsubstituted or substituted C_3-C_{10} cycloalkyl,
5) $-OR^{10}$,
6) $-N(R^{10})_2$, or
7) $R^{10}C(O)$-;

R^3 is independently selected from
1) H,
2) halo,
3) -CN,
4) $-NO_2$,
5) unsubstituted or substituted C_1-C_6 alkyl,
6) unsubstituted or substituted aryl,
7) unsubstituted or substituted heterocycle,
8) $-OR^{10}$,
9) OCF_3,
10) unsubstituted or substituted C_3-C_{10} cycloalkyl,
11) NR^6R^7,
12) $-C(O)R^{10}$,
13) $-C(O)NR^6R^7$, or
14) $-NR^6C(O)R^{10}$;

R^4 is independently selected from

1) H,
2) unsubstituted or substituted C_1-C_6 alkyl,
3) unsubstituted or substituted C_2-C_8 alkenyl,
4) unsubstituted or substituted C_2-C_8 alkynyl,
5) unsubstituted or substituted aryl,
6) unsubstituted or substituted heterocycle,
7) unsubstituted or substituted C_3-C_{10} cycloalkyl,
8) halo,
9) $-OR^{10}$,
10) CN, or
11) $-N(R^{10})_2$;

R^5 is independently selected from

1) H,
2) unsubstituted or substituted C_1-C_6 alkyl,
3) unsubstituted or substituted C_3-C_{10} cycloalkyl,
4) unsubstituted or substituted aryl,
5) $-C(O)R^6$,
6) $-C(O)OR^6$,
7) halo, or
8) $-OR^{10}$;

R^6 and R^7 are independently selected from: H, C_1-C_6 alkyl, C_{3-6} cycloalkyl, heterocycle, aryl, aroyl, heteroaroyl, arylsulfonyl, heteroarylsulfonyl, unsubstituted or substituted with:

a) C_{1-6} alkoxy,
b) C_1-C_{20} alkyl
c) aryl or heterocycle,
d) halogen,
e) HO,
f) $-C(O)R^{11}$,
g) $-SO_2R^{11}$, or
h) $N(R^{10})_2$; or

R^6 and R^7 may be joined in a ring;

R^{10} is independently selected from

1) H,
2) unsubstituted or substituted C_1-C_6 alkyl,
3) unsubstituted or substituted perfluoroalkyl,
4) unsubstituted or substituted aralkyl,
5) unsubstituted or substituted aryl, or
6) unsubstituted or substituted heterocycle;

R^{11} is independently selected from

1) unsubstituted or substituted C_1-C_6 alkyl,
2) unsubstituted or substituted aralkyl,
3) unsubstituted or substituted aryl, or
4) unsubstituted or substituted heterocycle;

G is selected from H_2 or O;

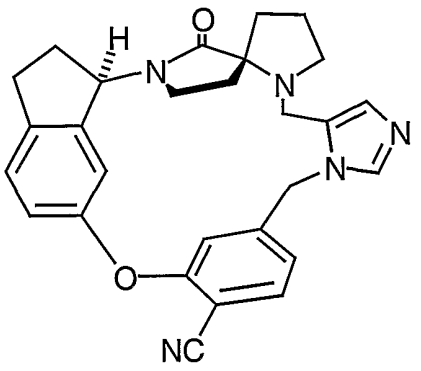
Y is selected from

1) a bond,
2) C_3-C_{10} cycloalkyl,
3) aryl, or
4) heterocycle;

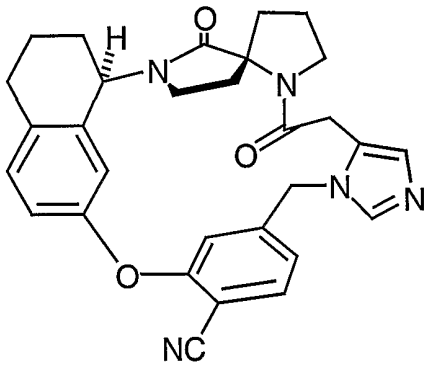
m is 0, 1 or 2;
n is 0, 1, 2, 3, 4, 5 or 6;
p is 0, 1, 2, 3, 4, 5 or 6;
q is 0, 1, 2, 3 or 4;
r is 0, 1, 2, 3 or 4;
t is 0, 1, 2, 3, or 4;
w is 0, 1, 2, 3 or 4, provided Y is not a bond;
x is 1 or 2; and
y is 1 or 2;

or a pharmaceutically acceptable salt or stereoisomer thereof.

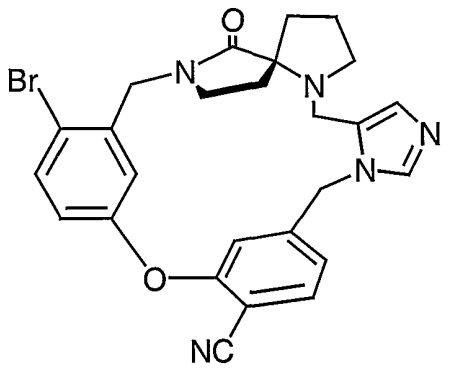
4. A compound which is selected from:



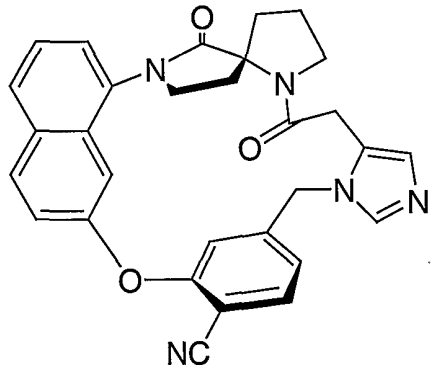
(17*R*, 20*R*)-19,20,21,22-tetrahydro-19-oxo-17*H*-15,17:18,20-diethano-6,10:12,16-dimetheno-20,21-propano-16*H*-imidazo[3,4-*h*][1,8,11,14]oxatriazacycloeicosine-9-carbonitrile;



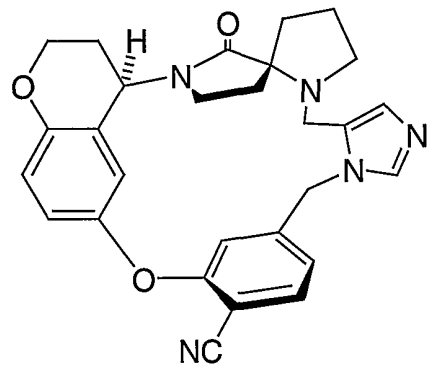
(*R*,*R*)-15,16,17,17a,19,20,22,23-octahydro-19,22-dioxo-5*H*,21*H*-18,20-ethano-12,14-etheno-6,10-metheno-20,21-propanobenz[*d*]imidazo[4,3-*l*] [1,6,9,13] oxatriazacyclononadecosine-9-carbonitrile;



(*R*)-15-bromo-19,20,21,22-tetrahydro-19-oxo-17*H*-18,20-ethano-6,10:12,16-dimetheno-20,21-propano-16*H*-imidazo[3,4-*h*][1,8,11,14]oxatriazacycloeicosine-9-carbonitrile;



(*R*)-19,20,22,23-tetrahydro-19,22-dioxo-5*H*,21*H*-18,20-ethano-12,14-etheno-6,10-metheno-20,21-propanobenz[*d*]imidazo[4,3-*l*][1,6,9,13]oxatriazacyclononadecosine-9-carbonitrile;



(*R*,*R*)-15,16,17,17a,19,20,21,22-octahydro-15-oxa-19-oxo-5*H*-18,20-ethano-12,14-etheno-6,10-metheno-20,21-propano-18*H*-benz[*d*]imidazo[4,3-*k*][1,6,9,12]oxatriazacyclooctadecosine-9-carbonitrile;

or a pharmaceutically acceptable salt, optical isomer or stereoisomer thereof.

5. A pharmaceutical composition comprising a pharmaceutical carrier, and dispersed therein, a therapeutically effective amount of a compound of Claim 1.

6. A pharmaceutical composition comprising a pharmaceutical carrier, and dispersed therein, a therapeutically effective amount of a compound of Claim 2.

7. A pharmaceutical composition comprising a pharmaceutical carrier, and dispersed therein, a therapeutically effective amount of a compound of Claim 4.

8. A method for inhibiting prenyl-protein transferase which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 1.

9. A method for inhibiting prenyl-protein transferase which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 2.

10. A method for inhibiting prenyl-protein transferase which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 4.

11. A method for treating cancer which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 1.

12. A method for treating cancer which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 2.

13. A method for treating cancer which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 4.

14. A method for treating neurofibromin benign proliferative disorder which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 1.

15. A method for treating blindness related to retinal vascularization which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 1.

16. A method for treating infections from hepatitis delta and related viruses which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 1.

17. A method for preventing restenosis which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 1.

18. A method for treating polycystic kidney disease which comprises administering to a mammal in need thereof a therapeutically effective amount of a compound of Claim 1.

19. A method of conferring radiation sensitivity on a tumor cell using a therapeutically effective amount of a compound of Claim 1 in combination with radiation therapy.

20. A method of using a therapeutically effective amount of a compound of Claim 1 in combination with an antineoplastic to treat cancer.

21. A method according to Claim 20 wherein the antineoplastic is paclitaxel.

22. A pharmaceutical composition made by combining the compound of Claim 1 and a pharmaceutically acceptable carrier.

23. A process for making a pharmaceutical composition comprising combining a compound of Claim 1 and a pharmaceutically acceptable carrier.

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