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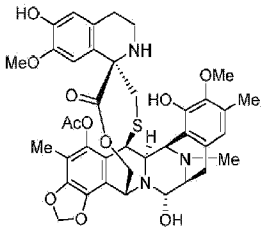
DESCRIPTION

FIELD OF THE INVENTION

[0001] The present invention relates to synthetic analogues of the ecteinascidins, particularly of ecteinascidin 736 (ET-736), pharmaceutical compositions containing them, methods for their manufacture and their use as antitumoral agents.

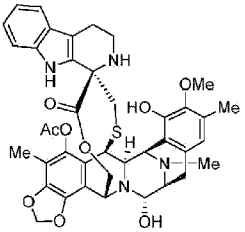
BACKGROUND OF THE INVENTION

[0002] The ecteinascidins are exceedingly potent antitumor agents isolated from the marine tunicate *Ecteinascidia turbinata.* One of these compounds, ET-743 of formula:



is being employed as an anticancer medicament, under the international nonproprietary name (INN) trabectedin, for the treatment of patients with advanced and metastatic soft tissue sarcoma (STS) after failure of anthracyclines and ifosfamide, or who are unsuited to receive such agents, and for the treatment of relapsed platinum-sensitive ovarian cancer in combination with pegylated liposomal doxorubicin.

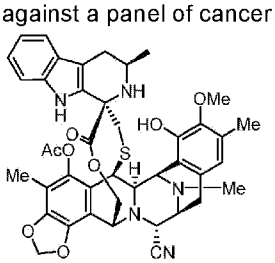
[0003] Ecteinascidin 736 (ET-736) was first discovered by Rinehart and features a tetrahydro-β-carboline unit in place of the tetrahydroisoquinoline unit more usually found in the ecteinascidin compounds isolated from natural sources; See for example Sakai et al., Proc. Natl. Acad. Sci. USA 1992, vol. 89, 11456-11460.

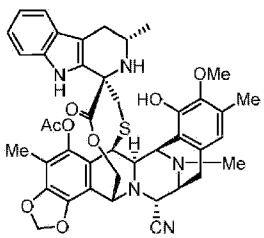


ET-736

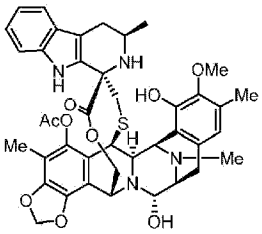
[0004] U.S. Patent No. 5,149,804 describes Ecteinascidin 736 (ET-736), isolated from the Caribbean tunicate *Ecteinascidia turbinata,* and it structure. ET-736 protects mice *in vivo* at very low concentrations against P388 lymphoma, B16 melanoma, and Lewis lung carcinoma.

[0005] WO03014127 describes several synthetic analogues of ET-736 and their cytotoxic activity against tumoral cells. In particular, WO03014127 describes compounds **A** to **D** together with their cytotoxic activity against a panel of cancer cell lines.

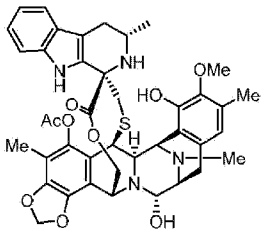




A



B

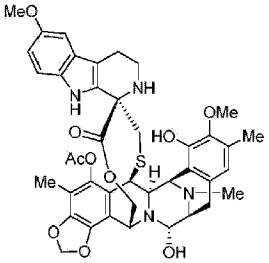


C

D

[0006] WO2011/147828 describes synthetic processes for the manufacture of ecteinascidin compounds.

[0007] Another compound described in this patent application, **PM01183**, is currently in clinical trials for the treatment of cancer. **PM01183** has the following chemical structure:

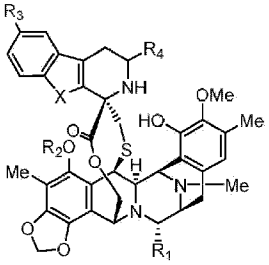


[0008] PM01183 has demonstrated a highly potent *in vitro* activity against solid and non-solid tumour cell lines as well as a significant *in vivo* activity in several xenografted human tumor cell lines in mice, such as those for breast, kidney and ovarian cancer. **PM01183** exerts its anticancer effects through the covalent modification of guanines in the DNA minor groove that eventually give rise to DNA double-strand break, *S*-phase arrest and apoptosis in cancer cells.

[0009] Despite the positive results obtained in clinical applications in chemotherapy, the search in the field of ecteinascidin compounds is still open to the identification of new compounds with optimal features of activity, selectivity toward the tumour, with a reduced systemic toxicity and/or improved pharmacokinetic properties.

SUMMARY OF THE INVENTION

[0010] In a first aspect of the present invention there is provided a compound of formula **IE** or a pharmaceutically acceptable salt or ester thereof:



IE

wherein:

X is -NH- or -O-;

R_1 is -OH or -CN;

R_2 is a -C(=O)R^a group;

R_3 is hydrogen or a -OR^b group;

R_4 is selected from -CH_2NH_2 and -$CH_2NHProt^{NH}$;

R^a is selected from hydrogen, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl;

R^b is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl; and

$Prot^{NH}$ is a protecting group for amino.

[0011] In a further aspect of the present invention, there is provided a pharmaceutical composition comprising a compound according to the present invention and a pharmaceutically acceptable carrier.

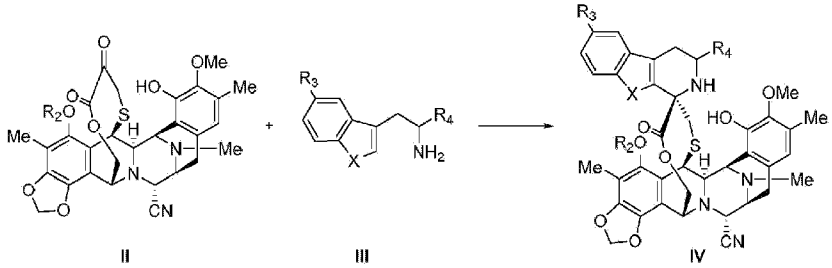
[0012] In a yet further aspect of the present invention, there is provided a dosage form comprising a pharmaceutical composition according to the present invention.

[0013] In a yet further aspect of the present invention, there is provided a compound, pharmaceutical composition or dosage form according to the present invention for use as a medicament.

[0014] In a yet further aspect of the present invention, there is provided a compound, pharmaceutical composition or dosage form according to the present invention for use in the treatment of cancer.

[0015] In a yet further aspect of the present invention, there is provided a kit comprising a therapeutically effective amount of a compound according to the present invention and a pharmaceutically acceptable carrier. The kit is for use in the treatment of cancer.

[0016] In a yet further aspect of the present invention, there is provided a process for obtaining compounds of formula **IE** or a pharmaceutically acceptable salt or ester thereof; comprising the step of reacting a compound of formula **II** with a compound of formula **III** to give a compound of formula **IV:**



II III IV

wherein (insofar as allowed by possible substituent groups):

X is -NH- or -O-;

R_2 is a -C(=O)R^a group;

R_3 is hydrogen or a -OR^b group;

R_4 is $-CH_2NHProt^{NH}$;

R^a is selected from hydrogen, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl;

R^b is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl; and $Prot^{NH}$ is a protecting group for amino.

[0017] The process may include the further step of replacing the cyano group in the compound of formula IV with a hydroxy group to give a compound of formula IE where R_1 is OH.

BRIEF DESCRIPTION OF THE FIGURES

[0018]

Figure 1. Tumor total diameter evaluation of HT1080 tumors in mice treated with placebo, compound **C, 4-S,** and **12-S.**

Figure 2. Tumor volume evaluation of MDA-MB-231 tumors in mice treated with placebo, compound **C, 4-S,** and **12-S.**

Figure 3. Tumor volume evaluation of H460 tumors in mice treated with placebo, compound **C, 4-S,** and **12-S.**

Figure 4. Tumor volume evaluation of H526 tumors in mice treated with placebo, compound **C, 4-S,** and **12-S.**

Figure 5. Tumor volume evaluation of H82 tumors in mice treated with placebo, compound **C, 4-S,** and **12-S.**

Figure 6. Tumor volume evaluation of A2780 tumors in mice treated with placebo, compound **C, 4-S,** and **12-S.**

Figure 7. Tumor volume evaluation of HGC-27 tumors in mice treated with placebo, compound **C, 4-S,** and **12-S.**

Figure 8. Tumor total diameter evaluation of HT1080 tumors in mice treated with placebo, **PM01183** and **4-R.**

Figure 9. Tumor total diameter evaluation of HT1080 tumors in mice treated with placebo, **PM01183** and Compound **D.**

Figure 10. Tumor volume evaluation of MDA-MB-231 tumors in mice treated with placebo, **PM01183** and **4-R.**

Figure 11. Tumor volume evaluation of MDA-MB-231 tumors in mice treated with placebo, **PM01183** and Compound **D.**

Figure 12. Tumor volume evaluation of H460 tumors in mice treated with placebo, **PM01183** and **4-R.**

Figure 13. Tumor volume evaluation of H460 tumors in mice treated with placebo, **PM01183** and Compound **D.**

Figure 14. Tumor volume evaluation of A2780 tumors in mice treated with placebo, **PM01183** and **4-R.**

Figure 15. Tumor volume evaluation of A2780 tumors in mice treated with placebo, **PM01183** and Compound **D.**

Figure 16. Tumor volume evaluation of HGC-27 tumors in mice treated with placebo, **PM01183** and **4-R.**

Figure 17. Tumor volume evaluation of HGC-27 tumors in mice treated with placebo, **PM01183** and Compound **D.**

Figure 18. Tumor total diameter evaluation of HT1080 tumors in mice treated with placebo, compound **D** and **12-R.**

Figure 19. Tumor volume evaluation of MDA-MB-231 tumors in mice treated with placebo, compound **D** and **12-R.**

Figure 20. Tumor volume evaluation of H460 tumors in mice treated with placebo, compound **D** and **12-R.**

Figure 21. Tumor volume evaluation of H526 tumors in mice treated with placebo, compound **D** and **12-R.**

Figure 22. Tumor volume evaluation of H82 tumors in mice treated with placebo, compound **D** and **12-R.**

Figure 23. Tumor volume evaluation of A2780 tumors in mice treated with placebo, compound **D** and **12-R.**

Figure 24. Tumor volume evaluation of HGC-27 tumors in mice treated with placebo, compound **D** and **12-R.**

Figure 25. Tumor total diameter evaluation of HT1080 tumors in mice treated with placebo, **PM01183** and **19-S.**

Figure 26. Tumor volume evaluation of MDA-MB-231 tumors in mice treated with placebo, **PM01183** and **19-S.**

Figure 27. Tumor volume evaluation of H460 tumors in mice trated with placebo, **PM01183** and **19-S.**

Figure 28. Tumor volume evaluation of A2780 tumors in mice treated with placebo, **PM01183** and **19-S.**

Figure 29. Tumor volume evaluation of HGC27 tumors in mice treated with placebo, **PM01183** and **19-S.**

Figure 30. Tumor total diameter evaluation of HT1080 tumors in mice treated with placebo, **PM01183** and **19-R.**

Figure 31. Tumor volume evaluation of MDA-MB-231 tumors in mice treated with placebo, **PM01183** and **19-R.**

Figure 32. Tumor volume evaluation of H460 tumors in mice treated with placebo, **PM01183** and **19-R.**

Figure 33. Tumor volume evaluation of A2780 tumors in mice treated with placebo, **PM01183** and **19-R.**

Figure 34. Tumor volume evaluation of HGC-27 tumors in mice treated with placebo, **PM01183** and **19-R.**

Figure 35. Tumor total diameter evaluation of HT1080 tumors in mice treated with placebo, compound **C** and **39-S.**

Figure 36. Tumor volume evaluation of MDA-MB-231 tumors in mice treated with placebo, compound **C** and **39-S.**

Figure 37. Tumor volume evaluation of H460 tumors in mice treated with placebo, compound **C** and **39-S.**

Figure 38. Tumor volume evaluation of H526 tumors in mice treated with placebo, compound **C** and **39-S.**

Figure 39. Tumor volume evaluation of H82 tumors in mice treated with placebo, compound **C** and **39-S.**

Figure 40. Tumor volume evaluation of A2780 tumors in mice treated with placebo, compound **C** and **39-S.**

Figure 41. Tumor volume evaluation of HGC27 tumors in mice treated with placebo, compound **C** and **39-S.**

Figure 42. Tumor total diameter evaluation of HT1080 tumors in mice treated with placebo, compound **D** and **47-R.**

Figure 43. Tumor volume evaluation of MDA-MB-231 tumors in mice treated with placebo, compound **D** and **47-R.**

Figure 44. Tumor volume evaluation of H460 tumors in mice treated with placebo, compound **D** and **47-R.**

Figure 45. Tumor volume evaluation of H526 tumors in mice treated with placebo, compound **D** and **47-R.**

Figure 46. Tumor volume evaluation of H82 tumors in mice treated with placebo, compound **D** and **47-R.**

Figure 47. Tumor volume evaluation of A2780 tumors in mice treated with placebo, compound **D** and **47-R.**

Figure 48. Tumor volume evaluation of HGC27 tumors in mice treated with placebo, compound **D** and **47-R.**

Figure 49. Tumor total diameter evaluation of HT1080 tumors in mice treated with placebo, **ET-736** and **32.**

Figure 50. Tumor volume evaluation of MDA-MB-231 tumors in mice treated with placebo, **ET-736** and **32.**

Figure 51. Tumor volume evaluation of H460 tumors in mice treated with placebo, **ET-736** and **32.**

Figure 52. Tumor volume evaluation of H526 tumors in mice treated with placebo, **ET-736** and **32.**

Figure 53. Tumor volume evaluation of H82 tumors in mice treated with placebo, **ET-736** and **32.**

Figure 54. Tumor volume evaluation of A2780 tumors in mice treated with placebo, **ET-736** and **32.**

Figure 55. Tumor volume evaluation of HGC27 tumors in mice treated with placebo, **ET-736** and **32.**

Figure 56. Tumor total diameter evaluation of HT1080 tumors in mice treated with placebo, **PM01183** and **35.**

Figure 57. Tumor volume evaluation of MDA-MB-231 tumors in mice treated with placebo, **PM01183** and **35.**

Figure 58. Tumor volume evaluation of H460 tumors in mice treated with placebo, **PM01183** and **35.**

Figure 59. Tumor volume evaluation of A2780 tumors in mice treated with placebo, **PM01183** and **35.**

Figure 60. Tumor volume evaluation of HGC27 tumors in mice treated with placebo, **PM01183** and **35.**

Figure 61. Tumor volume evaluation of PC-3 tumors in mice treated with placebo, **12-S** and **12-R.**

Figure 62. Tumor volume evaluation of PC-3 tumors in mice treated with placebo and **4-S.**

Figure 63. Tumor volume evaluation of DU-145 tumors in mice treated with placebo and **4-S.**

Figure 64. Tumor volume evaluation of 22Rv1 tumors in mice treated with placebo and **4-S.**

Figure 65. Tumor volume evaluation of PC-3 tumors in mice treated with placebo and **39-S.**

Figure 66. Tumor volume evaluation of DU-145 tumors in mice treated with placebo and **39-S.**

Figure 67. Tumor volume evaluation of 22Rv1 tumors in mice treated with placebo and **39-S.**

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[0019] The following apply to all aspects of the present invention:
In the compounds of the present invention, the alkyl groups may be branched or unbranched, and preferably have from 1 to about 12 carbon atoms. One more preferred class of alkyl groups has from 1 to about 6 carbon atoms. Even more preferred are alkyl groups having 1, 2, 3 or 4 carbon atoms. Methyl, ethyl, *n*-propyl, isopropyl and butyl, including *n*-butyl, isobutyl, *sec*-butyl and *tert*-butyl are particularly preferred alkyl groups in the compounds of the present invention.

[0020] In the compounds of the present invention, the alkenyl groups may be branched or unbranched, have one or more double bonds and from 2 to about 12 carbon atoms. One more preferred class of alkenyl groups

has from 2 to about 6 carbon atoms. Even more preferred are alkenyl groups having 2, 3 or 4 carbon atoms. Ethenyl, 1-propenyl, 2-propenyl, 1-methylethenyl, 1-butenyl, 2-butenyl, and 3-butenyl are particularly preferred alkenyl groups in the compounds of the present invention.

[0021] In the compounds of the present invention, the alkynyl groups may be branched or unbranched, have one or more triple bonds and from 2 to about 12 carbon atoms. One more preferred class of alkynyl groups has from 2 to about 6 carbon atoms. Even more preferred are alkynyl groups having 2, 3 or 4 carbon atoms.

[0022] Suitable aryl groups in the compounds of the present invention include single and multiple ring compounds, including multiple ring compounds that contain separate and/or fused aryl groups. Typical aryl groups contain from 1 to 3 separated and/or fused rings and from 6 to about 18 carbon ring atoms. Preferably aryl groups contain from 6 to about 10 carbon ring atoms. Specially preferred aryl groups included substituted or unsubstituted phenyl, substituted or unsubstituted naphthyl, substituted or unsubstituted biphenyl, substituted or unsubstituted phenanthryl and substituted or unsubstituted anthryl.

[0023] Suitable heterocyclic groups include heteroaromatic and heteroalicyclic groups containing from 1 to 3 separated and/or fused rings and from 5 to about 18 ring atoms. Preferably heteroaromatic and heteroalicyclic groups contain from 5 to about 10 ring atoms, most preferably 5, 6, or 7 ring atoms. Suitable heteroaromatic groups in the compounds of the present invention contain one, two or three heteroatoms selected from N, O or S atoms and include, e.g., coumarinyl including 8-coumarinyl, quinolyl including 8-quinolyl, isoquinolyl, pyridyl, pyrazinyl, pyrazolyl, pyrimidinyl, furyl, pyrrolyl, thienyl, thiazolyl, isothiazolyl, triazolyl, tetrazolyl, isoxazolyl, oxazolyl, imidazolyl, indolyl, isoindolyl, indazolyl, indolizinyl, phthalazinyl, pteridyl, purinyl, oxadiazolyl, thiadiazolyl, furazanyl, pyridazinyl, triazinyl, cinnolinyl, benzimidazolyl, benzofuranyl, benzofurazanyl, benzothiophenyl, benzothiazolyl, benzoxazolyl, quinazolinyl, quinoxalinyl, naphthyridinyl and furopyridyl. Suitable heteroalicyclic groups in the compounds of the present invention contain one, two or three heteroatoms selected from N, O or S and include, e.g., pyrrolidinyl, tetrahydrofuranyl, tetrahydrothienyl, tetrahydrothiopyranyl, piperidyl, morpholinyl, thiomorpholinyl, thioxanyl, piperazinyl, azetidinyl, oxetanyl, thietanyl, homopiperidyl, oxepanyl, thiepanyl, oxazepinyl, diazepinyl, thiazepinyl, 1,2,3,6-tetrahydropyridyl, 2-pirrolinyl, 3-pyrrolinyl, indolinyl, 2*H*-pyranyl, 4*H*-pyranyl, dioxanyl, 1,3-dioxolanyl, pyrazolinyl, dithianyl, dithiolanyl, dihydropyranyl, dihydrothienyl, dihydrofuranyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, 3-azabicyclo[3.1.0]hexyl, 3-azabicyclo[4.1.0]heptyl, 3*H*-indolyl, and quinolizinyl.

[0024] The groups above mentioned may be substituted at one or more available positions by one or more suitable groups such as OR', =O, SR', SOR', SO_2R', NO_2, NHR', NR'R', =N-R', NHCOR', $N(COR')_2$, $NHSO_2R'$, NR'C(=NR')NR'R', CN, halogen, COR', COOR', OCOR', OCONHR', OCONR'R', CONHR', CONR'R', protected OH, protected amino, protected SH, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, substituted or unsubstituted C_2-C_{12} alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group, where each of the R' groups is independently selected from the group consisting of hydrogen, OH, NO_2, NH_2, SH, CN, halogen, COH, COalkyl, CO_2H, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, substituted or unsubstituted C_2-C_{12} alkynyl, substituted or unsubstituted aryl, and substituted or unsubstituted heterocyclic group. Where such groups are themselves substituted, the substituents may be chosen from the foregoing list. In addition, where there are more than one R' groups on a substituent, each R' may be the same or different.

[0025] In the compounds for the present invention, the halogen substituents include F, Cl, Br, and I.

[0026] The terms "pharmaceutically acceptable salt" and "ester" refers to any pharmaceutically acceptable salt or ester which, upon administration to the patient is capable of providing (directly or indirectly) a compound as described herein. However, it will be appreciated that non-pharmaceutically acceptable salts also are disclosed since those may be useful in the preparation of pharmaceutically acceptable salts. The preparation of salts can be carried out by methods known in the art.

[0027] For instance, pharmaceutically acceptable salts of the compounds provided herein are synthesized from the parent compounds, which contain a basic or acidic moiety, by conventional chemical methods. Generally, such salts are, for example, prepared by reacting the free acid or base of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent or in a mixture of both. Generally, nonaqueous media like ether, ethyl acetate, ethanol, 2-propanol or acetonitrile are preferred. Examples of the acid addition salts include mineral acid addition salts such as, for example, hydrochloride, hydrobromide, hydroiodide, sulfate, nitrate, phosphate, and organic acid addition salts such as, for example, acetate, trifluoroacetate, maleate, fumarate, citrate, oxalate, succinate, tartrate, malate, mandelate, methanesulfonate and *p*-toluenesulfonate. Examples of the alkali addition salts include inorganic salts such as, for example, sodium, potassium, calcium and ammonium 11743329-1 salts, and organic alkali salts such as, for example, ethylenediamine, ethanolamine, *N,N*-dialkylenethanolamine, triethanolamine and basic aminoacids salts.

[0028] The compounds of the invention may be in crystalline or amorphous form either as free compounds or as solvates (e.g. hydrates) and it is intended that all forms are within the scope of the present invention. Methods of solvation are generally known within the art.

[0029] Stereoisomerism about the asymmetric carbons with unspecified stereochemistry is possible, therefore in such cases the asymmetric carbons can have (R) or (S) configuration. All diastereomers generated by a specific configuration of such asymmetric carbons in conjunction with the other asymmetric carbons present in the molecule, and mixtures thereof, are considered within the scope of the present invention. Stereoisomerism about the double bond (geometric isomerism) is also possible, therefore in some cases the molecule could exist as (*E*)-isomer or (*Z*)-isomer. If the molecule contains several double bonds, each double bond will have its own stereoisomerism, that could be the same or different than the stereoisomerism of the other double bonds of the molecule. Furthermore, compounds referred to herein may exist as atropoisomers. The single stereoisomers including diastereoisomers, geometric isomers and atropoisomers of the compounds referred to herein, and mixtures thereof fall within the scope of the present invention.

[0030] In addition, compounds referred to herein may exist in isotopically-labelled forms. All pharmaceutically acceptable salts, esters and isotopically labelled forms of the compounds referred to herein, and mixtures thereof, are considered within the scope of the present invention.

[0031] Protected forms of the compounds disclosed herein are considered within the scope of the present invention. Suitable protecting groups are well known for the skilled person in the art. A general review of protecting groups in organic chemistry is provided by Wuts, PGM and Greene TW in Protecting Groups in Organic Synthesis, 4th Ed. Wiley-Interscience, and by Kocienski PJ in Protecting Groups, 3rd Ed. Georg Thieme Verlag. These references provide sections on protecting groups for OH, amino and SH groups.

[0032] Within the scope of the present invention an OH protecting group is defined to be the O-bonded moiety resulting from the protection of the OH through the formation of a suitable protected OH group. Examples of such protected OH groups include ethers, silyl ethers, esters, sulfonates, sulfenates and sulfinates, carbonates, and carbamates. In the case of ethers the protecting group for the OH can be selected from methyl, methoxymethyl, methylthiomethyl, (phenyldimethylsilyl)methoxymethyl, benzyloxymethyl, *p*-methoxybenzyloxymethyl, [(3,4-dimethoxybenzyl)oxy]methyl, *p*-nitrobenzyloxymethyl, *o*-nitrobenzyloxymethyl, [(*R*)-1-(2-nitrophenyl)ethoxy]methyl, (4-methoxyphenoxy)methyl, guaiacolmethyl, [(*p*-phenylphenyl)oxy]methyl, *t*-butoxymethyl, 4-pentenyloxymethyl, siloxymethyl, 2-methoxyethoxymethyl, 2-cyanoethoxymethyl, bis(2-chloroethoxy)methyl, 2,2,2-trichloroethoxymethyl, 2-(trimethylsilyl)ethoxymethyl, menthoxymethyl, *O*-bis(2-acetoxy-ethoxy)methyl, tetrahydropyranyl, fluorous tetrahydropyranyl, 3-bromotetrahydropyranyl, tetrahydrothiopyranyl, 1-methoxycyclohexyl, 4-methoxytetrahydropyranyl, 4-methoxytetrahydrothiopyranyl, 4-methoxytetrahydrothiopyranyl *S,S*-dioxide, 1-[(2-chloro-4-methyl)-phenyl]-4-methoxypiperidin-4-yl, 1-(2-fluorophenyl)-4-methoxypiperidin-4-yl, 1-(4-chlorophenyl)-4-methoxypiperidin-4-yl, 1,4-dioxan-2-yl, tetrahydrofuranyl, tetrahydrothiofuranyl, 2,3,3*a*,4,5,6,7,7*a*-octahydro-7,8,8-trimethyl-4,7-methanobenzofuran-2-yl, 1-ethoxyethyl, 1-(2-chloroethoxy)ethyl, 2-hydroxyethyl, 2-bromoethyl, 1-[2-(trimethylsilyl)ethoxy]ethyl, 1-

methyl-1-methoxyethyl, 1-methyl-1-benzyloxyethyl, 1-methyl-1-benzyloxy-2-fluoroethyl, 1-methyl-1-phenoxyethyl, 2,2,2-trichloroethyl, 1,1-dianisyl-2,2,2-trichloroethyl, 1,1,1,3,3,3-hexafluoro-2-phenylisopropyl, 1-(2-cyanoethoxy)ethyl, 2-trimethylsilylethyl, 2-(benzylthio)ethyl, 2-(phenylselenyl)ethyl, *t*-butyl, cyclohexyl, 1-methyl-1'-cyclopropylmethyl, allyl, prenyl, cinnamyl, 2-phenallyl, propargyl, *p*-chlorophenyl, *p*-methoxyphenyl, *p*-nitrophenyl, 2,4-dinitrophenyl, 2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenyl, benzyl, *p*-methoxybenzyl, 3,4-dimethoxybenzyl, 2,6-dimethoxybenzyl, *o*-nitrobenzyl, *p*-nitrobenzyl, pentadienylnitrobenzyl, pentadienylnitropiperonyl, halobenzyl, 2,6-dichlorobenzyl, 2,4-dichlorobenzyl, 2,6-difluorobenzyl, *p*-cyanobenzyl, fluorous benzyl, 4-fluorousalkoxybenzyl, trimethylsilylxylyl, *p*-phenylbenzyl, 2-phenyl-2-propyl, *p*-acylaminobenzyl, *p*-azidobenzyl, 4-azido-3-chlorobenzyl, 2-trifluoromethylbenzyl, 4-trifluoromethylbenzyl, *p*-(methylsulfinyl)benzyl, *p*-siletanylbenzyl, 4-acetoxybenzyl, 4-(2-trimethylsilyl)ethoxymethoxybenzyl, 2-naphthylmethyl, 2-picolyl, 4-picolyl, 3-methyl-2-picolyl *N*-oxide, 2-quinolinylmethyl, 6-methoxy-2-(4-methylphenyl)-4-quinolinemethyl, 1-pyrenylmethyl, diphenylmethyl, 4-methoxydiphenylmethyl, 4-phenyldiphenylmethyl, *p,p'*-dinitrobenzhydryl, 5-dibenzosuberyl, triphenylmethyl, tris(4-*t*-butylphenyl)methyl, α-naphthyldiphenylmethyl, *p*-methoxyphenyldiphenylmethyl, di(*p*-methoxyphenyl)phenylmethyl, tri(*p*-methoxyphenyl)methyl, 4-(4'-bromophenacyloxy)phenyldiphenylmethyl, 4,4',4"-tris(4,5-dichlorophthalimidophenyl)methyl, 4,4',4"-tris(levulinoyloxyphenyl)methyl, 4,4',4"-tris(benzoyloxyphenyl)methyl, 4,4'-dimethoxy-3"-[*N*-(imidazolylmethyl)]trityl, 4,4'-dimethoxy-3"-[*N*-(imidazolylethyl)carbamoyl]trityl, bis(4-methoxyphenyl)-1'-pyrenylmethyl, 4-(17-tetrabenzo [*a,c,g,i*]fluorenylmethyl)-4,4" -dimethoxytrityl, 9-anthryl, 9-(9-phenyl)xanthenyl, 9-phenylthioxanthyl, 9-(9-phenyl-10-oxo)anthryl, 1,3-benzodithiolan-2-yl, 4,5-bis(ethoxycarbonyl)-[1,3]-dioxolan-2-yl, benzisothiazolyl *S,S*-dioxide. In the case of silyl ethers the protecting group for the OH can be selected from trimethylsilyl, triethylsilyl, triisopropylsilyl, dimethylisopropylsilyl, diethylisopropylsilyl, dimethylhexylsilyl, 2-norbornyldimethylsilyl, *t*-butyldimethylsilyl, *t*-butyldiphenylsilyl, tribenzylsilyl, tri-*p*-xylylsilyl, triphenylsilyl, diphenylmethylsilyl, di-t-butylmethylsilyl, bis(*t*-butyl)-1-pyrenylmethoxysilyl, tris(trimethylsilyl)silyl, (2-hydroxystyryl)dimethylsilyl, (2-hydroxystyryl)diisopropylsilyl, *t*-butylmethoxyphenylsilyl, *t*-butoxydiphenylsilyl, 1,1,3,3-tetraisopropyl-3-[2-(triphenylmethoxy) ethoxy]disiloxane-1-yl, and fluorous silyl. In the case of esters the protecting group for the OH together with the oxygen atom of the unprotected OH to which it is attached form an ester that can be selected from formate, benzoylformate, acetate, chloroacetate, dichloroacetate, trichloroacetate, trichloroacetamidate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, phenoxyacetate, *p*-chlorophenoxyacetate, phenylacetate, diphenylacetate, 3-phenylpropionate, bisfluorous chain type propanoyl, 4-pentenoate, 4-oxopentanoate, 4,4-(ethylenedithio)pentanoate, 5 [3-bis(4-methoxyphenyl)hydro-xymethylphenoxy]levulinate, pivaloate, 1-adamantoate, crotonate, 4-methoxycrotonate, benzoate, *p*-phenylbenzoate, 2,4,6-trimethylbenzoate, 4-bromobenzoate, 2,5-difluorobenzoate, *p*-nitrobenzoate, picolinate, nicotinate, 2-(azidomethyl)benzoate, 4-azidobutyrate, (2-azidomethyl)phenylacetate, 2-{[(tritylthio)oxy]methyl}benzoate, 2-{[(4-methoxytritylthio)oxy]methyl}benzoate, 2-{[methyl(tritylthio)amino]methyl}benzoate, 2-{ {[(4-methoxytrityl)thio]methylamino}methyl}benzoate, 2-(allyloxy)phenylacetate, 2-(prenyloxymethyl)benzoate, 6-(levulinyloxymethyl)-3-methoxy-2-nitrobenzoate, 6-(levulinyloxymethyl)-3-methoxy-4-nitrobenzoate, 4-benzyloxybutyrate, 4-trialkylsilyloxy-butyrate, 4-acetoxy-2,2-dimethylbutyrate, 2,2-dimethyl-4-pentenoate, 2-iodobenzoate, 4-nitro-4-methylpentanoate, *o*-(dibromomethyl)benzoate, 2-formylbenzenesulfonate, 4-(methylthio-methoxy)butyrate, 2-(methylthiomethoxymethyl)benzoate, 2-(chloroacetoxymethyl)benzoate, 2-[(2-chloroacetoxy)ethyl]benzoate, 2-[2-(benzyloxy)ethyl]benzoate, 2-[2-(4-methoxybenzyl-oxy)ethyl]benzoate, 2,6-dichloro-4-methylphenoxyacetate, 2,6-dichloro-4-(1,1,3,3-tetramethylbutyl)phenoxyacetate, 2,4-bis(1,1-dimethylpropyl)phenoxyacetate, chlorodiphenyl-acetate, isobutyrate, monosuccinoate, (*E*)-2-methyl-2-butenoate, *o*-(methoxycarbonyl)benzoate, α-naphthoate, nitrate, alkyl *N,N,N',N'*-tetramethylphosphorodiamidate, and 2-chlorobenzoate. In the case of sulfonates, sulfenates and sulfinates the protecting group for the OH together with the oxygen atom of the unprotected OH to which it is attached form a sulfonate, sulfenate or sulfinates that can be selected from sulfate, allylsulfonate, methanesulfonate, benzylsulfonate, tosylate, 2-[(4-nitrophenyl)ethyl]sulfonate, 2-trifluoromethylbenzenesulfonate, 4-monomethoxytritylsulfenate, alkyl 2,4-dinitrophenylsulfenate, 2,2,5,5-tetramethylpyrrolidin-3-one-1-sulfinate, and dimethylphosphinothioyl. In the case of carbonates the protecting group for the OH together with the oxygen atom of the unprotected OH to which it is attached form a carbonate that can be selected from methyl carbonate, methoxymethyl carbonate, 9-fluorenylmethyl carbonate, ethyl carbonate, bromoethyl carbonate, 2-(methylthiomethoxy)ethyl carbonate, 2,2,2-

trichloroethyl carbonate, 1,1-dimethyl-2,2,2-trichloroethyl carbonate, 2-(trimethylsilyl)ethyl carbonate, 2-[dimethyl(2-naphthylmethyl)silyl]ethyl carbonate, 2-(phenylsulfonyl)ethyl carbonate, 2-(triphenylphosphonio)ethyl carbonate, *cis*-[4-[[(methoxytrityl)sulfenyl]oxy]tetrahydrofuran-3-yl]oxy carbonate, isobutyl carbonate, *t*-butyl carbonate, vinyl carbonate, allyl carbonate, cinnamyl carbonate, propargyl carbonate, *p*-chlorophenyl carbonate, *p*-nitrophenyl carbonate, 4-ethoxy-1-naphthyl carbonate, 6-bromo-7-hydroxycoumarin-4-ylmethyl carbonate, benzyl carbonate, *o*-nitrobenzyl carbonate, *p*-nitrobenzyl carbonate, *p*-methoxybenzyl carbonate, 3,4-dimethoxybenzyl carbonate, anthraquinon-2-ylmethyl carbonate, 2-dansylethyl carbonate, 2-(4-nitrophenyl)ethyl carbonate, 2-(2,4-dinitrophenyl)ethyl carbonate, 2-(2-nitrophenyl)propyl carbonate, 2-(3,4-methylenedioxy-6-nitrophenyl)propyl carbonate, 2-cyano-1-phenylethyl carbonate, 2-(2-pyridyl)amino-1-phenylethyl carbonate, 2-[*N*-methyl-*N*-(2-pyridyl)]amino-1-phenylethyl carbonate, phenacyl carbonate, 3',5'-dimethoxybenzoin carbonate, methyl dithiocarbonate, and S-benzyl thiocarbonate. And in the case of carbamates the protecting group for OH together with the oxygen atom of the unprotected OH to which it is attached forms a carbamate that can be selected from dimethyl thiocarbamate, *N*-phenyl carbamate, and *N*-methyl-*N*-(o-nitrophenyl) carbamate.

[0033] Within the scope of the present invention an amino protecting group is defined to be the N-bonded moiety resulting from the protection of the amino group through the formation of a suitable protected amino group. Examples of protected amino groups include carbamates, ureas, amides, heterocyclic systems, *N*-alkyl amines, *N*-alkenyl amines, *N*-alkynyl amines, *N*-aryl amines, imines, enamines, N-metal derivatives, N-N derivatives, N-P derivatives, *N*-Si derivatives, and N-S derivatives. In the case of carbamates the protecting group for the amino group together with the amino group to which it is attached form a carbamate that can be selected from methyl carbamate, ethyl carbamate, 9-fluorenylmethyl carbamate, 2,6-di-*t*-butyl-9-fluorenylmethyl carbamate, 2,7-bis(trimethylsilyl)fluorenylmethyl carbamate, 9-(2-sulfo)fluorenylmethyl carbamate, 9-(2,7-dibromo)fluorenylmethyl carbamate, 17-tetrabenzo[*a*,*c*,*g*,*i*]fluorenylmethyl carbamate, 2-chloro-3-indenylmethyl carbamate, benz[*f*]inden-3-ylmethyl carbamate, 1,1-dioxobenzo[*b*]-thiophene-2-ylmethyl carbamate, 2-methylsulfonyl-3-phenyl-1-prop-2-enyl carbamate, 2.7-di-*t*-butyl-[9,(10,10-dioxo-10,10,10,10-tetrahydrothioxanthyl)]methyl carbamate, 2,2,2-trichloroethyl carbamate, 2-trimethylsilylethyl carbamate, (2-phenyl-2-trimethylsilyl)ethyl carbamate, 2-phenylethyl carbamate, 2-chloroethyl carbamate, 1,1-dimethyl-2-haloethyl carbamate, 1,1-dimethyl-2,2-dibromoethyl carbamate, 1,1-dimethyl-2,2,2-trichloroethyl carbamate, 2-(2'-pyridyl)ethyl carbamate, 2-(4'-pyridyl)ethyl carbamate, 2,2-bis(4'-nitrophenyl)ethyl carbamate, 2-[(2-nitrophenyl)dithio]-1-phenylethyl carbamate, 2-(*N*,*N*-dicyclohexylcarboxamido)ethyl carbamate, *t*-butyl carbamate, fluorous BOC carbamate, 1-adamantyl carbamate, 2-adamantyl carbamate, 1-(1-adamantyl)-1-methylethyl carbamate, 1-methyl-1-(4-byphenylyl)ethyl carbamate, 1-(3,5-di-*t*-butylphenyl)-1-methylethyl carbamate, triisopropylsilyloxy carbamate, vinyl carbamate, allyl carbamate, prenyl carbamate, 1-isopropylallyl carbamate, cinnamyl carbamate, 4-nitrocinnamyl carbamate, 3-(3'-pyridyl)prop-2-enyl carbamate, hexadienyl carbamate, propargyl carbamate, 1,4-but-2-ynyl biscarbamate, 8-quinolyl carbamate, *N*-hydroxypiperidinyl carbamate, alkyl dithiocarbamate, benzyl carbamate, 3,5-di-*t*-butylbenzyl carbamate, *p*-methoxybenzyl carbamate, *p*-nitrobenzyl carbamate, *p*-bromobenzyl carbamate, *p*-chlorobenzyl carbamate, 2,4-dichlorobenzyl carbamate, 4-methylsulfinylbenzyl carbamate, 4-trifluoromethylbenzyl carbamate, fluorous benzyl carbamate, 2-naphthylmethyl carbamate, 9-anthrylmethyl carbamate, diphenylmethyl carbamate, 4-phenylacetoxybenzyl carbamate, 4-azidobenzyl carbamate, 4-azido-methoxybenzyl carbamate, *m*-chloro-p-acyloxybenzyl carbamate, *p*-(dihydroxyboryl)-benzyl carbamate, 5-benzisoxazolylmethyl carbamate, 2-(trifluoromethyl)-6-chromonylmethyl carbamate, 2-methylthioethyl carbamate, 2-methylsulfonylethyl carbamate, 2-(*p*-toluenesulfonyl)ethyl carbamate, 2-(4-nitrophenylsulfonyl)ethyl carbamate, 2-(2,4-dinitrophenylsulfonyl)ethyl carbamate, 2-(4-trifluoromethylphenylsulfonyl)ethyl carbamate, [2-(1,3-dithianyl)]methyl carbamate, 2-phosphonioethyl carbamate, 2-[phenyl(methyl)sulfonio]ethyl carbamate, 1-methyl-1-(triphenylphosphonio)ethyl carbamate, 1,1-dimethyl-2-cyanoethyl carbamate, 2-dansylethyl carbamate, 2-(4-nitrophenyl)ethyl carbamate, 4-methylthiophenyl carbamate, 2,4-dimethylthiophenyl carbamate, *m*-nitrophenyl carbamate, 3,5-dimethoxybenzyl carbamate, 1-methyl-1-(3,5-dimethoxyphenyl)ethyl carbamate, α-methylnitropiperonyl carbamate, *o*-nitrobenzyl carbamate, 3,4-dimethoxy-6-nitrobenzyl carbamate, phenyl(*o*-nitrophenyl)methyl carbamate, 2-nitrophenylethyl carbamate, 6-nitroveratryl carbamate, 4-methoxyphenacyl carbamate, 3',5'-dimethoxybenzoin carbamate, 9-xanthenylmethyl carbamate, *N*-methyl-*N*-(*o*-nitrophenyl) carbamate, *t*-amyl carbamate, 1-methylcyclobutyl carbamate, 1-methylcyclohexyl carbamate, 1-methyl-1-cyclopropylmethyl carbamate, cyclobutyl carbamate,

cyclopentyl carbamate, cyclohexyl carbamate, isobutyl carbamate, isobornyl carbamate, cyclopropylmethyl carbamate, *p*-decyloxybenzyl carbamate, diisopropylmethyl carbamate, 2,2-dimethoxy-carbonylvinyl carbamate, *o*-(*N,N*-dimethylcarboxamido)benzyl carbamate, 1,1-dimethyl-3-(*N,N* dimethyl-carboxamido)propyl carbamate, butynyl carbamate, 1,1-dimethylpropynyl carbamate, 2-iodoethyl carbamate, 1-methyl-1-(4'-pyridyl)ethyl carbamate, 1-methyl-1-(*p*-phenylazophenyl)ethyl carbamate, *p*-(*p'*-methoxyphenylazo)benzyl carbamate, *p*-(phenylazo)benzyl carbamate, 2,4,6-trimethylbenzyl carbamate, isonicotinyl carbamate, 4-(trimethyl-ammonium)benzyl carbamate, *p*-cyanobenzyl carbamate, di(2-pyridyl)methyl carbamate, 2-furanylmethyl carbamate, phenyl carbamate, 2,4,6-tri-*t*-butylphenyl carbamate, 1-methyl-1-phenylethyl carbamate, and S-benzyl thiocarbamate. In the case of ureas the protecting groups for the amino group can be selected from phenothiazinyl-(10)-carbonyl, *N'*-*p*-toluenesulfonylaminocarbonyl, *N'*-phenylaminothiocarbonyl, 4-hydroxyphenylaminocarbonyl, 3-hydroxytryptaminocarbonyl, and *N'*-phenylaminothiocarbonyl. In the case of amides the protecting group for the amino together with the amino group to which it is attached form an amide that can be selected from formamide, acetamide, chloroacetamide, trichloroacetamide, trifluoroacetamide, phenylacetamide, 3-phenylpropanamide, pent-4-enamide, picolinamide, 3-pyridylcarboxamide, *N*-benzoylphenylalanyl amide, benzamide, *p*-phenylbenzamide, *o*-nitrophenylacetamide, 2,2-dimethyl-2-(*o*-nitrophenyl)acetamide, *o*-nitrophenoxyacetamide, 3-(*o*-nitrophenyl)propanamide, 2-methyl-2-(*o*-nitrophenoxy)propanamide, 3-methyl-3-nitrobutanamide, *o*-nitrocinnamide, *o*-nitrobenzamide, 3-(4-*t*-butyl-2,6-dinitrophenyl)-2,2-dimethylpropanamide, *o*-(benzoyloxymethyl)benzamide, 2-(acetoxymethyl)benzamide, 2-[(*t*-butyldiphenylsiloxy)methyl]benzamide, 3-(3',6'-dioxo-2',4',5'-trimethylcyclohexa-1',4'-diene)-3,3-dimethylpropionamide, *o*-hydroxy-*trans*-cinnamide, 2-methyl-2-(*o*-phenylazophenoxy)propanamide, 4-chlorobutanamide, acetoacetamide, 3-(*p*-hydroxyphenyl)propanamide, (*N'*-dithiobenzyloxycarbonylamino)acetamide, and N-acetylmethionine amide. In the case of heterocyclic systems the protecting group for the amino group together with the amino group to which it is attached form a heterocyclic system that can be selected from 4,5-diphenyl-3-oxazolin-2-one, *N*-phthalimide, *N*-dichlorophthalimide, *N*-tetrachlorophthalimide, *N*-4-nitrophthalimide, *N*-thiodiglycoloyl, *N*-dithiasuccinimide, *N*-2,3-diphenylmaleimide, *N*-2,3-dimethylmaleimide, *N*-2,5-dimethylpyrrole, *N*-2,5-bis(triisopropylsiloxy)pyrrole, *N*-1,1,4,4-tetramethyldisilylazacyclopentane adduct, *N*-1,1,3,3-tetramethyl-1,3-disilaisoindoline, *N*-diphenylsilyldiethylene, *N*-5-substituted-1,3-dimethyl-1,3,5-triazacyclohexan-2-one, *N*-5-substituted-1,3 -benzyl-1,3,5 -triazacyclohexan-2-one, 1-substituted 3,5-dinitro-4-pyridone, and 1,3,5-dioxazine. In the case of *N*-alkyl, *N*-alkenyl, *N*-alkynyl or *N* aryl amines the protecting group for the amino group can be selected from *N*-methyl, *N*-*t*-butyl, *N*-allyl, *N*-prenyl, *N*-cinnamyl, *N*-phenylallyl, *N*-propargyl, *N*-methoxymethyl, *N*-[2-(trimethylsilyl)ethoxy]methyl, *N*-3-acetoxypropyl, *N*-cyanomethyl, *N*-2-azanorbornenes, *N*-benzyl, *N*-4-methoxybenzyl, *N*-2,4-dimethoxybenzyl, *N*-2-hydroxybenzyl, *N*-ferrocenylmethyl, *N*-2,4-dinitrophenyl, *o*-methoxyphenyl, *p*-methoxyphenyl, *N*-9-phenylfluorenyl, *N*-fluorenyl, *N*-2-picolylamine N'-oxide, *N*-7-methoxycoumar-4-ylmethyl, *N*-diphenylmethyl, *N*-bis(4-methoxyphenyl)methyl, *N*-5-dibenzosuberyl, *N*-triphenylmethyl, *N*-(4-methylphenyl)diphenylmethyl, and *N*-(4-methoxyphenyl)diphenylmethyl. In the case of imines the protecting group for the amino group can be selected from *N*-1,1-dimethylthiomethylene, *N*-benzylidene, *N*-*p*-methoxybenzylidene, *N*-diphenylmethylene, *N*-[2-pyridyl)mesityl]methylene, *N*-(*N',N'*-dimethylaminomethylene), *N*-(*N',N'*-dibenzylaminomethylene), *N*-(*N'*-*t*-butylaminome-thylene), *N,N'*-isopropylidene, *N*-*p*-nitrobenzylidene, *N*-salicylidene, *N*-5-chlorosalicylidene, *N*-(5-chloro-2-hydroxyphenyl)phenylmethylene, *N*-cyclohexylidene, and *N*-*t*-butylidene. In the case of enamines the protecting group for the amino group can be selected from *N*-(5,5-dimethyl-3-oxo-1-cyclohexenyl), *N*-2,7-dichloro-9-fluorenylmethylene, *N*-1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl, *N*-(1,3-dimethyl-2,4,6-(1*H*,3*H*,5*H*)-trioxopyrimidine-5-ylidene)-methyl, *N*-4,4,4-trifluoro-3-oxo-1-butenyl, and *N*-(1-isopropyl-4-nitro-2-oxo-3-pyrrolin-3-yl). In the case of N-metal derivatives the protecting group for the amino group can be selected from N-borane, *N*-diphenylborinic ester, *N*-diethylborinic ester, *N*-9-borabicyclononane, *N*-difluoroborinic ester, and 3,5-bis(trifluoromethyl)phenylboronic acid; and also including *N*-phenyl(pentacarbonylchromium)carbenyl, *N*-phenyl(pentacarbonyl-tungsten)carbenyl, *N*-methyl(pentacarbonylchromium)carbenyl, *N*-methyl(pentacarbonyltungsten)carbenyl, *N*-copper chelate, *N*-zinc chelate, and a 18-crown-6-derivative. In the case of N-N derivatives the protecting group for the amino group together with the amino group to which it is attached form a N-N derivative that can be selected from *N*-nitroamino, *N*-nitrosoamino, amine *N*-oxide, azide, triazene derivative, and *N*-trimethylsilylmethyl-*N*-benzylhydrazine. In the case of N-P derivatives the protected

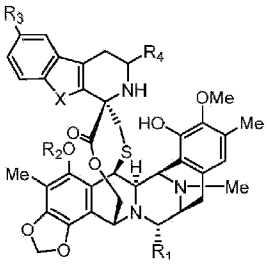
group for the amino group together with the amino group to which it is attached form a N-P derivative that can be selected from diphenylphosphinamide, dimethylthiophosphinamide, diphenylthiophosphinamide, dialkyl phosphoramidate, dibenzyl phosphoramidate, diphenyl phosphoramidate, and iminotriphenylphosphorane. In the case of N-Si derivatives the protecting group for the NH_2 can be selected from *t*-butyldiphenylsilyl and triphenylsilyl. In the case of N-S derivatives the protected amino group can be selected from N-sulfenyl or N-sulfonyl derivatives. The N-sulfenyl derivatives can be selected from benzenesulfenamide, 2-nitrobenzenesulfenamide, 2,4-dinitrobenzenesulfenamide, pentachlorobenzenesulfenamide, 2-nitro-4-methoxybenzenesulfenamide, triphenylmethylsulfe-namide, 1-(2,2,2-trifluoro-1,1-diphenyl)ethylsulfenamide, and *N*-3 -nitro-2-pyridinesulfenamide. The N-sulfonyl derivatives can be selected from methanesulfonamide, trifluoromethanesulfonamide, *t*-butylsulfonamide, benzylsulfonamide, 2-(trimethylsilyl) ethane sulfonamide, *p*-toluenesulfonamide, benzenesulfonamide, *o*-anisylsulfonamide, 2-nitrobenzenesulfonamide, 4-nitrobenzenesulfonamide, 2,4-dinitrobenzenesulfonamide, 2-naphthalene sulfonamide, 4-(4',8'-dimethoxynaphthylmethyl)benzenesulfonamide, 2-(4-methylphenyl)-6-methoxy-4-methylsulfonamide, 9-anthracenesulfonamide, pyridine-2-sulfonamide, benzothiazole-2-sulfonamide, phenacylsulfonamide, 2,3,6-trimethyl-4-methoxybenzenesulfonamide, 2,4,6-trimethoxybenzenesulfonamide, 2,6-dimethyl-4-methoxybenzenesulfonamide, pentamethylbenzenesulfonamide, 2,3,5,6-tetramethyl-4-methoxybenzenesulfonamide, 4-methoxybenzenesulfonamide, 2,4,6-trimethylbenzenesulfonamide, 2,6-dimethoxy-4-methylbenzenesulfonamide, 3-methoxy-4-*t*-butylbenzenesulfonamide, and 2,2,5,7,8-pentamethylchroman-6-sulfonamide.

[0034] Within the scope of the present invention a protecting group for SH is defined to be the S-bonded moiety resulting from the protection of the SH group through the formation of a suitable a protected SH group. Examples of such protected SH groups include thioethers, disulfides, silyl thioethers, thioesters, thiocarbonates, and thiocarbamates. In the case of thioethers the protecting group for the SH can be selected from *S*-alkyl, *S*-benzyl, *S*-*p*-methoxybenzyl, *S*-*o*-hydroxybenzyl, *S*-*p*-hydroxybenzyl, *S*-*o*-acetoxybenzyl, *S*-p-acetoxybenzyl, *S*-*p*-nitrobenzyl, *S*-*o*-nitrobenzyl, *S*-2,4,6-trimethylbenzyl, *S*-2,4,6,-trimethoxybenzyl, *S*-4-picolyl, *S*-2-picolyl-*N*-oxide, *S*-2-quinolinylmethyl, *S*-9-anthrylmethyl, *S*-9-fluorenylmethyl, *S*-xanthenyl, *S*-ferrocenylmethyl, *S*-diphenylmethyl, *S*-bis(4-methoxyphenyl)methyl, *S*-5-dibenzosuberyl, *S*-triphenylmethyl, 4-methoxytrityl, *S*-diphenyl-4-pyridylmethyl, *S*-phenyl, *S*-2,4-dinitrophenyl, *S*-2-quinolyl, *S*-*t*-butyl, *S*-1-adamantyl, *S*-methoxymethyl, *S*-isobutoxymethyl, *S*-benzyloxymethyl, *S*-1-ethoxyethyl, *S*-2-tetrahydropyranyl, *S*-benzylthiomethyl, *S*-phenylthiomethyl, *S*-acetamidomethyl (Acm), *S*-trimethylacetamidomethyl, *S*-benzamidomethyl, *S*-allyloxycarbonylaminomethyl, *S*-*N*-[2,3,5,6-tetrafluoro-4-(*N*'-piperidino)-phenyl-*N*-allyloxycarbonylaminomethyl, *S*-phthalimidomethyl, *S*-phenylacetamidomethyl, *S*-acetylmethyl, *S*-carboxymethyl, *S*-cyanomethyl, *S*-(2-nitro-1-phenyl)ethyl, *S*-2-(2,4-dinitrophenyl)ethyl, *S*-2-(4'-pyridyl)ethyl, *S*-2-cyanoethyl, *S*-2-(trimethylsilyl)ethyl, *S*-2,2-bis(carboethoxy)ethyl, *S*-(1-*m*-nitrophenyl-2-benzoyl)ethyl, *S*-2-phenylsulfonylethyl, *S*-1-(4-methylphenylsulfonyl)-2-methylprop-2-yl, and *S*-*p*-hydroxyphenacyl. In the case of disulfides the protected SH group can be selected from S-ethyl disulfide, *S*-*t*-butyl disulfide, *S*-2-nitrophenyl disulfide, *S*-2,4-dinitrophenyl disulfide, *S*-2-phenylazophenyl disulfide, *S*-2-carboxyphenyl disulfide, and *S*-3-nitro-2-pyridyl disulfide. In the case of silyl thioethers the protecting group for the SH can be selected from the list of groups that was listed above for the protection of OH with silyl ethers. In the case of thioesters the protecting group for the SH can be selected from *S*-acetyl, *S*-benzoyl, *S*-2-methoxyisobutyryl, *S*-trifluoroacetyl, *S*-*N*-[[*p*-biphenylyl)-isopropyloxy]carbonyl]-*N*-methyl-γ-aminothiobutyrate, and *S*-*N*-(*t*-butoxycarbonyl)-*N*-methyl-γ-aminothiobutyrate. In the case of thiocarbonate protecting group for the SH can be selected from *S*-2,2,2-trichloroethoxycarbonyl, S-t-butoxycarbonyl, *S*-benzyloxycarbonyl, *S*-*p*-methoxybenzyloxycarbonyl, and *S*-fluorenylmethylcarbonyl. In the case of thiocarbamate the protected SH group can be selected from *S*-(*N*-ethylcarbamate) and *S*-(*N*-methoxymethylcarbamate).

[0035] The mention of these groups should not be interpreted as a limitation of the scope of the invention, since they have been mentioned as a mere illustration of protecting groups for OH, amino and SH groups, but further groups having said function may be known by the skilled person in the art, and they are to be understood to be also encompassed by the present invention.

[0036] To provide a more concise description, some of the quantitative expressions given herein are not qualified with the term "about". It is understood that, whether the term "about" is used explicitly or not, every quantity given herein is meant to refer to the actual given value, and it is also meant to refer to the approximation to such given value that would reasonably be inferred based on the ordinary skill in the art, including equivalents and approximations due to the experimental and/or measurement conditions for such given value.

[0037] The compound of the invention is a compound of formula **IE** or a pharmaceutically acceptable salt or ester thereof:



IE

wherein:

X is -NH- or -O-;

R_1 is -OH or -CN;

R_2 is a -C(=O)R^a group;

R_3 is hydrogen or a -OR^b group;

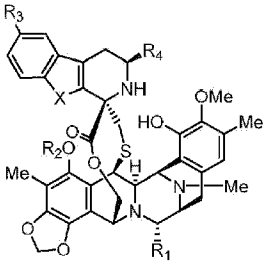
R_4 is selected from -CH_2NH_2 and -$CH_2NHProt^{NH}$;

R^a is selected from hydrogen, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl;

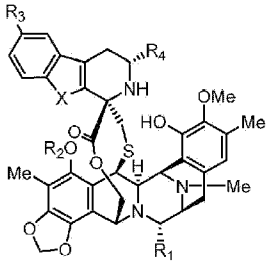
R^b is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl; and

$Prot^{NH}$ is a protecting group for amino.

[0038] Preferred compounds of the compounds of formula **IE** are those having general formula **IEa** or **IEb**, or a pharmaceutically acceptable salt or ester thereof:



IEa



IEb

[0039] Preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein: X is -NH-; and R_1; R_2; R_3; R_4; R^a; R^b; and $Prot^{NH}$ are as defined as above.

[0040] Preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein: X is -O-; and R_1; R_2; R_3; R_4; R^a; R^b; and $Prot^{NH}$ are as defined as above.

[0041] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb**, wherein:

R_1 is -OH;

and X; R_2; R_3; R_4; R^a; R^b; and $Prot^{NH}$ are as defined as above.

[0042] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb**, wherein:

R_2 is a -C(=O)R^a group where R^a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R^a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted n-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted sec-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

and X; R_1; R_3; R_4; R^b; and $Prot^{NH}$ are as defined as above.

[0043] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb**, wherein:

R_3 is hydrogen or a -OR^b group for compounds of formula **IE, IEa** and **IEb**; where R^b is a substituted or unsubstituted Ci-Ce alkyl. Particularly preferred R^b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

and X; R_1; R_2; R_4; R^a; and $Prot^{NH}$ are as defined as above.

[0044] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb**, wherein:

R_4 may be -CH_2NH_2;

and X; R_1; R_2; R_3; R^a; and R^b are as defined as above.

[0045] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb**, wherein:

X is -NH-;

R_1 is -OH;

and R_2; R_3; R_4; R^a; R^b; and $Prot^{NH}$ are as defined as above.

[0046] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb**, wherein:

X is -NH-;

R_2 is a -C(=O)R^a for compounds of formula **IE, IEa** and **IEb**; where R^a is a substituted or unsubstituted Ci-Ce alkyl. Particularly preferred R^a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

and R_1; R_3; R_4; R^b; and $Prot^{NH}$ are as defined as above.

[0047] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein:

X is -NH-;

R_3 is hydrogen or a -OR^b group for compounds of formula **IE, IEa** and **IEb;** where R^b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R^b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

and R_1; R_2; R_4; R^a; and $Prot^{NH}$ are as defined as above.

[0048] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein:

X is -NH-;

R_4 may be -CH_2NH_2;

and R_1; R_2; R_3; R^a; and R^b are as defined as above.

[0049] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein:

X is -NH-;

R_1 is -OH;

R_2 is a -C(=O)R^a group for compounds of formula **IE, IEa** and **IEb;** where R^a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R^a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

and R_3; R_4; R^b; and $Prot^{NH}$ are as defined as above.

[0050] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein:

X is -NH-;

R_1 is -OH;

R_3 is hydrogen or a $-OR^b$ group for compounds of formula **IE, IEa** and **IEb;** where R^b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R^b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

and R_2; R_4; R^a; and $Prot^{NH}$ are as defined as above.

[0051] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein:

X is -NH-;

R_1 is -OH;

R_4 may be $-CH_2NH_2$;

and R_2; R_3; R^a; and R^b are as defined as above.

[0052] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein:

X is -NH-;

R_2 is a $-C(=O)R^a$ group for compounds of formula **IE, IEa** and **IEb;** where R^a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R^a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

R_3 is hydrogen or a $-OR^b$ group for compounds of formula **IE, IEa** and **IEb;** where R^b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R^b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

and R_1; R_4; and $Prot^{NH}$ are as defined as above.

[0053] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein:

X is -NH-;

R_2 is a -C(=O)R^a group for compounds of formula **IE, IEa** and **IEb;** an where R^a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R^a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

R_4 may be -CH_2NH_2;

and R_1; R_3; and R^b are as defined as above.

[0054] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein:

X is -NH-;

R_3 is hydrogen or a -OR^b group for compounds of formula **IE, IEa** and **IEb;** where R^b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R^b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

R_4 may be -CH_2NH_2;

and R_1; R_2; and R^a; are as defined as above.

[0055] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein:

X is -NH-;

R_1 is -OH;

R_2 is a -C(=O)R^a group for compounds of formula **IE, IEa** and **IEb;** where R^a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R^a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

R_3 is hydrogen or a -OR^b group for compounds of formula **IE, IEa** and **IEb;** where R^b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R^b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

and R_4; and $Prot^{NH}$ are as defined as above.

[0056] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein:

X is -NH-;

R_1 is -OH;

R_2 is a -C(=O)R^a group for compounds of formula **IE, IEa** and **IEb;** where R^a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R^a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

R_4 may be -CH_2NH_2;

and R_3; and R^b are as defined as above.

[0057] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein:

X is -NH-;

R_2 is a -C(=O)R^a group for compounds of formula **IE, IEa** and **IEb;** where R^a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R^a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted sec-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

R_3 is hydrogen or a -OR^b group for compounds of formula **IE, IEa** and **IEb;** where R^b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R^b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

R_4 may be -CH_2NH_2;

and R_1 is as defined as above.

[0058] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein:

X is -NH-;

R_1 is -OH;

R_2 is a -C(=O)R^a group for compounds of formula **IE, IEa** and **IEb;** awhere R^a is a substituted or unsubstituted Ci-Ce alkyl. Particularly preferred R^a is selected from substituted or unsubstituted methyl, substituted or

unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

R_3 is hydrogen or a $-OR^b$ group for compounds of formula **IE, IEa** and **IEb;** where R^b is a substituted or unsubstituted Ci-Ce alkyl. Particularly preferred R^b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

R_4 may be $-CH_2NH_2$.

[0059] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein:

X is -O-;

R_1 is -OH;

and R_2; R_3; R_4; R^a; R^b; and $Prot^{NH}$ are as defined as above.

[0060] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein:

X is -O-;

R_2 is a $-C(=O)R^a$ group for compounds of formula **IE, IEa** and **IEb;** where R^a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R^a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

and R_1; R_3; R_4; R^b; and $Prot^{NH}$ are as defined as above.

[0061] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein:

X is -O-;

R_3 is hydrogen or a $-OR^b$ group for compounds of formula **IE, IEa** and **IEb;** where R^b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R^b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 is hydrogen and methoxy, being hydrogen the most preferred R_3 group;

and R_1; R_2; R_4; R^a; and $Prot^{NH}$ are as defined as above.

[0062] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein:

X is -O-;

R_4 may be $-CH_2NH_2$;

and R_1; R_2; R_3; R^a; and R^b are as defined as above.

[0063] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein:

X is -O-;

R_1 is -OH;

R_2 is a $-C(=O)R^a$ group for compounds of formula **IE, IEa** and **IEb;** where R^a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R^a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted tert-butyl. Most preferred R_2 is acetyl;

and R_3; R_4; R^b; and $Prot^{NH}$ are as defined as above.

[0064] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein:

X is -O-;

R_1 is -OH;

R_3 is hydrogen or a $-OR^b$ group for compounds of formula **IE, IEa** and **IEb;** where R^b is a substituted or unsubstituted Ci-Ce alkyl. Particularly preferred R^b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

and R_2; R_4; R^a; and $Prot^{NH}$ are as defined as above.

[0065] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein:

X is -O-;

R_1 is -OH;

R_4 may be $-CH_2NH_2$;

and R_2; R_3; R^a; and R^b are as defined as above.

[0066] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein:

X is -O-;

R_2 is a -C(=O)R^a group for compounds of formula **IE, IEa** and **IEb;** where R^a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R^a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted sec-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

R_3 is hydrogen or a -OR^b group for compounds of formula **IE, IEa** and **IEb;** where R^b is a substituted or unsubstituted Ci-Ce alkyl. Particularly preferred R^b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

and R_1; R_4; and $Prot^{NH}$ are as defined as above.

[0067] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein:

X is -O-;

R_2 is a -C(=O)R^a group for compounds of formula **IE, IEa** and **IEb;** where R^a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R^a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

R_4 may be -CH_2NH_2;

and R_1; R_3; and R^b are as defined as above.

[0068] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein:

X is -O-;

R_3 is hydrogen or a -OR^b group for compounds of formula **IE, IEa** and **IEb;** where R^b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R^b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

R_4 may be -CH_2NH_2;

and R_1; R_2; and R^a; are as defined as above.

[0069] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein:

X is -O-;

R_1 is -OH;

R_2 is a -C(=O)R^a group for compounds of formula **IE, IEa** and **IEb;** where R^a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R^a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

R_3 is hydrogen or a -OR^b group for compounds of formula **IE, IEa** and **IEb;** where R^b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R^b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

and R_4; and $Prot^{NH}$ are as defined as above.

[0070] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein:

X is -O-;

R_1 is -OH;

R_2 is a -C(=O)R^a group for compounds of formula **IE, IEa** and **IEb;** where R^a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R^a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

R_4 may be -CH_2NH_2;

and R_3; and R^b are as defined as above.

[0071] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein:

X is -O-;

R_2 is a -C(=O)R^a group for compounds of formula **IE, IEa** and **IEb;** where R^a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R^a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted

or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

R_3 is hydrogen or a -OR^b group for compounds of formula **IE, IEa** and **IEb;** where R^b is a substituted or unsubstituted Ci-Ce alkyl. Particularly preferred R^b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted sec-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

R_4 may be -CH_2NH_2;

and R_1 is as defined as above.

[0072] Further preferred compounds include compounds of general formula **IE, IEa** and **IEb,** wherein:

X is -O-;

R_1 is -OH;

R_2 is a -$C(=O)R^a$ group for compounds of formula **IE, IEa** and **IEb;** where R^a is a substituted or unsubstituted Ci-Ce alkyl. Particularly preferred R^a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted sec-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl;

R_3 is hydrogen or a -OR^b group for compounds of formula **IE, IEa** and **IEb;** where R^b is a substituted or unsubstituted Ci-Ce alkyl. Particularly preferred R^b is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group;

R_4 may be -CH_2NH_2.

[0073] The following preferred substituents (where allowed by possible substituent groups) apply to compounds of formula **IE, IEa** and **IEb:**
In compounds of the present invention, particularly preferred R_1 is -OH.

[0074] In compounds of the present invention, particularly preferred R_2 is a -$C(=O)R^a$ group where R^a is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R^a is selected from substituted or unsubstituted methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted n-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. Most preferred R_2 is acetyl.

[0075] In compounds of the present invention, particularly preferred R_3 is hydrogen or a -OR^b group where R^b is a substituted or unsubstituted C_1-C_6 alkyl. Particularly preferred R^b is selected from substituted or unsubstituted

methyl, substituted or unsubstituted ethyl, substituted or unsubstituted *n*-propyl, substituted or unsubstituted isopropyl, substituted or unsubstituted *n*-butyl, substituted or unsubstituted isobutyl, substituted or unsubstituted *sec*-butyl and substituted or unsubstituted *tert*-butyl. More preferred R_3 are hydrogen and methoxy, being hydrogen the most preferred R_3 group.

[0076] In compounds of general formula **IE**, **IEa** and **IEb** particularly preferred R_4 is CH_2NH_2.

[0077] Being particularly preferred compounds of formula **IEb**.

[0078] In compounds of the present invention, particularly preferred X is -NH-. Alternatively, in compounds of the present invention, particularly preferred X is -O-. Preferred compounds according to the present invention include:

- Compounds of formula **IEb** wherein
  R_4 is $-CH_2NH_2$.

[0079] Particularly preferred compounds according to the present invention include:

- Compounds of formula **IE**, **IEa** and **IEb** wherein

  X is -NH-.

  Being more preferred compounds of formula **IEb**, and/or compounds where R_4 is - CH_2NH_2.

- Compounds of formula **IE**, **IEa** and **IEb** wherein

  X is -O-.

  Being more preferred compounds of formula **IEb** and/or compounds where R_4 is - CH_2NH_2.

- Compounds of formula **IE, IEa** and **IEb** wherein

  R_2 is a $-C(=O)R^a$ group for compounds of formula **IE, IEa** and **IEb;**

  R_3 is hydrogen or a $-OR^b$ group for compounds of formula **IE, IEa** and **IEb;**

  R^a is selected from hydrogen, and substituted or unsubstituted C_1-C_6 alkyl; and

  R^b is substituted or unsubstituted C_1-C_6 alkyl.

  Being more preferred compounds of formula **IEb** and/or compounds where R_4 is - CH_2NH_2.

[0080] More preferred compounds according to the present invention include

- Compounds of formula **IE, IEa** and **IEb** wherein

  X is -NH-;

  R_2 is a $-C(=O)R^a$ group for compounds of formula **IE, IEa** and **IEb;**

  R_3 is hydrogen or a $-OR^b$ group for compounds of formula **IE, IEa** and **IEb;**

R^a is selected from hydrogen and substituted or unsubstituted C_1-C_6 alkyl; and

R^b is substituted or unsubstituted C_1-C_6 alkyl.

Being particularly more preferred compounds of formula **IEb** and/or compounds where R_4 is $-CH_2NH_2$.

- Compounds of formula **IE, IEa** and **IEb** wherein

  X is -O-;

  R_2 is a $-C(=O)R^a$ group for compounds of formula **IE, IEa** and **IEb;**

  R_3 is hydrogen or a $-OR^b$ group for compounds of formula **IE, IEa** and **IEb;**

  R^a is selected from hydrogen and substituted or unsubstituted C_1-C_6 alkyl; and

  R^b is substituted or unsubstituted C_1-C_6 alkyl.

  Being particularly more preferred compounds of formula **IEb** and/or compounds where R_4 is CH_2NH_2.

- Compounds of formula **IE, IEa** and **IEb** wherein

  R_2 is a $-C(=O)R^a$ group for compounds of formula **IE, IEa** and **IEb;**

  R_3 is hydrogen or a $-OR^b$ group for compounds of formula **IE, IEa** and **IEb;**

  R^a is substituted or unsubstituted C_1-C_6 alkyl; and

  R^b is substituted or unsubstituted C_1-C_6 alkyl.

  Being particularly more preferred compounds of formula **IEb** and/or compounds where R_4 is $-CH_2NH_2$.

[0081] Particularly more preferred compounds according to the present invention include:

- Compounds of formula **IE, IEa** and **IEb** wherein

  X is -NH-;

  R_2 is a $-C(=O)R^a$ group for compounds of formula **IE, IEa** and **IEb;**

  R_3 is hydrogen or methoxy for compounds of formula **IE, IEa** and **IEb;** and

  R^a is substituted or unsubstituted C_1-C_6 alkyl.

  Being even more preferred compounds of formula **IEb** and/or compounds where R_4 is $-CH_2NH_2$.

- Compounds of formula **IE, IEa** and **IEb** wherein

  X is -O-;

  R_2 is a $-C(=O)R^a$ group for compounds of formula **IE, IEa** and **IEb;**

  R_3 is hydrogen or methoxy for compounds of formula **IE, IEa** and **IEb;** and

R^a is substituted or unsubstituted C_1-C_6 alkyl.

Being even more preferred compounds of formula **IEb** and/or compounds where R_4 is $-CH_2NH_2$.

- Compounds of formula **IE, IEa** and **IEb** wherein

  R_2 is a $-C(=O)R^a$ group for compounds of formula **IE, IEa** and **IEb;**

  R_3 is hydrogen or a methoxy for compounds of formula **IE, IEa** and **IEb;** and

  R^a is selected from methyl, ethyl, *n*-propyl, isopropyl and butyl, including *n*-butyl, *sec*-butyl, isobutyl and *tert*-butyl.

  Being even more preferred compounds of formula **IEb** and/or compounds where R_4 is $-CH_2NH_2$.

[0082] Even more preferred compounds according to the present invention include:

- Compounds of formula **IE, IEa** and **IEb** wherein

  X is -NH-;

  R_2 is acetyl;

  R_3 is hydrogen; and

  R_4 is $-CH_2NH_2$.

  Being most preferred compounds of formula **IEb.**

- Compounds of formula **IE, IEa** and **IEb** wherein

  X is -O-;

  R_2 is acetyl;

  R_3 is hydrogen; and

  R_4 is $-CH_2NH_2$.

  Being most preferred compounds of formula **IEb.**

- Compounds of formula **IE, IEa** and **IEb** wherein

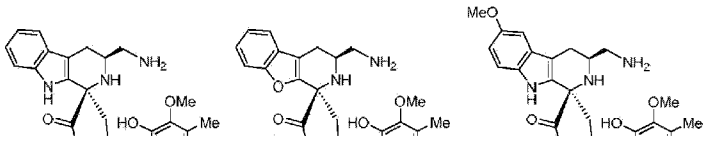
  R_2 is acetyl;

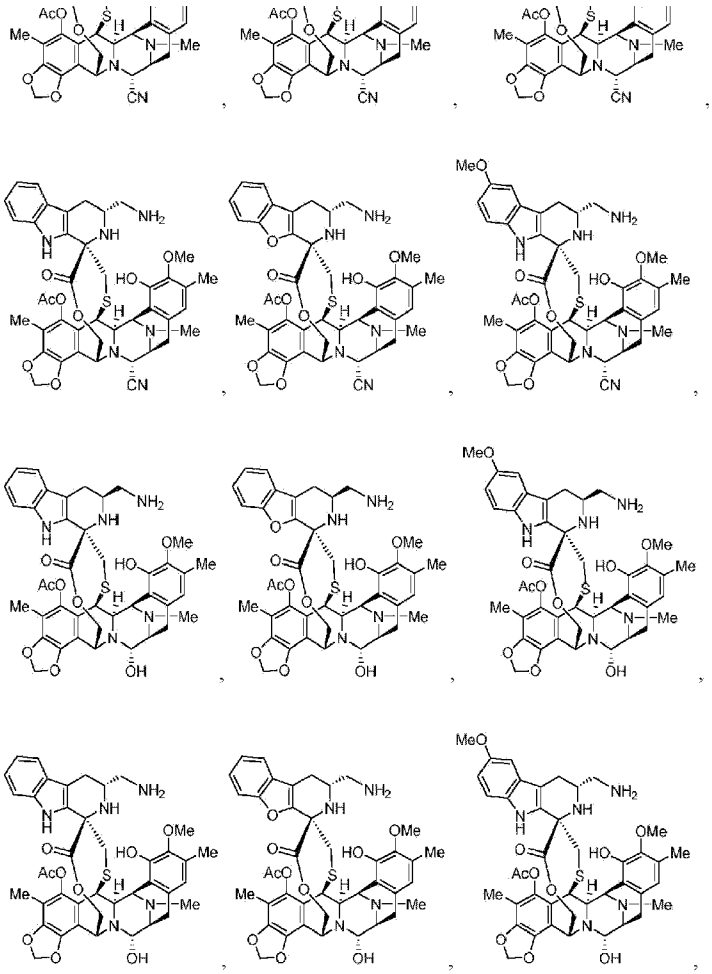
  R_3 is hydrogen; and

  R_4 is $-CH_2NH_2$.

  Being most preferred compounds of formula **IEb.**

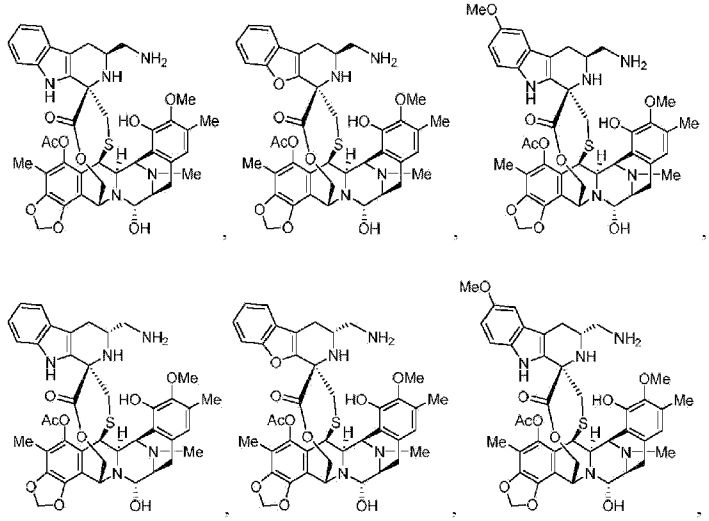
- A compound according to the present invention of formula:





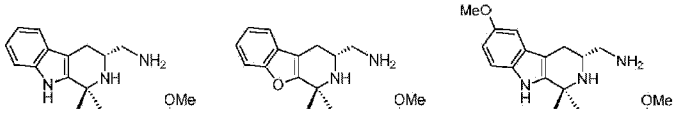
or a pharmaceutically acceptable salt or ester thereof.

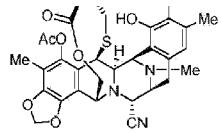
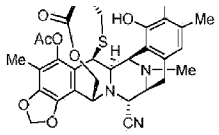
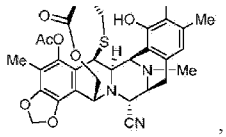
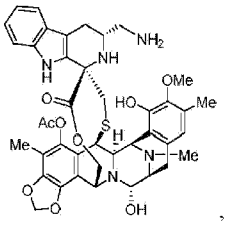
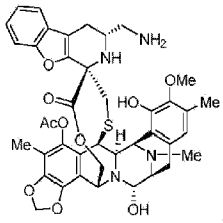
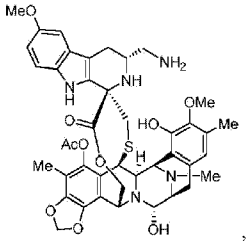
[0083] Being particularly preferred a compound of formula:



or a pharmaceutically acceptable salt or ester thereof.

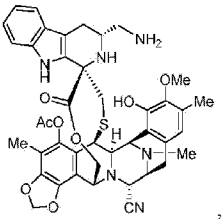
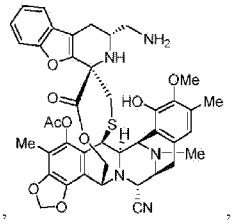
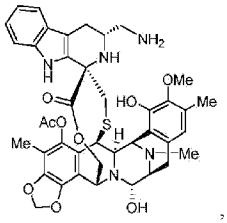
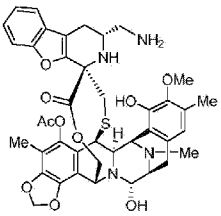
- A compound according to the present invention of formula:



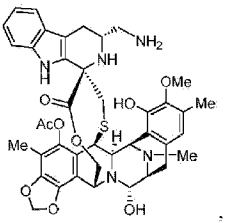
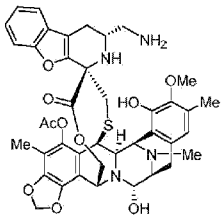
or a pharmaceutically acceptable salt or ester thereof.

[0084] Being particularly preferred a compound of formula:

or a pharmaceutically acceptable salt or ester thereof.

[0085] Being more preferred a compound of formula:

or a pharmaceutically acceptable salt or ester thereof.

[0086] In additional preferred embodiments, the preferences described above for the different substituents are combined. The present invention is also directed to such combinations of preferred substitutions (where allowed by possible substituent groups) in compounds of formula **IE, IEa** and **IEb** according to the present invention.

[0087] An important feature of the above-described compounds is their bioactivity and in particular their cytotoxic activity. In this regard, we have surprisingly found that the compounds of the present invention show an enhanced antitumor activity, as it is shown in Examples 27 and 29 to 40.

Compositions comprising a compound of formula IE, IEa and IEb of the invention and uses thereof

[0088] In a further embodiment of the present invention, there is provided a pharmaceutical composition comprising a compound according to the present invention and a pharmaceutically acceptable carrier. Examples of the administration form include without limitation oral, topical, parenteral, sublingual, rectal, vaginal, ocular and intranasal. Parenteral administration includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. Preferably the compositions are administered parenterally. Pharmaceutical compositions of the invention can be formulated so as to allow a compound according to the present invention to be bioavailable upon administration of the composition to an animal, preferably human. Compositions can take the form of one or more dosage units, where for example, a tablet can be a single dosage unit, and a container of a compound according to the present invention may contain the compound in liquid or in aerosol form and may hold a single or a plurality of dosage units.

[0089] The pharmaceutically acceptable carrier or vehicle can be particulate, so that the compositions are, for example, in tablet or powder form. The carrier(s) can be liquid, with the compositions being, for example, an oral syrup or injectable liquid. In addition, the carrier(s) can be gaseous, or liquid so as to provide an aerosol composition useful in, for example inhalatory administration. Powders may also be used for inhalation dosage forms. The term "carrier" refers to a diluent, adjuvant or excipient, with which the compound according to the present invention is administered. Such pharmaceutical carriers can be liquids, such as water and oils including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. The carriers can be saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea, disaccharides, and the like. In addition, auxiliary, stabilizing, thickening, lubricating and coloring agents can be used. In one embodiment, when administered to an animal, the compounds and compositions according to the present invention, and pharmaceutically acceptable carriers are sterile. Water is a preferred carrier when the compounds according to the present invention are administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical carriers also include excipients such as starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol and the like. The present compositions, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

[0090] When intended for oral administration, the composition is preferably in solid or liquid form, where semi-solid, semi-liquid, suspension and gel forms are included within the forms considered herein as either solid or liquid.

[0091] As a solid composition for oral administration, the composition can be formulated into a powder, granule, compressed tablet, pill, capsule, chewing gum, wafer or the like form. Such a solid composition typically contains one or more inert diluents. In addition, one or more for the following can be present: binders such as carboxymethylcellulose, ethyl cellulose, microcrystalline cellulose, or gelatin; excipients such as starch, lactose or dextrins, disintegrating agents such as alginic acid, sodium alginate, corn starch and the like; lubricants such as magnesium stearate; glidants such as colloidal silicon dioxide; sweetening agent such as sucrose or saccharin; a flavoring agent such as peppermint, methyl salicylate or orange flavoring; and a coloring agent.

[0092] When the composition is in the form of a capsule (e.g. a gelatin capsule), it can contain, in addition to materials of the above type, a liquid carrier such as polyethylene glycol, cyclodextrins or a fatty oil.

[0093] The composition can be in the form of a liquid, e.g. an elixir, syrup, solution, emulsion or suspension. The liquid can be useful for oral administration or for delivery by injection. When intended for oral administration, a composition can comprise one or more of a sweetening agent, preservatives, dye/colorant and flavor enhancer. In a composition for administration by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent can also be included.

[0094] The preferred route of administration is parenteral administration including, but not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, intracerebral,

intraventricular, intrathecal, intravaginal or transdermal. The preferred mode of administration is left to the discretion of the practitioner, and will depend in part upon the site of the medical condition (such as the site of cancer). In a more preferred embodiment, the compounds according to the present invention are administered intravenously. Infusion times of up to 24 hours are preferred to be used, more preferably 1 to 12 hours, with 1 to 6 hours being most preferred. Short infusion times which allow treatment to be carried out without an overnight stay in a hospital are especially desirable. However, infusion may be 12 to 24 hours or even longer if required. Infusion may be carried out at suitable intervals of, for example, 1 to 4 weeks.

[0095] The liquid compositions of the invention, whether they are solutions, suspensions or other like form, can also include one or more of the following: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides, polyethylene glycols, glycerin, or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; and agents for the adjustment of tonicity such as sodium chloride or dextrose. A parenteral composition can be enclosed in an ampoule, a disposable syringe or a multiple-dose vial made of glass, plastic or other material. Physiological saline is a preferred adjuvant.

[0096] The amount of the compound according to the present invention that is effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* or *in vivo* assays can optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the compositions will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgement of the practitioner and each patient's circumstances.

[0097] The compositions comprise an effective amount of a compound of the present invention such that a suitable dosage will be obtained. The correct dosage of the compounds will vary according to the particular formulation, the mode of application, and its particular site, host and the disease being treated, e.g. cancer and, if so, what type of tumor. Other factors like age, body weight, sex, diet, time of administration, rate of excretion, condition of the host, drug combinations, reaction sensitivities and severity of the disease should be taken into account. Administration can be carried out continuously or periodically within the maximum tolerated dose.

[0098] Typically, the amount is at least about 0.01% of a compound of the present invention, and may comprise at least 80%, by weight of the composition. When intended for oral administration, this amount can be varied to range from about 0.1% to about 80% by weight of the composition. Preferred oral compositions can comprise from about 4% to about 50% of the compound of the present invention by weight of the composition.

[0099] Preferred compositions of the present invention are prepared so that a parenteral dosage unit contains from about 0.01% to about 10 % by weight of the compound of the present invention. More preferred parenteral dosage unit contains about 0.5 % to about 5 % by weight of the compound of the present invention.

[0100] For intravenous administration, the composition is suitable for doses from about 0.1 mg/kg to about 250 mg/kg of the animal's body weight, preferably from about 0.1 mg/kg and about 20 mg/kg of the animal's body weight, and more preferably from about 1 mg/kg to about 10 mg/kg of the animal's body weight.

[0101] The compound of the present invention, can be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings.

[0102] In specific embodiments, it can be desirable to administer one or more compounds of the present invention, or compositions locally to the area in need of treatment. In one embodiment, administration can be by direct injection at the site (or former site) of a cancer, tumor or neoplastic or pre-neoplastic tissue.

[0103] Pulmonary administration can also be employed, e.g. by use of an inhaler or nebulizer, and formulation with an aerosolizing agent, or via perfusion in a fluorocarbon or synthetic pulmonary surfactant. In certain

embodiments, the compound of the present invention can be formulated as a suppository, with traditional binders and carriers such as triglycerides.

[0104] The present compositions can take the form of solutions, suspensions, emulsions, tablets, pills, pellets, capsules, capsules containing liquids, powders, sustained-release formulations, suppositories, emulsions, aerosols, sprays, suspensions, or any other form suitable for use. Other examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin.

[0105] The pharmaceutical compositions can be prepared using methodology well known in the pharmaceutical art. For example, a composition intended to be administered by injection can be prepared by combining a compound of the present invention with water, or other physiologically suitable diluent, such as phosphate buffered saline, so as to form a solution. A surfactant can be added to facilitate the formation of a homogeneous solution or suspension.

[0106] Preferred compositions according to the present invention include:

- Pharmaceutical compositions comprising a compound of the present invention and a disaccharide. Particularly preferred disaccharides are selected from lactose, trehalose, sucrose, maltose, isomaltose, cellobiose, isosaccharose, isotrehalose, turanose, melibiose, gentiobiose, and mixtures thereof.
- Lyophilised pharmaceutical compositions comprising a compound of the present invention and a disaccharide. Particularly preferred disaccharides are selected from lactose, trehalose, sucrose, maltose, isomaltose, cellobiose, isosaccharose, isotrehalose, turanose, melibiose, gentiobiose, and mixtures thereof.

[0107] The ratio of the active substance to the disaccharide in embodiments of the present invention is determined according to the solubility of the disaccharide and, when the formulation is freeze dried, also according to the freeze-dryability of the disaccharide. It is envisaged that this active substance:disaccharide ratio (w/w) can be about 1:10 in some embodiments, about 1:20 in other embodiments, about 1:50 in still other embodiments. It is envisaged that other embodiments have such ratios in the range from about 1:5 to about 1:500, and still further embodiments have such ratios in the range from about 1:10 to about 1:500.

[0108] The composition comprising a compound of the present invention may be lyophilized. The composition comprising a compound of the present invention is usually presented in a vial which contains a specified amount of such compound.

[0109] We have found that the compounds of the present invention and compositions of the present invention are particularly effective in the treatment of cancer.

[0110] Thus, as described earlier, the present invention provides a compound or composition for use in the treatment of cancer, and more preferably a cancer selected from lung cancer, including non-small cell lung cancer and small cell lung cancer, colon cancer, breast cancer, pancreas cancer, sarcoma, ovarian cancer, prostate cancer and gastric cancer.

[0111] Thus, the compounds and compositions according to the present invention are useful for inhibiting the multiplication, or proliferation, of a tumor cell or cancer cell, or for treating cancer in an animal.

[0112] The compounds and compositions according to the present invention show excellent activity in the treatment of cancers such as lung cancer including non-small cell lung cancer and small cell lung cancer, colon cancer, breast cancer, pancreas cancer, sarcoma, ovarian cancer, prostate cancer and gastric cancer. Most preferred cancers are selected from lung cancer including non-small cell lung cancer and small cell lung cancer, breast cancer, pancreas cancer and colorectal cancer.

[0113] In the present application, by "cancer" it is meant to include tumors, neoplasias and any other malignant disease having as cause malignant tissue or cells.

[0114] The term "treating", as used herein, unless otherwise indicated, means reversing, attenuating, alleviating or inhibiting the progress of the disease or condition to which such term applies, or one or more symptoms of such disorder or condition. The term "treatment", as used herein, unless otherwise indicated, refers to the act of treating as "treating" is defined immediately above.

[0115] The compounds and compositions according to the present invention can be administered to an animal that has also undergone surgery as treatment for the cancer. In one embodiment of the present invention, the additional treatment is radiation therapy.

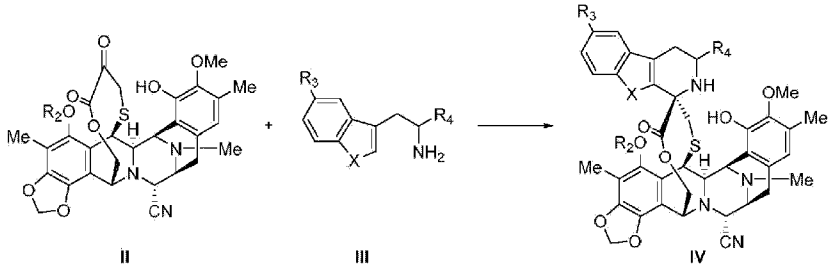
[0116] In a specific embodiment of the present invention, the compound or composition according to the present invention is administered concurrently with radiation therapy. In another specific embodiment, the radiation therapy is administered prior or subsequent to administration of the compound or composition of the present invention, preferably at least an hour, three hours, five hours, 12 hours, a day, a week, a month, more preferably several months (e.g. up to three months) prior or subsequent to administration of a compound or composition of the present invention.

[0117] Any radiation therapy protocol can be used depending upon the type of cancer to be treated. For example, but not by way of limitation, x-ray radiation can be administered; in particular, high-energy megavoltage (radiation of greater than 1 MeV energy) can be used for deep tumors, and electron beam and orthovoltage x-ray radiation can be used for skin cancers. Gamma-ray emitting radioisotopes, such as radioactive isotopes of radium, cobalt and other elements, can also be administered.

[0118] In a further embodiment of the present invention, there is provided a kit comprising a therapeutically effective amount of a compound according to the present invention and a pharmaceutically acceptable carrier.

[0119] In one embodiment, the kit according to this embodiment is for use in the treatment of cancer, and more preferably a cancer selected from lung cancer, including non-small cell lung cancer and small cell lung cancer, colon cancer, breast cancer, pancreas cancer, sarcoma, ovarian cancer, prostate cancer and gastric cancer.

[0120] In a further embodiment of the present invention, there is provided a process for obtaining a compound of formula **IE, IEa** and **IEb,** or a pharmaceutically acceptable salt or ester thereof, comprising the step of reacting a compound of formula **II** with a compound of formula **III** to give a compound of formula **IV:**



II III IV

wherein (where allowed by possible substituent groups):

X is -NH- or -O-;

R_2 is a $-C(=O)R^a$ group;

R_3 is hydrogen or a $-OR^b$ group;

R_4 is $-CH_2NHProt^{NH}$;

R^a is selected from hydrogen, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, substituted or unsubstituted C_2-C_{12} alkynyl;

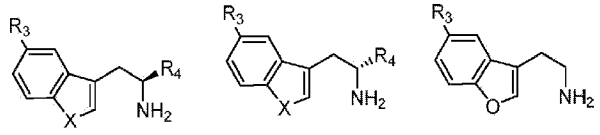
R^b is selected from substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, and substituted or unsubstituted C_2-C_{12} alkynyl; and

$Prot^{NH}$ is a protecting group for amino.

[0121] It is particularly preferred that the process further comprises the step of deprotecting R_4 to provide a compound of formula **IE, IEa** and **IEb** wherein R_4 is $-CH_2NH_2$ and R_1 is cyano.

[0122] In a more preferred embodiment, the process further comprises the step of replacing the cyano group in the compound of formula **IV** or in the compound of formula **IE, IEa** and **IEb** where R_4 is $-CH_2NH_2$ and R_1 is cyano with a hydroxy group to give a compound of formula **IE, IEa** and **IEb** where R_1 is OH:
Preferred processes according to the present invention include:

- A process that employs a compound of formula **II** wherein:
  R_2 is a $-C(=O)R^a$ group where R^a is substituted or unsubstituted C_1-C_{12} alkyl. Particularly preferred R^a is a substituted or unsubstituted C_1-C_6 alkyl. More preferred R^a is a substituted or unsubstituted alkyl group selected from methyl, ethyl, *n*-propyl, isopropyl, and butyl, including *n*-butyl, *sec*-butyl, isobutyl and *tert*-butyl, being methyl the most preferred R^a group.
- A process wherein the compound of formula **III** is selected from a compound of formula **IIIa, IIIb** and **IIIc:**



IIIa **IIIb** **IIIc**

wherein

X is selected from -NH- and -O-;

R_3 is selected from hydrogen and OR^b where R^b is substituted or unsubstituted C_1-C_{12} alkyl. Particularly preferred R^b is a substituted or unsubstituted C_1-C_6 alkyl. More preferred R^b is a substituted or unsubstituted alkyl group selected from methyl, ethyl, *n*-propyl, isopropyl, and butyl, including *n*-butyl, *sec*-butyl, isobutyl and *tert*-butyl. More preferred R^3 is hydrogen or methoxy. Most preferred R^3 is hydrogen.

It is particularly preferred that the compound of formula **III** is a compound of formula **IIIa** or **IIIb.**

- A process that employs a compound of formula **III, IIIa** or **IIIb** wherein R_4 is - $CH_2NHProt^{NH}$.

  Being preferred a process that employs a compound of formula **IIIa** or **IIIb** wherein R_4 is as defined above.

  Being more preferred a process that employs a compound of formula **IIIb** wherein R_4 is as defined above.

EXAMPLES

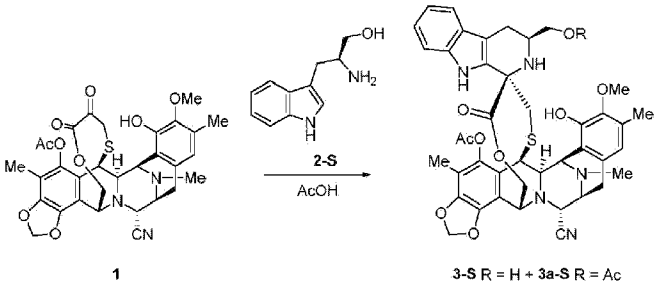
[0123] Compound 1 was prepared as described in Example 20 of WO 01/87895.

[0124] Reference compounds **A, B, C, D, E, F, ET-736,** and **PM01183** were prepared as described in WO 03/014127 (Compounds **19, 18, 44, 43, 2,1, 26,** and **27** respectively).

Reference Example 1.

[0125]

A)



[0126] To a solution of **1** (0.5 g, 0.80 mmol) in acetic acid (20 mL, 0.04 M) was added L-tryptophanol **(2-S)** (533 mg, 3.0 mmol, Sigma-Aldrich). The reaction mixture was stirred at 23 °C for 16 h and then acetic acid was evaporated. An aqueous saturated solution of NaHCOs was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, 1:1) gave compounds **3-S** (616 mg, 97%) and **3a-S** (12 mg, 2%).

3-S

[0127] R_f= 0.50 (Hexane:EtOAc, 1:1).

[0128] ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.71 (s, 1H), 7.36 (dd, *J* = 7.9, 1.0 Hz, 1H), 7.27 (dd, *J* = 8.2, 0.9 Hz, 1H), 7.13 (ddd, *J* = 8.3, 7.0, 1.2 Hz, 1H), 7.03 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H), 6.62 (s, 1H), 6.26 (d, *J* = 1.4 Hz, 1H), 6.04 (d, *J* = 1.3 Hz, 1H), 5.75 (s, 1H), 5.14 (dd, *J* = 11.7, 1.2 Hz, 1H), 4.60 (s, 1H), 4.41 (s, 1H), 4.36-4.24 (m, 2H), 4.21 (d, *J* = 2.7 Hz, 1H), 3.82 (s, 3H), 3.52 (s, 1H), 3.50-3.47 (m, 1H), 3.45 (dq, *J* = 8.4, 2.2 Hz, 1H), 3.35 (t, *J* = 10.1 Hz, 1H), 3.01-2.78 (m, 5H), 2.62 (dd, *J*= 15.3, 4.7 Hz, 1H), 2.41 (s, 1H), 2.38 (s, 3H), 2.37-2.31 (m, 1H), 2.28 (s, 3H), 2.17 (s, 3H), 2.06 (s, 3H).

[0129] ESI-MS *m/z:* 794.2 $(M+H)^+$.

3a-S

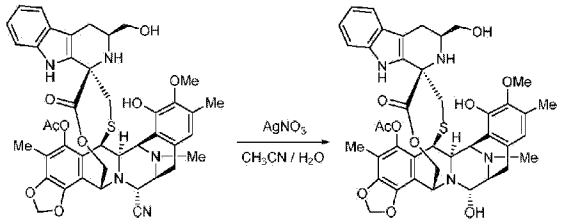
[0130] R_f= 0.70 (Hexane:EtOAc, 1:1).

[0131] ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.83 (s, 1H), 7.38 (dt, *J* = 7.9, 0.9 Hz, 1H), 7.25 (dt, *J* = 8.3, 0.9 Hz, 1H), 7.11 (ddd, *J* = 8.2, 7.1, 1.2 Hz, 1H), 7.02 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H), 6.62 (s, 1H), 6.24 (d, *J* = 1.4 Hz, 1H), 6.03 (d, *J* = 1.3 Hz, 1H), 5.79 (s, 1H), 5.13 (d, *J* = 11.7 Hz, 1H), 4.60 (s, 1H), 4.39 (s, 1H), 4.36-4.22 (m, 3H), 4.17-4.09 (m, 1H), 3.91 (dd, *J* = 10.5, 8.6 Hz, 1H), 3.83 (s, 3H), 3.51-3.41 (m, 2H), 3.04-2.92 (m, 3H), 2.72 (dd, *J* = 15.1, 4.0 Hz, 1H), 2.54-2.41 (m, 2H), 2.38 (s, 3H), 2.35-2.30 (m, 1H), 2.29 (s, 3H), 2.21-2.16 (m, 1H), 2.18 (s, 3H), 2.12 (s, 3H); 2.05 (s, 3H).

[0132] ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.2, 170.7, 168.6, 147.5, 145.8, 143.0, 141.1, 140.4, 135.6, 130.1, 129.5, 126.7, 122.2, 121.2, 120.9, 119.4, 118.4, 118.2, 118.2, 113.6, 113.5, 110.9, 110.0, 109.1, 102.1, 91.4, 67.2, 63.4, 61.3, 60.4, 59.7, 59.1, 54.8, 54.6, 47.7, 42.0, 41.6, 31.6, 24.0, 22.6, 21.0, 15.9, 14.2,9.7.

[0133] ESI-MS *m/z:* 836.2 $(M+H)^+$.

B)



3-S 4-S

[0134] To a solution of 3-S (616 mg, 0.77 mmol) in CH_3CN:H_2O (1.39:1, 51 mL, 0.015 M) was added $AgNO_3$ (3.40 g, 23.3 mmol). After 3 h at 23 °C, the reaction mixture was quenched with a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to give 4-S (471 mg, 78%).

[0135] R_f= 0.50 (CH_2Cl_2:CH_3OH, 9:1).

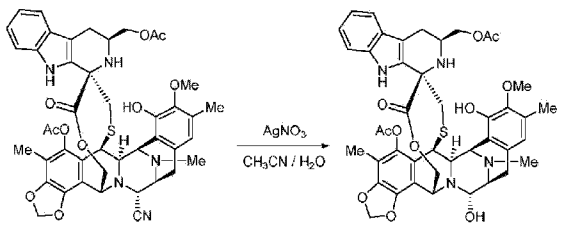
[0136] ^1H NMR (500 MHz, $CDCl_3$): δ 7.71 (s, 1H), 7.36 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.26 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.12 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H), 7.03 (ddd, *J* = 8.0, 7.1, 1.0 Hz, 1H), 6.64 (s, 1H), 6.23 (d, *J* = 1.3 Hz, 1H), 6.01 (d, *J* = 1.4 Hz, 1H), 5.75 (s, 1H), 5.25 (d, *J* = 11.4 Hz, 1H), 4.92 (s, 1H), 4.52 (br s, 3H), 4.22 (dd, *J* = 11.4, 2.2 Hz, 1H), 4.19 (s, 1H), 3.83 (s, 3H), 3.54 (br s, 2H), 3.35 (t, *J* = 10.2 Hz, 1H), 3.26 (s, 1H), 3.01-2.93 (m, 3H), 2.88 (br s, 3H), 2.63 (dd, *J* = 15.2, 4.8 Hz, 1H), 2.38 (s, 3H), 2.36-2.31 (m, 2H), 2.28 (s, 3H), 2.05 (s, 3H).

[0137] ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.9, 168.6, 147.5, 145.4, 142.9, 141.2, 140.7, 135.5, 130.4, 126.8, 122.3, 122.0, 121.3, 119.4, 118.4, 115.2, 112.8, 111.0, 110.0, 109.6, 101.8, 81.9, 76.8, 65.2, 62.8, 62.5, 60.4, 58.1, 57.9, 55.9, 55.1, 53.4, 51.6, 41.8, 41.3, 39.6, 24.1, 23.8, 20.5, 15.8, 9.7.

[0138] ESI-MS *m/z:* 767.3 $(M-H_2O+H)^+$.

[0139] (+)-HR-ESI-TOF-MS *m/z* 767.2788 $[M-H_2O+H]^+$ (Calcd. for $C_{41}H_{43}N_4O_9S$: 767.2745).

B')



3a-S 4a-S

[0140] To a solution of **3a-S** (30 mg, 0.035 mmol) in CH_3CN:H_2O (1.39:1, 2.4 mL, 0.015 M) was added AgNOs (180 mg, 1.07 mmol). After 3 h at 23 °C, the reaction mixture was quenched with a mixture 1:1 of saturated aqueous solutions of NaCl and NaHCOs, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted

with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to give **4a-S** (24 mg, 83%).

[0141] R_f= 0.60 (CH_2Cl_2:CH_3OH, 9:1).

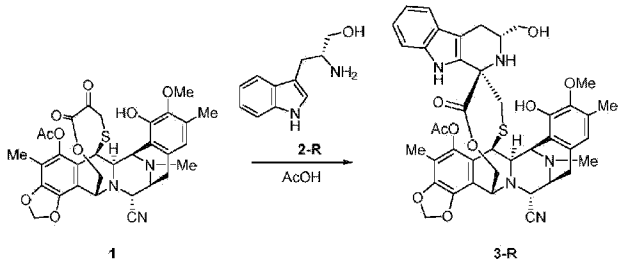
[0142] ^1H NMR (400 MHz, $CDCl_3$): δ 7.81 (s, 1H), 7.37 (d, *J* = 7.8 Hz, 1H), 7.30-7.21 (m, 1H), 7.06 (dddt, *J* = 34.7, 8.0, 7.1, 1.1 Hz, 2H), 6.63 (s, 1H), 6.22 (d, *J* = 1.3 Hz, 1H), 6.02 (dd, *J* = 12.9, 1.4 Hz, 1H), 5.74 (s, 1H), 5.25-5.21 (m, 1H), 4.89 (d, *J* = 8.7 Hz, 1H), 4.55-4.45 (m, 2H), 4.30-4.18 (m, 1H), 4.14 (dd, *J* = 10.5, 4.2 Hz, 1H), 4.00-3.88 (m, 2H), 3.82 (s, 3H), 3.56-3.44 (m, 2H), 3.23 (d, *J* = 9.0 Hz, 1H), 2.95 (d, *J* = 15.7 Hz, 2H), 2.87-2.78 (m, 2H), 2.71 (dd, *J* = 15.0, 3.9 Hz, 1H), 2.48 (dd, *J* = 15.1, 9.6 Hz, 1H), 2.37 (s, 3H), 2.35-2.29 (m, 1H), 2.28 (s, 3H), 2.22-2.16 (m, 1H), 2.15 (s, 3H), 2.12 (s, 3H), 2.03 (s, 3H).

[0143] ESI-MS *m/z:* 809.2 $(M-H_2O+H)^+$.

Reference Example 2

[0144]

A)



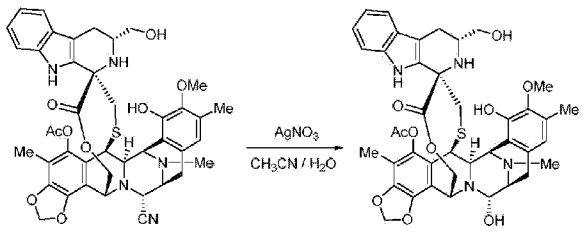
[0145] To a solution of **1** (0.5 g, 0.80 mmol) in acetic acid (20 mL, 0.04 M) was added D-tryptophanol **(2-R)** (533 mg, 3.0 mmol, Sigma-Aldrich). The reaction mixture was stirred at 23 °C for 16 h and then acetic acid was evaporated. An aqueous saturated solution of NaHCOs was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, 1:1) gave compound **3-R** (479 mg, 75%).

[0146] R_f= 0.44 (Hexane:EtOAc, 1:1).

[0147] ^1H NMR (400 MHz, $CDCl_3$): δ 7.61 (s, 1H), 7.39 (d, *J* = 7.8 Hz, 1H), 7.29 (d, *J* = 9.6 Hz, 1H), 7.12 (t, *J* = 7.3 Hz, 1H), 7.03 (t, *J* = 7.3 Hz, 1H), 6.60 (s, 1H), 6.25 (s, 1H), 6.03 (s, 1H), 5.75 (s, 1H), 5.04 (d, *J* = 11.7 Hz, 1H), 4.62 (s, 1H), 4.37 (s, 1H), 4.32-4.25 (m, 1H), 4.22 (d, *J* = 2.7 Hz, 1H), 4.19-4.09 (m, 1H), 3.82 (s, 3H), 3.77 (s, 1H), 3.64 (d, *J* = 9.0 Hz, 1H), 3.49-3.41 (m, 2H), 3.02-2.90 (m, 2H), 2.60-2.52 (m, 2H), 2.45 (d, *J* = 14.7 Hz, 2H), 2.40 (s, 3H), 2.28 (s, 3H), 2.22-2.14 (m, 2H), 2.18 (s, 3H), 2.10 (m, 3H).

[0148] ESI-MS *m/z:* 794.3 $(M+H)^+$.

B)



3-R 4-R

[0149] To a solution of **3-R** (479 mg, 0.60 mmol) in CH_3CN:H_2O (1.39:1, 40 mL, 0.015 M) was added AgNOs (3.03 g, 18.1 mmol). After 3 h at 23 °C, the reaction mixture was quenched with a mixture 1:1 of saturated aqueous solutions of NaCl and NaHCOs, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to afford **4-R** (428 mg, 91%).

[0150] R_f= 0.45 (CH_2Cl_2:CH_3OH, 9:1).

[0151] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62 (s, 1H), 7.39 (d, *J* = 8.1 Hz, 1H), 7.28 (d, *J* = 8.1 Hz, 1H), 7.11 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H), 7.02 (ddd, *J* = 7.9, 7.1, 1.0 Hz, 1H), 6.61 (s, 1H), 6.22 (d, *J* = 1.3 Hz, 1H), 5.99 (d, *J* = 1.3 Hz, 1H), 5.73 (s, 1H), 5.17 (dd, *J* = 11.5, 1.2 Hz, 1H), 4.86 (s, 1H), 4.56-4.47 (m, 2H), 4.17 (dd, *J* = 5.1, 1.6 Hz, 1H), 4.08 (dd, *J* = 11.5, 2.1 Hz, 1H), 3.81 (s, 3H), 3.78 (d, *J* = 3.8 Hz, 1H), 3.64 (dd, *J* = 10.8, 3.8 Hz, 2H), 3.51 (d, *J* = 5.1 Hz, 1H), 3.48-3.43 (m, 2H), 3.24 (d, *J* = 8.6 Hz, 1H), 3.00-2.80 (m, 2H), 2.57 (s, 1H), 2.55-2.43 (m, 1H), 2.40 (s, 3H), 2.27 (s, 3H), 2.19-2.12 (m, 1H), 2.16 (s, 3H), 2.08 (s, 3H).

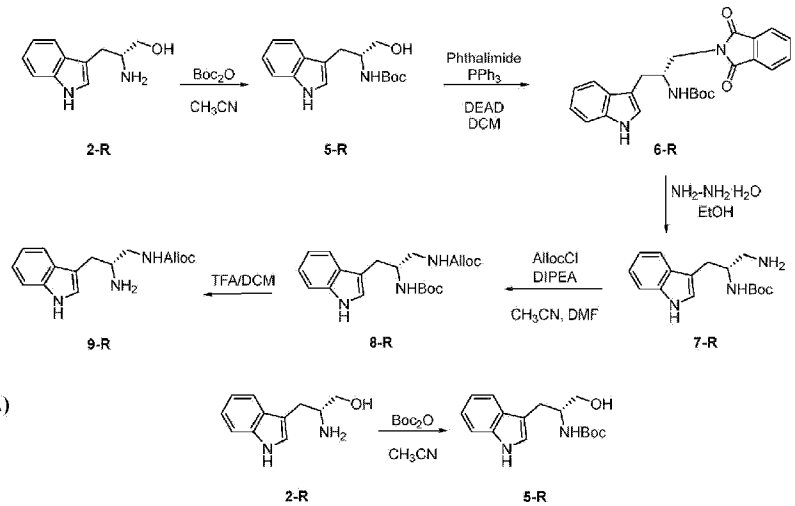
[0152] ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.8, 168.6, 147.6, 145.4, 143.0, 141.3, 140.7, 136.0, 131.1, 130.0, 129.6, 126.6, 122.1, 121.6, 121.2, 119.4, 118.4, 115.6, 112.9, 111.1, 110.6, 101.8, 81.7, 65.8, 62.7, 61.8, 60.4, 60.3, 57.9, 57.8, 56.1, 55.0, 52.1, 42.2, 41.3, 41.1, 23.8, 23.4, 20.5, 15.7, 9.8.

[0153] ESI-MS *m/z:* 767.6 $(M\text{-}H_2O+H)^+$.

[0154] (+)-HR-ESI-TOF-MS m/z: 767.2799 $[M\text{-}H_2O+H]^+$ (Calcd. for $C_{41}H_{43}N_4O_9S$: 767.2745).

Example 3. Synthesis of allyl *N*-[(*R*)-(2-amino-3-(*1H*-indol-3-yl)propyl)]carbamate (9-R)

[0155]



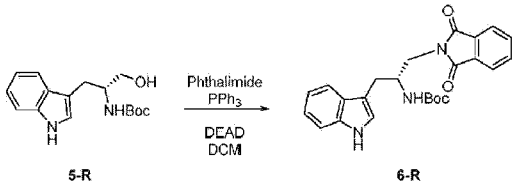
A)

[0156] To a solution of D-tryptophanol (**2-R**) (2.0 g, 10.4 mmol) in CH_3CN (42 mL, 4 mL/mmol) was added di-tert-butyl dicarbonate (4.6 g, 20.8 mmol). The reaction mixture was stirred at 23 °C for 3 h and concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH from 99:1 to 85:15) to afford **5-R** (2.2 g, 73%).

[0157] R_f= 0.5 (CH_2Cl_2:CH_3OH, 9:1).

[0158] ^1H NMR (400 MHz, $CDCl_3$): δ 8.13 (s, 1H), 7.67 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.38 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.29-7.10 (m, 2H), 7.06 (s, 1H), 4.82 (s, 1H), 4.00 (s, 1H), 3.71 (dd, *J* = 11.0, 3.8 Hz, 1H), 3.62 (dd, *J* = 11.0, 5.5 Hz, 1H), 3.01 (d, *J* = 6.7 Hz, 2H), 2.14 (s, 1H), 1.44 (s, 9H).

B)



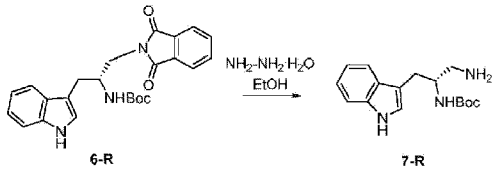
5-R 6-R

[0159] To a solution of **5-R** (2.4 g, 8.2 mmol) in CH_2Cl_2 (50 mL, 6 mL/mmol) was added phthalimide (2.7 g, 18.2 mmol), triphenylphosphine (4.8 g, 18.2 mmol) and the mixture was cooled at 0 °C. A solution of diethyl azodicarboxylate solution in CH_2Cl_2 (25 mL, 3 mL/mmol) was added for 15 min. The reaction was stirred at 23 °C for 16 h, concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to afford **6-R** (3.3 g, 96%).

[0160] R_f= 0.7 (CH_2Cl_2:CH_3OH, 9:1).

[0161] ^{1}HNMR(400 MHz, $CDCl_3$): δ 8.50 (s, 1H), 7.81 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.66 (dd, *J* = 5.6, 3.2 Hz, 2H), 7.60 (d, *J* = 7.8 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 1H), 7.19-7.04 (m, 3H)., 4.81 (s, 1H), 4.40 (s, 1H), 3.83 (dd, *J* = 13.9, 3.7 Hz, 1H), 3.72 (dd, *J* = 13.9, 9.9 Hz, 1H), 3.08-3.01 (m, 2H), 1.23 (s, 9H).

C)



6-R 7-R

[0162] To a solution of **6-R** (3.25 g, 7.74 mmol) in ethanol (231 mL, 30 mL/mmol) was added hydrazine monohydrate (37 mL, 774 mmol). The reaction mixture was stirred at 80 °C in sealed tube for 2.25 h, concentrated under vacuum. Flash chromatography (EtOAc:CH_3OH, from 100:1 to 50:50) afforded **7-R** (2.15 g, 96%).

[0163] R_f= 0.2 (EtOAc:CH_3OH, 6:4).

[0164] ^1H NMR (400 MHz, CD_3OD): δ 7.60 (d, *J* = 7.9 Hz, 1H), 7.33 (d, *J* = 8.1 Hz, 1H), 7.13-7.04 (m, 2H), 7.05-6.96 (m, 1H), 4.02-3.94 (m, 1H), 2.99-2.87 (m, 3H), 2.78 (dd, *J* = 13.1, 9.7 Hz, 1H), 1.39 (s, 9H).

[0165] ESI-MS *m/z:* 290.2 $(M+H)^+$.

D)

7-R 8-R

[0166] To a solution of **7-R** (2.15 g, 7.4 mmol) in CH_3CN (74 mL, 10 mL/mmol) and DMF (7.4 mL, 1 mL/mmol) was added *N,N*-diisopropylethylamine (1.06 mL, 5.9 mmol) and allyl chloroformate (7.9 mL, 74 mmol). The reaction was stirred at 23 °C for 16 h. The mixture was diluted with EtOAc, NH_4Cl was added and the mixture

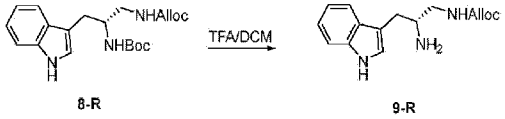
was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (Hexane:EtOAc, from 100:1 to 1:100) to afford **8-R** (1.69 g, 61%).

[0167] R_f= 0.4 (Hexane:EtOAc, 1:1).

[0168] ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 (s, 1H), 7.62 (d, *J* = 7.8 Hz, 1H), 7.35 (dd, *J* = 8.1, 0.9 Hz, 1H), 7.16 (dddd, *J* = 27.8, 8.0, 7.0, 1.1 Hz, 2H), 7.04 (d, *J* = 2.4 Hz, 1H), 5.90 (ddt, *J* = 17.3, 10.7, 5.6 Hz, 1H), 5.34-5.22 (m, 1H), 5.20 (dt, *J* = 10.5, 1.4 Hz, 1H), 5.12 (s, 1H), 4.82 (s, 1H), 4.55 (dq, *J* = 5.4, 1.7 Hz, 2H), 4.02 (s, 1H), 3.35 (dt, *J* = 10.0, 4.7 Hz, 1H), 3.21 (s, 1H), 2.95 (ddd, *J* = 21.6, 15.4, 9.1 Hz, 2H), 1.42 (s, 9H).

[0169] ESI-MS *m/z:* 274.3 $(M\text{-}Boc+H)^+$.

E)



8-R 9-R

[0170] To a solution of **8-R** (1.30 g, 3.50 mmol) in CH_2Cl_2 (58 mL, 16.6 mL/mmol) was added trifluoroacetic acid (30 mL, 8.3 mL/mmol). The reaction mixture was stirred at 23 °C for 1.5 h, concentrated under vacuum to give crude **9-R** which was used in the next steps without further purification.

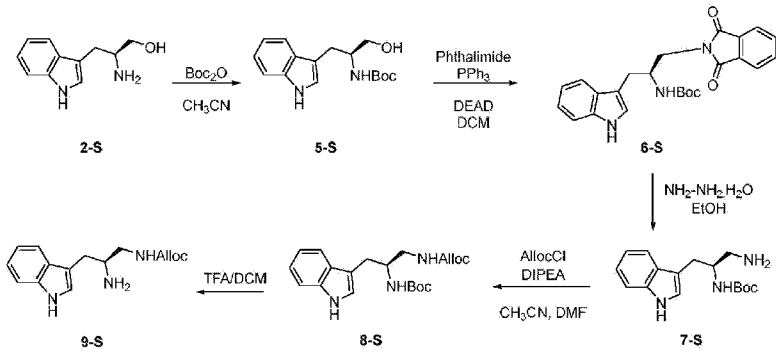
[0171] R_f= 0.2 (CH_2Cl_2:CH_3OH, 9:1).

[0172] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 (s, 1H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.17 (s, 1H), 7.09 (t, *J* = 7.5 Hz, 1H), 7.03 (t, *J* = 7.5 Hz, 1H), 5.87 (ddt, *J* = 16.4, 10.8, 5.6 Hz, 1H), 5.34-5.13 (m, 2H), 4.50 (d, *J* = 5.5 Hz, 2H), 3.62 (bs, 1H), 3.42 (dd, *J* = 14.9, 3.9 Hz, 1H), 3.36-3.20 (m, 1H), 3.11-3.00 (m, 2H).

[0173] ESI-MS *m/z:* 274.3 $(M+H)^+$.

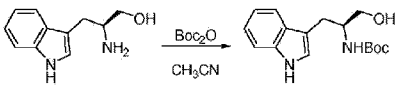
Example 4. Synthesis of allyl *N*-[(*S*)-(2-amino-3-(*1H*-indol-3-yl)propyl)]carbamate (9-S)

[0174]



2-S 5-S 6-S 7-S 8-S 9-S

A)



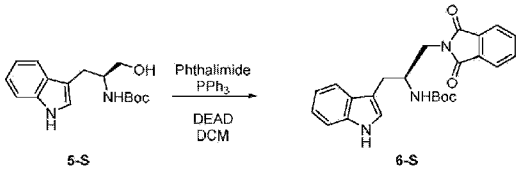
2-S 5-S

[0175] To a solution of L-tryptophanol (**2-S**) (2.0 g, 10.4 mmol) in CH_3CN (42 mL, 4 mL/mmol) was added Di-*tert*-butyl dicarbonate (4.6 g, 20.8 mmol). The reaction mixture was stirred at 23 °C for 3 h, concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to afford **5-S** (2.24 g, 73%).

[0176] R_f= 0.5 (CH_2Cl_2:CH_3OH, 9:1).

[0177] ^1H NMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.65 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.37 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.23-7.11 (m, 2H), 7.06 (s, 1H), 4.81 (s, 1H), 3.99 (s, 1H), 3.70 (dd, *J* = 11.0, 3.8 Hz, 1H), 3.61 (dd, *J* = 11.0, 5.5 Hz, 1H), 3.00 (d, *J* = 6.7 Hz, 2H), 2.01 (s, 1H), 1.42 (s, 9H).

B)



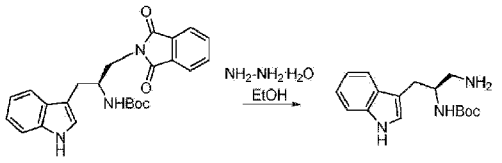
5-S 6-S

[0178] To a solution of **5-S** (1.2 g, 4.13 mmol) in CH_2Cl_2 (24.8 mL, 6 mL/mmol) was added phthalimide (1.33 g, 9.1 mmol), triphenylphosphine (2.4 g, 9.1 mmol) and the mixture was cooled at 0 °C. A solution of diethyl azodicarboxylate solution (3 mL, 10.32 mmol) in CH_2Cl_2 (12.4 mL, 3 mL/mmol) was added for 15 min. The reaction was stirred at 23 °C for 16 h, concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to afford **6-S** (2.8 g, >100%).

[0179] R_f= 0.7 (CH_2Cl_2:CH_3OH, 9:1).

[0180] ^1H NMR (400 MHz, $CDCl_3$): δ 8.49 (s, 1H), 7.80 (dd, *J* = 5.4, 3.1 Hz, 2H), 7.66 (dd, *J* = 5.6, 3.2 Hz, 2H), 7.60 (d, *J* = 7.8 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.21-7.04 (m, 3H)., 4.74 (s, 1H), 4.42 (s, 1H), 3.83 (dd, *J* = 13.9, 3.7 Hz, 1H), 3.72 (dd, *J* = 13.9, 9.9 Hz, 1H), 3.10-3.01 (m, 2H), 1.23 (s, 9H).

C)



6-S 7-S

[0181] To a solution of **6-S** (0.86 g, 2.07 mmol) in ethanol (72 mL, 36 mL/mmol) was added hydrazine monohydrate (10 mL, 207 mmol). The reaction mixture was stirred at 80 °C in sealed tube for 2.25 h, concentrated under vacuum. Flash chromatography (EtOAc:CH_3OH, from 100:1 to 50:50) to afford **7-S** (1.0 g, 84%).

[0182] R_f= 0.2 (EtOAc:CH_3OH, 6:4).

[0183] ^1H NMR (400 MHz, CD_3OD): δ 7.61 (d, *J* = 7.9 Hz, 1H), 7.35 (d, *J* = 8.1 Hz, 1H), 7.13-6.97 (m, 2H), 7.09 (s, 1H), 4.06-3.96 (m, 1H), 3.01-2.76 (m, 4H), 1.38 (s, 9H).

[0184] ESI-MS *m/z:* 290.3 $(M+H)^+$.

D)

7-S 8-S

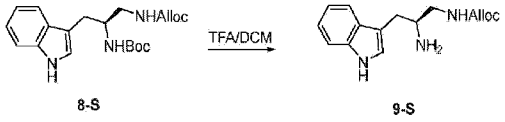
[0185] To a solution of **7-S** (0.95 g, 3.3 mmol) in CH_3CN (33 mL, 10 mL/mmol) and DMF (3.3 mL, 1 mL/mmol) was added *N,N*-diisopropylethylamine (0.5 mL, 2.6 mmol) and allyl chloroformate (3.5 mL, 33 mmol). The reaction was stirred at 23 °C for 20 h. The mixture was diluted with EtOAc, NH_4Cl was added and the mixture was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (Hexane:EtOAc, from 100:1 to 1:100) to afford **8-S** (0.88 g, 73%).

[0186] R_f= 0.5 (Hexane:EtOAc, 1:1).

[0187] ^1H NMR (400 MHz, $CDCl_3$): δ 8.17 (s, 1H), 7.63 (d, *J* = 7.8 Hz, 1H), 7.20 (dd, *J* = 8.1, 0.9 Hz, 1H), 7.13 (dddd, *J* = 27.8, 8.0, 7.0, 1.1 Hz, 2H), 7.06 (d, *J* = 2.4 Hz, 1H), 5.90 (ddt, *J* = 17.3, 10.7, 5.6 Hz, 1H), 5.31-5.18 (m, 2H), 5.09 (s, 1H), 4.80 (s, 1H), 4.59-4.52 (m, 2H), 4.03 (s, 1H), 3.37 (dt, *J* = 10.0, 4.7 Hz, 1H), 3.21 (s, 1H), 3.05-2.87 (m, 2H), 1.42 (s, 9H).

[0188] ESI-MS *m/z:* 274.3 $(M\text{-}Boc+H)^+$.

E)



8-S 9-S

[0189] To a solution of **8-S** (0.875 g, 2.3 mmol) in CH_2Cl_2 (38 mL, 16.6 mL/mmol) was added trifluoroacetic acid (19 mL, 8.3 mL/mmol). The reaction mixture was stirred at 23 °C for 2 h, concentrated under vacuum to give crude **9-S** which was used in the next steps without further purification.

[0190] R_f= 0.2 (CH_2Cl_2:CH_3OH, 9:1).

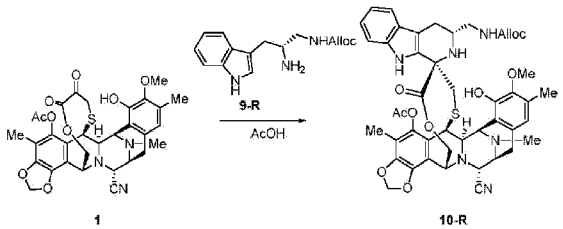
[0191] ^1H NMR (400 MHz, CD_3OD): δ 7.56 (d, *J* = 7.8 Hz, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.21 (s, 1H), 7.13 (t, *J* = 7.5 Hz, 1H), 7.05 (t, *J* = 7.5 Hz, 1H), 5.94 (ddt, *J* = 16.4, 10.8, 5.6 Hz, 1H), 5.34-5.16 (m, 2H), 4.56 (d, *J* = 5.5 Hz, 2H), 3.60 (bs, 1H), 3.43 (dd, *J* = 14.9, 3.9 Hz, 1H), 3.37-3.31 (m, 1H), 3.14-2.99 (m, 2H).

[0192] ESI-MS *m/z:* 274.3 $(M+H)^+$.

Example 5

[0193]

A)



1 10-R

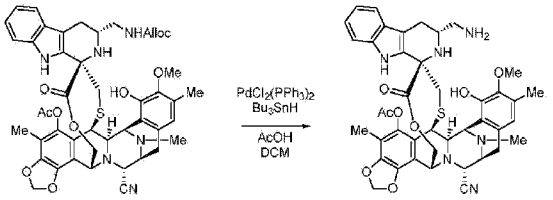
[0194] To a solution of **1** (1.45 g, 2.33 mmol) in acetic acid (58 mL, 0.08 M) was added **9-R** (0.95 g, 3.50 mmol). The reaction mixture was stirred a 50 °C for 18 h and then acetic acid was evaporated. An aqueous saturated solution of NaHCOs was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4. Flash chromatography (Hexane:EtOAc, 1:1) gives compound **10-R** (1.3 g, 64%).

[0195] R_f= 0.5 (Hexane:EtOAc, 1:1).

[0196] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66 (s, 1H), 7.36 (d, *J* = 7.9 Hz, 1H), 7.27 (d, *J* = 7.9 Hz, 1H), 7.10 (ddd, *J* = 8.3, 7.0, 1.3 Hz, 1H), 7.01 (td, *J* = 7.5, 7.0, 1.0 Hz, 1H), 6.62 (s, 1H), 6.23 (d, *J* = 1.4 Hz, 1H), 6.01 (d, *J* = 1.4 Hz, 1H), 5.99-5.89 (m, 1H), 5.79 (s, 1H), 5.44-5.21 (m, 2H), 5.14-4.99 (m, 2H), 4.63 (ddd, *J* = 7.3, 4.4, 1.5 Hz, 2H), 4.36 (s, 1H), 4.33-4.24 (m, 1H), 4.29-4.26 (m, 1H), 4.21 (d, *J* = 2.7 Hz, 1H), 4.19-4.13 (m, 3H), 3.80 (s, 3H), 3.56 (s, 1H), 3.48-3.43 (m, 3H), 3.27 (dt, *J* = 13.2, 4.0 Hz, 1H), 3.04-2.88 (m, 2H), 2.56 (dd, *J* = 15.2, 3.8 Hz, 1H), 2.49-2.35 (m, 2H), 2.31 (s, 3H), 2.28 (s, 3H), 2.17 (s, 3H), 2.07 (s, 3H).

[0197] ESI-MS *m/z:* 877.3 $(M+H)^+$.

B)



10-R 11-R

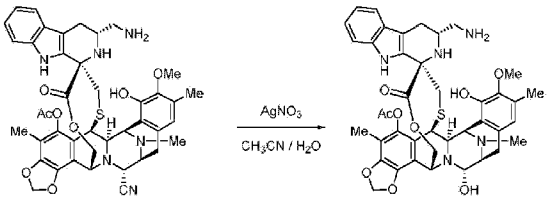
[0198] To a solution of **10-R** (600 mg, 0.68 mmol) in CH_2Cl_2 (12 mL, 18 mL/mmol) was added bis(triphenylphosphine)palladium(II) dichloride (77 mg, 0.1 mmol) and acetic acid (0.4 mL, 6.8 mmol). Tributyltin hydride (1.1 mL, 4.08 mmol) was added at 0 °C, the reaction mixture was stirred at 0 °C for 0.5 h and concentrated under vacuum. The crude obtained was diluted with EtOAc, saturated aqueous solution of NH_4Cl was added, and the mixture was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4. filtered, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, from 100:1 to 1:100 and EtOAc:CH_3OH, from 100:1 to 1:100) to afford **11-R** (440 mg, 82%).

[0199] R_f= 0.5 (CH_2Cl_2:CH_3OH, 1:1).

[0200] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.64 (s, 1H), 7.38 (d, *J* = 7.9 Hz, 1H), 7.29 (d, *J* = 8.1 Hz, 1H), 7.11 (ddt, *J* = 8.3, 7.0, 1.4 Hz, 1H), 7.03 (ddt, J = 8.3, 7.0, 1.4 Hz, 1H), 6.58 (s, 1H), 6.24 (d, *J* = 1.5 Hz, 1H), 6.02 (d, *J* = 1.5 Hz, 1H), 5.02 (d, *J* = 11.8 Hz, 1H), 4.63 (s, 1H), 4.36 (s, 1H), 4.28 (d, *J* = 5.1 Hz, 1H), 4.21 (d, *J* = 2.2 Hz, 1H), 4.16 (s, 1H), 3.80 (s, 3H), 3.51-3.39 (m, 4H), 3.32-3.13 (m, 3H), 2.95 (d, *J* = 8.9 Hz, 2H), 2.89-2.76 (m, 2H), 2.73-2.57 (m, 1H), 2.42 (d, *J* = 14.8 Hz, 1H), 2.36 (s, 3H), 2.25 (s, 3H), 2.16 (s, 3H), 2.09 (s, 3H).

[0201] ESI-MS *m/z:* 793.2 $(M+H)^+$.

C)



11-R 12-R

[0202] To a solution of **11-R** (850 mg, 1.07 mmol) in CH_3CN:H_2O (1.39:1, 70 mL, 0.015 M) was added $AgNO_3$ (3.64 g, 21.4 mmol). After 17 h at 23 °C, the reaction was quenched with a mixture 1:1 of saturated aqueous solutions of NaCl and NaHCOs, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to give **12-R** (553 mg, 66%).

[0203] R_f= 0.3 (CH_2Cl_2:CH_3OH, 9:1).

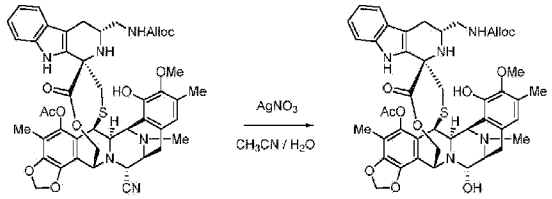
[0204] ^1H NMR (500 MHz, $CDCl_3$): δ 7.60 (s, 1H), 7.38 (d, *J* = 7.9 Hz, 1H), 7.28 (d, *J* = 7.9 Hz, 1H), 7.11 (ddt, *J* = 8.3, 7.1, 1.2 Hz, 1H), 7.02 (ddt, *J* = 8.3, 7.1, 1.2 Hz, 1H), 6.58 (s, 1H), 6.22 (s, 1H), 6.00 (s, 1H), 5.16 (d, *J* = 11.5 Hz, 1H), 4.87 (s, 1H), 4.54 (s, 1H), 4.51 (d, *J* = 3.3 Hz, 1H), 4.17 (d, *J* = 5.4 Hz, 1H), 4.07 (dd, *J* = 11.3, 2.2 Hz, 1H), 3.81 (s, 3H), 3.52 (d, *J* = 5.1 Hz, 1H), 3.24 (d, *J* = 8.8 Hz, 2H), 2.99-2.78 (m, 4H), 2.66 (dd, *J* = 14.9, 3.5 Hz, 1H), 2.49-2.39 (m, 2H), 2.38 (s, 3H), 2.28 (m, 2H), 2.25 (s, 3H), 2.21-2.16 (m, 2H), 2.15 (s, 3H), 2.08 (s, 3H).

[0205] ^{13}C NMR (101 MHz, CD_3OD): δ 171.7, 169.4, 148.7, 145.9, 143.7, 141.4, 140.9, 136.9, 130.8, 130.0, 129.7, 126.0, 121.4, 121.0, 119.7, 119.1, 118.4, 117.5, 114.9, 110.8, 107.5, 106.4, 102.1, 91.3, 63.2, 60.0, 59.0, 58.6, 55.3, 54.6, 52.7, 52.4, 48.4, 45.8, 42.5, 40.2, 24.5, 23.2, 19.2, 15.0, 8.2.

[0206] ESI-MS *m/z:* 766.2 $(M\text{-}H_2O+H)^+$.

[0207] (+)-HR-ESI-TOF-MS *m/z*: 766.2972 $[M\text{-}H_2O+H]^+$ (Calcd. for $C_{41}H_{44}N_5O_8S^+$: 766.2905).

C')



10-R 13-R

[0208] To a solution of **10-R** (700 mg, 0.8 mmol) in CH_3CN:H_2O (1.39:1, 87.5 mL, 0.015 M) was added $AgNO_3$ (2.66 g, 16 mmol). After 20 h at 23 °C, the reaction mixture was quenched with a mixture 1:1 of saturated aqueous solutions of NaCl and NaHCOs, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2: CH_3OH, from 99:1 to 85:15) to give **13-R** (438 mg, 63%).

[0209] Rf= 0.40 (CH_2Cl_2:CH_3OH, 9:1).

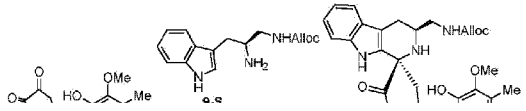
[0210] ^1H NMR (400 MHz, $CDCl_3$): δ 7.64 (s, 1H), 7.37 (d, *J* = 7.9 Hz, 1H), 7.32-7.20 (m, 1H), 7.11 (t, *J* = 7.7 Hz, 1H), 7.01 (t, *J* = 7.4 Hz, 1H), 6.62 (s, 1H), 6.21 (s, 1H), 6.05-5.90 (m, 1H), 5.99 (s, 1H), 5.75 (d, *J* = 6.0 Hz, 1H), 5.40-5.07 (m, 4H), 4.88 (d, *J* = 14.7 Hz, 1H), 4.68-4.50 (m, 3H), 4.28-4.13 (m, 1H), 4.08 (dt, *J* = 11.4, 2.4 Hz, 1H), 3.83 (s, 3H), 3.68-3.40 (m, 4H), 3.37-3.19 (m, 2H), 2.98-2.79 (m, 2H), 2.59-2.36 (m, 3H), 2.29 (s, 3H), 2.27 (s, 3H), 2.14 (s, 3H), 2.10-2.16 (m, 1H), 2.08 (s, 3H).

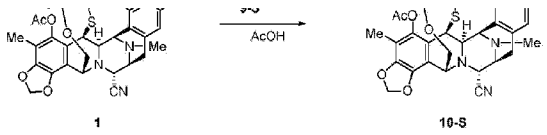
[0211] ESI-MS *m/z:* 850.3 $(M\text{-}H_2O+H)^+$.

Example 6

[0212]

A)





1 10-S

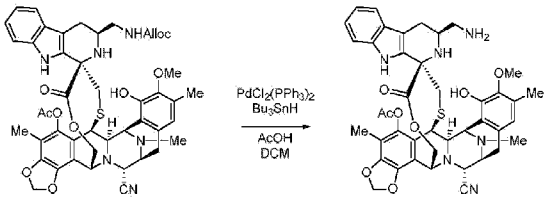
[0213] To a solution of **1** (955 mg, 1.5 mmol) in acetic acid (37.5 mL, 0.08 M) was added **9-S** (627 mg, 2.29 mmol). The reaction mixture was stirred a 50 °C for 18 h and then acetic acid was evaporated. An aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4. Flash chromatography (Hexane:EtOAc, 1:1) gives compound **10-S** (756 mg, 58%).

[0214] R_f= 0.4 (Hexane: EtOAc, 1:1).

[0215] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (s, 1H), 7.36 (d, *J* = 7.9 Hz, 1H), 7.24 (d, *J* = 7.9 Hz, 1H), 7.10 (ddd, *J* = 8.3, 7.0, 1.3 Hz, 1H), 7.01 (td, *J* = 7.5, 7.0, 1.0 Hz, 1H), 6.68 (s, 1H), 6.23 (d, *J* = 1.4 Hz, 1H), 6.01 (d, *J* = 1.4 Hz, 1H), 6.07-5.93 (m, 1H), 5.82 (s, 1H), 5.41-5.19 (m, 2H), 5.1 (d, *J* = 11.7 Hz, 1H), 4.66 (dt, *J* = 5.9, 1.3 Hz, 1H), 4.57 (s, 1H), 4.37 (s, 1H), 4.33-4.20 (m, 3H), 3.81 (s, 3H), 3.46 (d, *J* = 4.2 Hz, 2H), 3.22-3.13 (m, 1H), 3.11-2.88 (m, 4H), 2.66 (dd, *J* = 15.2, 4.2 Hz, 1H), 2.51 (dd, *J* = 15.3, 6.0 Hz, 1H), 2.43-2.32 (m, 2H), 2.31 (s, 3H), 2.26 (s, 3H), 2.19 (s, 3H), 2.04 (s, 3H).

[0216] ESI-MS *m/z:* 877.3 $(M+H)^+$.

B)



10-S 11-S

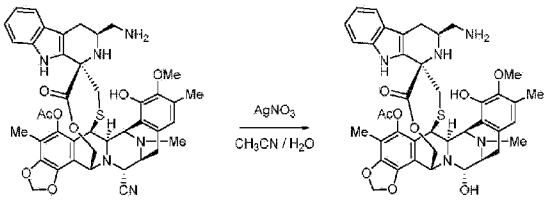
[0217] To a solution of **10-S** (650 mg, 0.72 mmol) in CH_2Cl_2 (13.3 mL, 18 mL/mmol) was added bis(triphenylphosphine)palladium(II) dichloride (83 mg, 0.11 mmol) and acetic acid (0.42 mL, 7.4 mmol). Tributyltin hydride (1.2 mL, 4.4 mmol) was added at 0 °C, the reaction mixture was stirred at 23 °C for 0.5 h, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, from 100:1 to 1:100 and EtOAc:CH_3OH, from 100:1 to 1:100) to afford **11-S** (445 mg, 78%).

[0218] R_f= 0.5 (CH_2Cl_2:CH_3OH, 1:1).

[0219] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74 (s, 1H), 7.36 (d, *J* = 7.9 Hz, 1H), 7.26 (d, *J* = 8.1 Hz, 1H), 7.12 (ddt, *J* = 8.3, 7.0, 1.4 Hz, 1H), 7.02 (ddt, *J* = 8.3, 7.0, 1.4 Hz, 1H), 6.62 (s, 1H), 6.26 (d, *J* = 1.5 Hz, 1H), 6.04 (d, *J* = 1.5 Hz, 1H), 5.12 (d, *J* = 11.8 Hz, 1H), 4.59 (s, 1H), 4.42 (s, 1H), 4.36-4.17 (m, 3H), 3.81 (s, 3H), 3.51-3.39 (m, 3H), 2.98-2.75 (m, 4H), 2.69-2.60 (m, 2H), 2.47 (d, *J* = 16.1 Hz, 1H), 2.38 (s, 3H), 2.35-2.17 (m, 2H), 2.28 (s, 3H), 2.13 (s, 3H), 2.04 (s, 3H).

[0220] ESI-MS *m/z:* 793.3 $(M+H)^+$.

C)



11-S 12-S

[0221] To a solution of **11-S** (435 mg, 0.55 mmol) in CH_3CN:H_2O (1.39:1, 38.5 mL, 0.015 M) was added $AgNO_3$ (1.84 g, 11 mmol). After 24 h at 23 °C, the reaction was quenched with a mixture 1:1 of saturated aqueous solutions of NaCl and NaHCOs, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to give **12-S** (152 mg, 35%).

[0222] R_f= 0.2 (CH_2Cl_2:CH_3OH, 9:1).

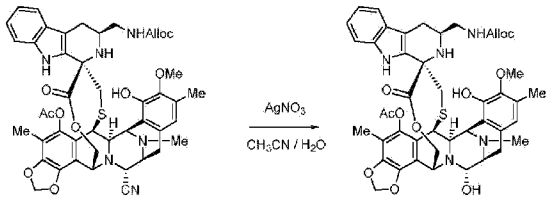
[0223] ^{1}H NMR (500 MHz, CD_3OD): δ 7.34 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.28 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.04 (ddt, *J* = 8.2, 7.0, 1.1 Hz, 1H), 6.95 (ddt, *J* = 8.2, 7.0, 1.2 Hz, 1H), 6.55 (s, 1H), 6.31-6.25 (m, 1H), 6.15-6.05 (m, 1H), 5.31 (d, *J* = 11.4 Hz, 1H), 4.91 (s, 1H), 4.64 (s, 1H), 4.40-4.19 (m, 3H), 3.76 (s, 3H), 3.64 (d, *J* = 5.2 Hz, 1H), 3.44 (d, *J* = 9.0 Hz, 1H), 3.03-2.85 (m, 4H), 2.85-2.65 (m, 2H), 2.59 (d, *J* = 15.6 Hz, 1H), 2.52-2.39 (m, 2H), 2.37 (s, 3H), 2.27 (s, 3H), 2.09 (s, 3H), 2.00 (s, 3H).

[0224] ^{13}C NMR (126 MHz, CD_3OD): δ 171.4, 169.3, 148.6, 145.8, 143.5, 141.2, 140.8, 136.5, 131.2, 130.3, 129.5, 126.3, 121.6, 121.2, 119.8, 119.4, 118.6, 117.5, 114.9, 111.0, 107.5, 107.4, 102.2, 91.1, 63.5, 60.5, 59.2, 58.5, 55.3, 54.7, 53.4, 52.7, 48.6, 44.7, 42.7, 39.9, 24.3, 23.4, 19.2, 15.1, 8.2.

[0225] ESI-MS *m/z:* 766.2 $(M\text{-}H_2O+H)^+$.

[0226] (+)-HR-ESI-TOF-MS *m/z*: 766.2958 $[M\text{-}H_2O+H]^+$ (Calcd. for $C_{41}H_{44}N_5O_8S$: 766.2905).

C')



10-S 13-S

[0227] To a solution of **10-S** (5 mg, 0.006 mmol) in CH_3CN:H_2O (1.39:1, 0.5 mL, 0.015 M) was added $AgNO_3$ (29 mg, 0.17 mmol). After 20 h at 23 °C, the reaction mixture was quenched with a mixture 1:1 of saturated aqueous solutions of NaCl and NaHCOs, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to give **13-S** (5 mg, 100%).

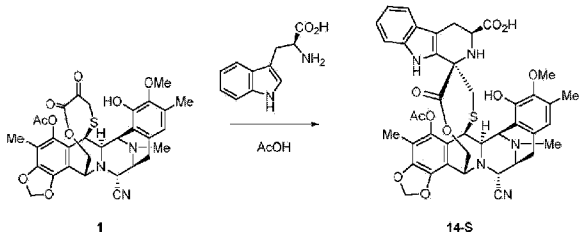
[0228] R_f= 0.40 (CH_2Cl_2:CH_3OH, 9:1).

[0229] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (s, 1H), 7.37 (d, *J* = 7.9 Hz, 1H), 7.32-7.20 (m, 1H), 7.12 (t, *J* = 7.7 Hz, 1H), 7.02 (t, *J* = 7.4 Hz, 1H), 6.84 (s, 1H), 6.24 (s, 1H), 6.08-5.97 (m, 1H), 6.01 (s, 1H), 5.87 (s, 1H), 5.42-5.19 (m, 4H), 4.88 (s, 1H), 4.69-4.65 (m, 2H), 4.58 (s, 1H), 4.28-4.13 (m, 2H), 3.84 (s, 3H), 3.68-3.40 (m, 2H), 3.24-3.15 (m, 2H), 3.08-2.90 (m, 2H), 2.73-2.57 (m, 2H), 2.53-2.37 (m, 3H), 2.34 (s, 3H), 2.25 (s, 3H), 2.14 (s, 3H), 2.10-2.16 (m, 1H), 2.03 (s, 3H). ESI-MS *m/z:* 850.3 $(M\text{-}H_2O+H)^+$.

Reference Example 7. Synthesis of Reference Compounds 14-S and 15-S

[0230]

A)



1 14-S

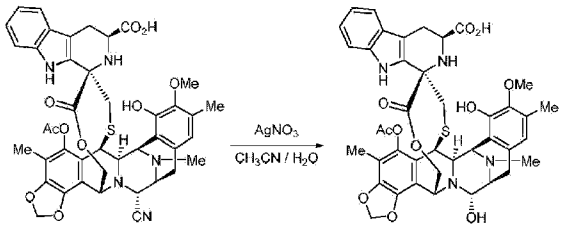
[0231] To a solution of **1** (50 mg, 0.08 mmol) in acetic acid (1 mL, 0.08 M) was added L-tryptophan (50 mg, 0.24 mmol). The reaction mixture was stirred at 50 °C for 17 h and then acetic acid was evaporated. An aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 80:20) gave compound **14-S** (58 mg, 90%).

[0232] R_f= 0.20 (CH_2Cl_2:CH_3OH, 10:1).

[0233] ^1H NMR (400 MHz, $CDCl_3$): δ 7.77 (s, 1H), 7.39 (d, *J* = 7.9 Hz, 1H), 7.25 (d, *J* = 7.9 Hz, 1H), 7.13 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H), 7.04 (td, *J* = 7.5, 7.1, 1.0 Hz, 1H), 6.56 (s, 1H), 6.24 (d, *J* = 1.3 Hz, 1H), 6.03 (d, *J* = 1.3 Hz, 1H), 5.15 (d, *J* = 11.7 Hz, 1H), 4.62 (s, 1H), 4.43 (s, 1H), 4.35 (dd, *J* = 11.7, 2.1 Hz, 1H), 4.28 (dd, *J* = 5.2, 1.6 Hz, 1H), 4.20 (s, 1H), 3.78 (s, 3H), 3.52-3.41 (m, 4H), 3.07-2.88 (m, 2H), 2.91-2.80 (m, 2H), 2.42-2.21 (m, 2H), 2.35 (s, 3H), 2.27 (s, 3H), 2.14 (s, 3H), 2.04 (s, 3H).

[0234] ESI-MS *m/z:* 808.6 $(M+H)^+$.

B)



14-S 15-S

[0235] To a solution of **14-S** (52 mg, 0.066 mmol) in CH_3CN:H_2O (2:1, 4.5 mL, 0.015 M) was added $AgNO_3$ (164 mg, 1.45 mmol). After 20 h at 23 °C, a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$ was added, stirred for 15 min, diluted with CH_2Cl_2, stirred for 30 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 70:30) to afford **15-S** (18 mg, 35%).

[0236] R_f= 0.15 (CH_2Cl_2:CH_3OH, 9:1).

[0237] ^1H NMR (400 MHz, CD_3OD): δ 7.76 (s, 1H), 7.40 (d, *J* = 7.8 Hz, 1H), 7.25 (d, *J* = 7.8 Hz, 1H), 7.14 (t, *J*= 7.4 Hz, 1H), 7.04 (t, *J* = 7.4 Hz, 1H), 6.58 (s, 1H), 6.23 (d, *J* = 1.3 Hz, 1H), 6.01 (d, *J* = 1.3 Hz, 1H), 5.28 (d, *J* = 12.7 Hz, 1H), 4.95 (s, 1H), 4.53 (s, 1H), 4.28 (dd, *J* = 11.4, 2.0 Hz, 1H), 4.21 (s, 1H), 3.80 (s, 3H), 3.58 (s, 1H), 3.52-3.47 (m, 2H), 3.28 (s, 1H), 3.03 (dd, *J* = 15.8, 5.2 Hz, 1H), 2.91-2.82 (m, 3H), 2.44 (d, *J* = 15.4 Hz, 1H),

2.36 (s, 3H), 2.35-2.31 (m, 1H), 2.28 (s, 3H), 2.15 (s, 3H), 2.03 (s, 3H).

[0238] ^{13}C NMR (101 MHz, $CDCl_3$): δ 173.7, 171.2, 168.7, 147.5, 145.7, 142.8, 141.2, 140.8, 135.6, 129.8, 126.3, 122.8, 121.5, 121.2, 119.9, 118.6, 117.7, 115.0, 111.1, 101.9, 81.5, 66.8, 62.9, 60.4, 57.9, 55.8, 55.1, 52.3, 42.3, 41.3, 38.3, 31.9, 29.4, 28.9, 24.5, 24.0, 23.8, 22.7, 20.5, 16.0, 9.7.

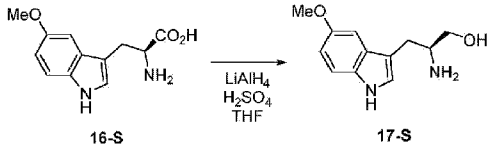
[0239] ESI-MS *m/z:* 781.6 $(M-H_2O+H)^+$.

[0240] (+)-HR-ESI-TOF-MS *m/z:* 781.2610 $[M-H_2O+H]^+$ (Calcd. for $C_{41}H_{41}N_4O_{10}S$: 781.2538).

Example 8.

A) Synthesis of (*S*)-5-methoxy-tryptophanol (17-S)

[0241]



16-S 17-S

[0242] To a solution of $LiAlH_4$ (23.4 mL, 1.0 M in THF, 23.4 mmol) at -40 °C was added carefully H_2SO_4 (0.31 mL, 5.57 mmol) and a suspension of 5-methoxy-L-tryptophan (**16-S**) (1.0 g, 4.26 mmol, Chem-Impex) in THF (13.4 mL, 0.3 M). The reaction mixture was left evolution at 23 °C, heated for 3 h at 80 °C and 18 h at 23 °C. Cool at -21 °C the reaction mixture was quenched carefully with NaOH 2N until basic pH. EtOAc was added and the mixture filtered through Celite® and washed with CH_3OH. The crude was concentrated under vacuum to give **17-S** as a crude which was used in the next step without further purification.

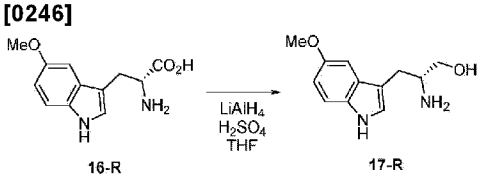
[0243] R_f= 0.2 (CH_2Cl_2:CH_3OH, 4:1).

[0244] ^1H NMR (400 MHz, $CDCl_3$): δ 7.19 (dt, *J* = 8.8, 0.7 Hz, 1H), 7.06-7.00 (m, 2H), 6.72 (dd, *J* = 8.8, 2.4 Hz, 1H), 3.77 (s, 3H), 3.63-3.48 (m, 1H), 3.42-3.33 (m, 1H), 3.17-3.06 (m, 1H), 2.86 (ddt, *J* = 14.3, 6.1, 0.8 Hz, 1H), 2.66 (dd, *J* = 14.3, 7.5 Hz, 1H).

[0245] ESI-MS *m/z:* 221.4 $(M+H)^+$.

B) Synthesis of (*R*)-5-methoxy-tryptophanol (17-R)

[0246]



16-R 17-R

[0247] To a solution of $LiAlH_4$ (11.7 mL, 1.0 M in THF, 11.7 mmol) at -40 °C was added carefully H_2SO_4 (0.31

mL, 5.75 mmol) and a suspension of 5-methoxy-D-tryptophan (**16-R**) (0.5 g, 2.13 mmol, Aldrich) in THF (6.7 mL, 0.3 M). The reaction mixture was left evolution at 23 °C, heated for 3.5 h at 80 °C and 18 h at 23 °C. Cool at -21 °C the reaction mixture was quenched carefully with NaOH 2N until basic pH. EtOAc was added and the mixture filtered through Celite® and washed with CH_3OH. The crude was concentrated under vacuum to give **17-R** as a crude which was used in the next step without further purification.

[0248] R_f= 0.2 (CH_2Cl_2:CH_3OH, 4:1).

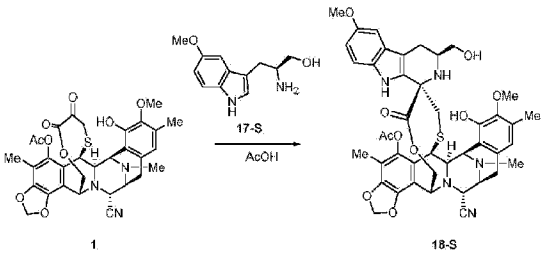
[0249] ^{1}H NMR (400 MHz, CD_3OD): δ 7.20 (d, *J* = 8.9 Hz, 1H), 7.06-6.96 (m, 2H), 6.71 (dd, *J* = 8.8, 2.5 Hz, 1H), 3.75 (s, 3H), 3.62-3.52 (m, 1H), 3.37 (dd, *J* = 10.8, 7.0 Hz, 1H), 3.09 (br s, 1H), 2.82 (dd, *J* = 14.3, 5.9 Hz, 1H), 2.62 (dd, *J* = 14.4, 7.6 Hz, 1H).

[0250] ESI-MS *m/z:* 221.6 $(M+H)^+$.

Reference Example 9

[0251]

A)



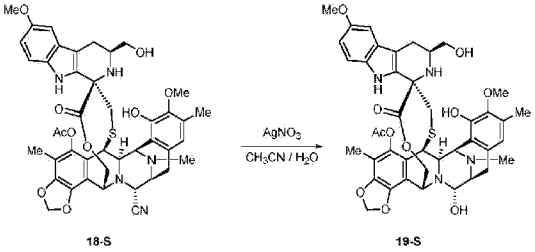
[0252] To a solution of **1** (530 mg, 0.85 mmol) in acetic acid (10.6 mL, 0.08 M) was added **17-S** (469 mg, 2.13 mmol). The reaction mixture was stirred at 50 °C for 18 h and then acetic acid was evaporated. An aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, 1:1) gave compound **18-S** (420 mg, 60%).

[0253] R_f= 0.3 (Hexane:EtOAc, 1:1).

[0254] ^{1}H NMR (400 MHz, CD_3OD): δ 7.13 (d, *J* = 8.8 Hz, 1H), 6.80 (d, *J* = 2.4 Hz, 1H), 6.66 (dd, *J*= 8.8, 2.5 Hz, 1H), 6.51 (s, 1H), 6.27 (s, 1H), 6.11 (s, 1H), 5.21 (d, *J* = 11.7 Hz, 1H), 4.67 (s, 1H), 4.49-4.29 (m, 4H), 3.75 (s, 3H), 3.73 (s, 3H), 3.47 (t, *J* = 5.8 Hz, 3H), 3.37 (d, *J* = 5.1 Hz, 1H), 3.01-2.81 (m, 2H), 2.75 (d, *J* = 7.4 Hz, 1H), 2.66 (dd, *J* = 15.1, 4.1 Hz, 1H), 2.55-2.35 (m, 4H), 2.34 (s, 3H), 2.28 (s, 3H), 2.11 (s, 3H), 1.99 (s, 3H).

[0255] ESI-MS *m/z:* 824.3 $(M+H)^+$.

B)



[0256] To a solution of **18-S** (420 mg, 0.519 mmol) in CH_3CN:H_2O (1.39:1, 36 mL, 0.015 M) was added $AgNO_3$ (2.60 g, 15.3 mmol). After 3 h at 23 °C, the reaction mixture was quenched with a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to obtain **19-S** (250 mg, 60%).

[0257] R_f= 0.45 (CH_2Cl_2:CH_3OH, 9:1).

[0258] ^{1}H NMR (500 MHz, CD_3OD): δ 7.15 (dd, *J* = 8.9, 0.6 Hz, 1H), 6.82 (dd, *J* = 2.4, 0.6 Hz, 1H), 6.68 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.54 (s, 1H), 6.27 (d, *J* = 1.3 Hz, 1H), 6.08 (d, *J* = 1.3 Hz, 1H), 5.30 (d, *J* = 11.5 Hz, 1H), 4.62 (s, 1H), 4.34 (dd, *J* = 11.4, 2.0 Hz, 1H), 4.31-4.27 (m, 2H), 3.76 (s, 3H), 3.75 (s, 3H), 3.66-3.58 (m, 1H), 3.55-3.45 (m, 2H), 3.42 (d, *J* = 7.8 Hz, 1H), 2.93-2.73 (m, 3H), 2.68 (dd, *J* = 15.1, 4.2 Hz, 1H), 2.54 (d, *J* = 15.4 Hz, 1H), 2.42 (dd, *J* = 15.1, 10.1 Hz, 2H), 2.35 (s, 3H), 2.29 (s, 3H), 2.09 (s, 3H), 2.00 (s, 3H).

[0259] ^{13}C NMR (126 MHz, CD_3OD): δ 172.7, 170.8, 155.1, 149.9, 147.2, 145.0, 142.6, 142.2, 133.1, 132.4, 132.1, 131.3, 128.1, 122.5, 121.6, 120.3, 116.4, 113.0, 112.9, 111.4, 109.0, 103.6, 100.8, 92.5, 66.6, 65.0, 61.7, 60.4, 59.9, 56.7, 56.1, 54.8, 54.1, 51.7, 44.1, 41.3, 30.7, 25.4, 24.7, 20.6, 16.3, 9.5.

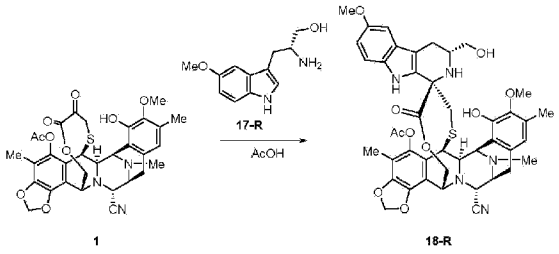
[0260] ESI-MS *m/z:* 798.1 $(M-H_2O+H)^+$.

[0261] (+)-HR-ESI-TOF-MS *m/z*: 797.2899 $[M-H_2O+H]^+$ (Calcd. for $C_{42}H_{45}N_4O_{10}S$ 797.2851).

Reference Example 10

[0262]

A)

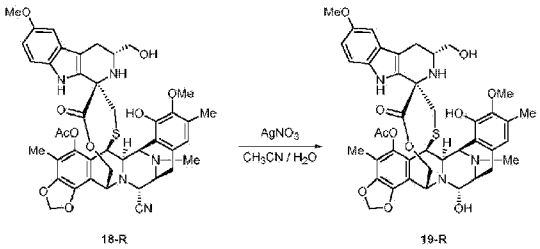


[0263] To a solution of **1** (311 mg, 0.50 mmol) in acetic acid (6.25 mL, 0.08 M) was added **17-R** (220 mg, 1.0 mmol). The reaction mixture was stirred at 50 °C for 18 h and then acetic acid was evaporated. An aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, 1:1) gave compound **18-R** (280 mg, 68%).

[0264] R_f= 0.3 (Hexane: EtOAc, 1:1).

[0265] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53 (s, 1H), 7.18 (d, *J* = 8.7 Hz, 1H), 6.82 (d, *J* = 2.4 Hz, 1H), 6.78 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.60 (s, 1H), 6.23 (s, 1H), 6.02 (s, 1H), 5.76 (s, 1H), 5.04 (d, *J* = 11.7 Hz, 1H), 4.62 (s, 1H), 4.36 (s, 1H), 4.28 (d, *J* = 5.0 Hz, 1H), 4.24-4.09 (m, 3H), 3.81 (s, 3H), 3.79 (s, 3H), 3.64 (s, 1H), 3.47-3.40 (m, 3H), 3.01-2.90 (m, 2H), 2.53 (d, *J* = 6.9 Hz, 2H), 2.45-2.41 (m, 1H), 2.40 (s, 3H), 2.27 (s, 3H), 2.22-2.14 (m, 1H), 2.18 (s, 3H), 2.06 (s, 3H). ESI-MS *m/z:* 824.3 $(M+H)^+$.

B)



18-R 19-R

[0266] To a solution of **18-R** (330 mg, 0.40 mmol) in CH_3CN:H_2O (1.39:1, 28 mL, 0.015 M) was added $AgNO_3$ (2.04 g, 12.0 mmol). After 3 h at 23 °C, the reaction was quenched with a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to obtain **19-R** (224 mg, 69%).

[0267] R_f= 0.44 (CH_2Cl_2:CH_3OH, 9:1).

[0268] ^{1}H NMR (500 MHz, CD_3OD): δ 7.14 (dd, *J* = 8.8, 0.5 Hz, 1H), 6.83 (d, *J* = 2.5 Hz, 1H), 6.68 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.59 (s, 1H), 6.26 (d, *J* = 1.4 Hz, 1H), 6.07 (d, *J* = 1.4 Hz, 1H), 5.21 (d, *J* = 11.5 Hz, 1H), 4.68-4.55 (m, 1H), 4.32-4.25 (m, 2H), 4.12 (dd, *J* = 11.5, 2.1 Hz, 1H), 3.75 (s, 3H), 3.74 (s, 3H), 3.60 (d, *J* = 5.2 Hz, 1H), 3.57-3.45 (m, 3H), 3.41 (d, *J* = 8.8 Hz, 1H), 2.97-2.83 (m, 3H), 2.73 (dd, *J* = 15.0, 3.4 Hz, 1H), 2.69 (d, *J* = 14.9 Hz, 1H), 2.34 (s, 3H), 2.30 (s, 3H), 2.20 (dd, *J* = 15.1, 10.4 Hz, 1H), 2.12 (s, 3H), 2.11-2.08 (m, 1H), 2.05 (s, 3H).

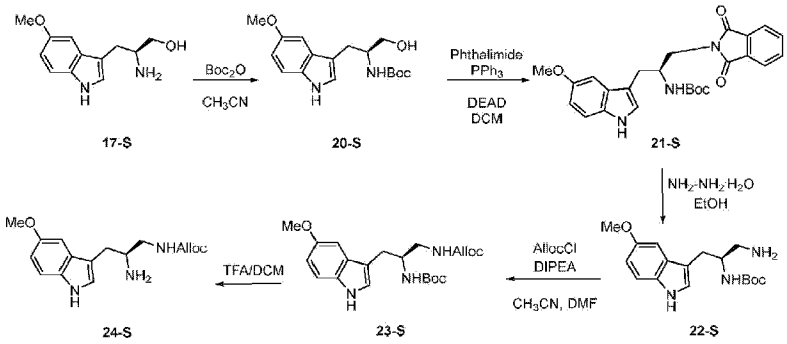
[0269] ^{13}C NMR (126 MHz, CD_3OD): δ 173.0, 170.8, 155.0, 149.8, 147.3, 145.0, 142.8, 142.3, 133.5, 133.1, 132.2, 132.1, 131.1, 130.5, 127.8, 122.5, 121.7, 120.0, 116.4, 113.5, 112.9, 111.4, 110.2, 103.5, 100.9, 92.6, 66.8, 64.5, 61.3, 60.4, 60.0, 56.8, 56.1, 55.9, 54.1, 44.1, 41.3, 25.6, 24.5, 20.6, 16.2, 9.6.

[0270] ESI-MS *m/z:* 797.4 $(M\text{-}H_2O+H)^+$.

[0271] (+)-HR-ESI-TOF-MS *m/z*: 797.2896 $[M\text{-}H_2O+H]^+$ (Calcd. for $C_{42}H_{45}N_4O_{10}S$ 797.2851).

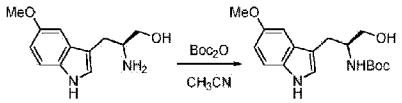
Example 11. Synthesis of allyl *N*-[(*S*)-2-amino-3-(5-methoxy-*1H*-indol-3-yl)propyl)]carbamate (24-S)

[0272]



17-S 20-S 21-S 22-S 23-S 24-S

A)



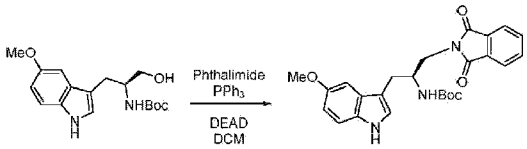
17-S 20-S

[0273] To a solution of **17-S** (6.9 g, 31.4 mmol) in CH_3CN (126 mL, 4 mL/mmol) was added di-*tert*-butyl dicarbonate (13.7 g, 62.8 mmol). The reaction mixture was stirred at 23 °C for 5.5 h, concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) gives **20-S** (4.5 g, 45%).

[0274] R_f= 0.6 (CH_2Cl_2:CH_3OH, 9:1).

[0275] ^1H NMR (400 MHz, $CDCl_3$): δ 8.04 (s, 1H), 7.25 (d, *J* = 8.4 Hz, 1H), 7.10 (d, *J* = 2.4 Hz, 1H), 7.03 (s, 1H), 6.87 (dd, *J* = 8.8, 2.5 Hz, 1H), 4.83 (s, 1H), 3.98 (s, 1H), 3.87 (s, 3H), 3.73-3.58 (m, 2H), 2.96 (d, *J* = 6.6 Hz, 2H), 1.42 (s, 9H).

B)



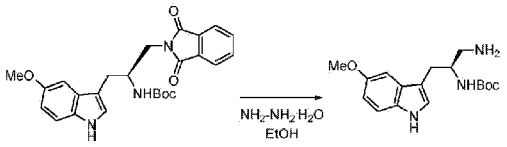
20-S 21-S

[0276] To a solution of **20-S** (4.5 g, 14 mmol) in CH_2Cl_2 (84 mL, 6 mL/mmol) was added phthalimide (4.5 g, 30.9 mmol), triphenylphosphine (8.1 g, 30.9 mmol) and the mixture was cooled at 0 °C. A solution of 40% of diethyl azodicarboxylate in CH_2Cl_2 (10.4 mL, 35 mmol) was added for 15 min. The reaction was stirred at 23 °C for 18 h, concentrated under vacuum. The residue obtained was purified by flash chromatography (Hexane:EtOAc, from 99:1 to 85:15) to yield **21-S** (5.8 g, 92%).

[0277] R_f= 0.55 (Hexane:EtOAc, 1:1).

[0278] ^1H NMR (400 MHz, $CDCl_3$): δ 8.48 (s, 1H), 7.78 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.69-7.61 (m, 2H), 7.21 (d, *J* = 8.8 Hz, 1H), 7.06 (dd, *J* = 18.5, 2.4 Hz, 2H), 6.81 (dd, *J* = 8.8, 2.4 Hz, 1H), 4.87 (s, 1H); 4.39 (s, 1H), 3.87 (s, 3H), 3.83-3.66 (m, 2H), 2.98 (d, *J* = 6.1 Hz, 2H), 1.20 (s, 9H).

C)



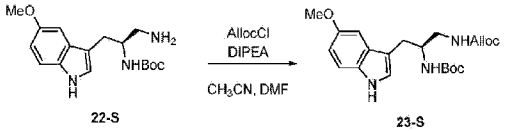
21-S 22-S

[0279] To a solution of **21-S** (6.29 g, 14 mmol) in ethanol (420 mL, 30 mL/mmol) was added hydrazine monohydrate (61.1 mL, 1260 mmol). The reaction mixture was stirred at 80 °C in sealed tube for 2 h, concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 100:1 to 50:50) affords **22-S** (4.2 g, 95%).

[0280] R_f= 0.1 (CH_2Cl_2:CH_3OH, 8:2).

[0281] ^1H NMR (400 MHz, $CDCl_3$): δ 7.22 (d, *J* = 8.8 Hz, 1H), 7.12 (d, *J* = 2.4 Hz, 1H), 7.06 (s, 1H), 6.76 (dd, *J* = 8.8, 2.4 Hz, 1H), 4.06-3.97 (m, 1H), 3.82 (s, 3H), 3.06-2.82 (m, 4H), 1.37 (s, 9H).

D)



22-S 23-S

[0282] To a solution of **22-S** (4.0 g, 12.52 mmol) in CH_3CN (125 mL, 10 mL/mmol) and DMF (12 mL, 1 mL/mmol) was added *N,N*-diisopropylethylamine (1.8 mL, 10 mmol) and allyl chloroformate (13.3 mL, 125 mmol). The reaction was stirred at 23 °C for 5 h. The mixture was diluted with EtOAc and NH_4Cl was added and the mixture was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (Hexane:EtOAc, from 100:1 to 1:100) to obtain **23-S** (2.65 g, 52%).

[0283] R_f= 0.5 (Hexane:EtOAc, 1:1).

[0284] ^1H NMR (400 MHz, $CDCl_3$): δ 8.11 (s, 1H), 7.28-7.20 (m, 1H), 7.04 (d, *J* = 13.1 Hz, 2H), 6.85 (dd, *J* = 8.9, 2.4 Hz, 1H), 5.97-5.82 (m, 1H), 5.33-5.24 (m, 1H), 5.19 (dt, *J* = 10.4, 1.3 Hz, 1H), 5.11 (s, 1H), 4.82 (s, 1H), 4.55 (d, *J* = 5.6 Hz, 2H), 4.01 (s, 1H), 3.86 (s, 3H), 3.37 (d, *J* = 13.7 Hz, 1H), 3.21 (s, 1H), 2.89 (dd, *J* = 14.5, 7.0 Hz, 1H), 1.41 (s, 9H).

E)

23-S 24-S

[0285] To a solution of **23-S** (2.60 g, 6.44 mmol) in CH_2Cl_2 (106 mL, 16.6 mL/mmol) was added trifluoroacetic acid (54 mL, 8.3 mL/mmol). The reaction mixture was stirred at 23 °C for 1.5 h, concentrated under vacuum to afford **24-S** (3.9 g, 100%).

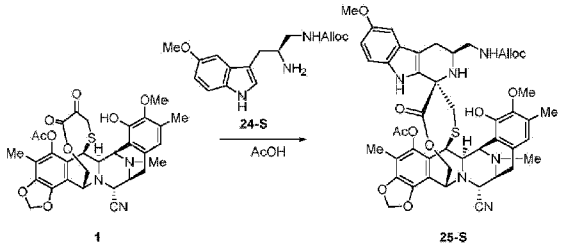
[0286] R_f= 0.1 (CH_2Cl_2:CH_3OH, 9:1).

[0287] ^1H NMR (400 MHz, CD_3OD): δ 8.27 (s, 1H), 7.25 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.10 (s, 1H), 6.96 (d, *J* = 2.3 Hz, 1H), 6.87 (dd, *J* = 9.0, 2.4 Hz, 1H), 5.81 (ddt, *J* = 16.3, 10.9, 5.7 Hz, 1H), 5.23 (dd, *J* = 19.3, 13.6 Hz, 2H), 4.49 (d, *J* = 5.9 Hz, 2H), 3.82 (s, 3H), 3.81-3.55 (m, 1H), 3.62-3.39 (m, 2H), 3.08 (qd, *J* = 15.1, 7.3 Hz, 2H).

Example 12

[0288]

A)



1 25-S

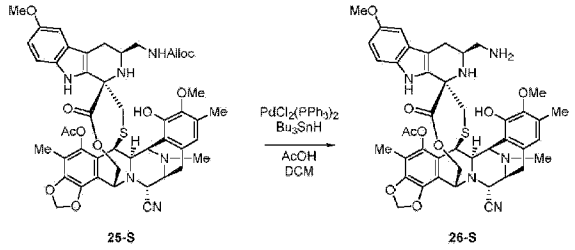
[0289] To a solution of **1** (120 mg, 0.19 mmol) in acetic acid (6 mL, 0.08 M) was added **24-S** (117 mg, 0.35 mmol). The reaction mixture was stirred at 23 °C for 18 h and then acetic acid was evaporated. An aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, 1:1) gives compound **25-S** (95 mg, 54%).

[0290] R_f= 0.4 (Hexane:EtOAc, 1:1).

[0291] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.64 (s, 1H), 7.14 (d, *J* = 8.8 Hz, 1H), 6.80 (s, 1H), 6.77 (d, *J* = 8.8 Hz, 1H), 6.68 (s, 1H), 6.24 (s, 1H), 6.03 (s, 1H), 6.02-5.93 (m, 1H), 5.76 (s, 1H), 5.38 (d, *J* = 10.5 Hz, 1H), 5.26 (d, *J* = 10.5 Hz, 1H), 5.11 (d, *J* = 11.7 Hz, 1H), 4.66 (d, *J* = 5.6 Hz, 2H), 4.57 (s, 1H), 4.37 (s, 1H), 4.33-4.19 (m, 3H), 3.82 (s, 3H), 3.79 (s, 3H), 3.46 (s, 2H), 3.17 (s, 1H), 3.10-2.90 (m, 3H), 2.68-2.45 (m, 2H), 2.38-2.33 (m, 1H), 2.32 (s, 3H), 2.27 (s, 3H), 2.16 (s, 3H), 2.04 (s, 2H).

[0292] ESI-MS *m/z:* 907.1 $(M+H)^+$.

B)



25-S 26-S

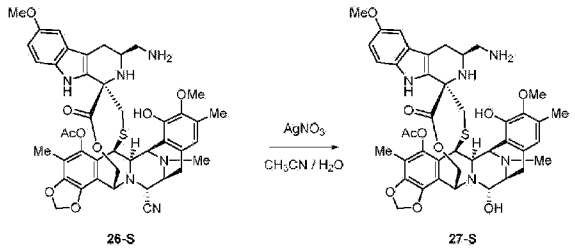
[0293] To a solution of **25-S** (90 mg, 0.1 mmol) in CH_2Cl_2 (2 mL, 18 mL/mmol) was added bis(triphenylphosphine)palladium(II)dichloride (12 mg, 0.1 mmol) and acetic acid (0.056 mL, 0.99 mmol). Tributyltin hydride (0.16 mL, 0.60 mmol) was added at 0 °C, the reaction mixture was stirred at 0 °C for 0.5 h, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, from 100:1 to 1:100 and EtOAc:CH_3OH, from 100:1 to 1:100) to afford **26-S** (75 mg, 92%).

[0294] R_f= 0.25 (CH_2Cl_2:CH_3OH, 1:1).

[0295] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62 (s, 1H), 7.15 (d, *J* = 9.3 Hz, 1H), 6.81-6.76 (m, 2H), 6.72 (s, 1H), 6.25 (d, *J* = 1.2 Hz, 1H), 6.03 (d, *J* = 1.2 Hz, 1H), 5.12 (d, *J* = 11.7 Hz, 1H), 4.57 (s, 1H), 4.41 (s, 1H), 4.36-4.24 (m, 2H), 4.20 (d, *J* = 11.7 Hz, 1H), 3.82 (s, 3H), 3.79 (s, 3H), 3.44 (dd, *J* = 22.0, 7.1 Hz, 2H), 3.08-2.78 (m, 4H), 2.73-2.64 (m, 2H), 2.41-2.22 (m, 3H), 2.28 (s, 3H), 2.25-2.15 (m, 1H), 2.14 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H).

[0296] ESI-MS *m/z:* 823.3 $(M+H)^+$.

C)



26-S 27-S

[0297] To a solution of **26-S** (70 mg, 0.085 mmol) in CH_3CN:H_2O (1.39: 1, 6 mL, 0.015 M) was added $AgNO_3$ (335 mg, 1.7 mmol). After 18 h at 23 °C, the reaction was quenched with a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to give **27-S** (23 mg, 33%).

[0298] R_f= 0.2 (CH_2Cl_2:CH_3OH, 9:1).

[0299] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62 (s, 1H), 7.15 (d, *J* = 7.8 Hz, 1H), 6.78 (s, 1H), 6.75 (d, *J* = 7.8 Hz, 1H), 6.21 (d, *J* = 1.5 Hz, 1H), 6.01 (d, *J* = 1.5 Hz, 1H), 5.78 (s, 1H), 5.22 (d, *J* = 11.5 Hz, 1H), 4.90 (s, 1H), 4.58-4.42

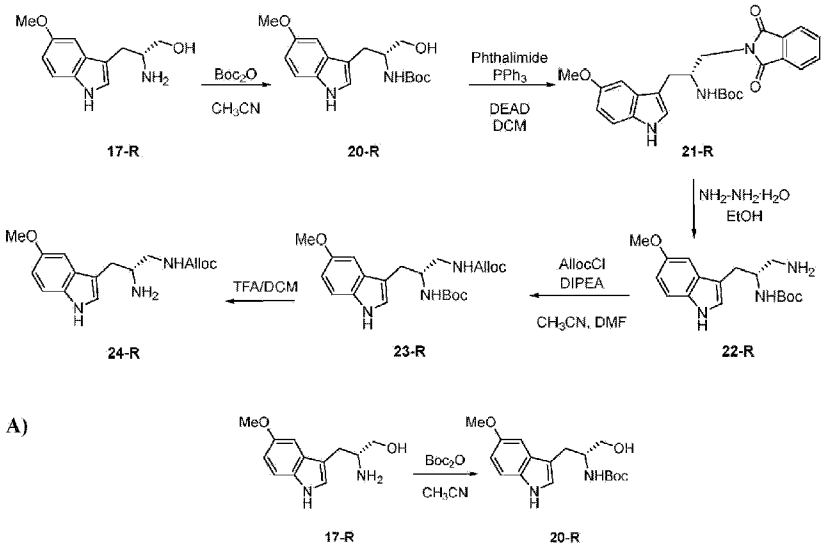
(m, 3H), 4.29-4.10 (m, 2H), 3.84-3.80 (m, 1H), 3.83 (s, 3H), 3.79 (s, 3H), 3.53-3.48 (m, 2H), 3.22 (d, *J* = 8.7 Hz, 1H), 3.12 (s, 1H), 3.02 (d, *J* = 12.8 Hz, 1H), 2.89-2.64 (m, 3H), 2.46 (s, 3H), 2.42-2.34 (m, 2H), 2.27 (s, 3H), 2.12 (s, 3H), 2.03 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 172.1, 168.7, 154.0, 147.6, 145.6, 143.0, 141.2, 140.8, 131.6, 130.6, 129.6, 127.1, 121.8, 120.9, 118.4, 115.2, 112.5, 111.8, 101.8, 100.2, 81.5, 62.6, 60.6, 58.0, 57.8, 56.0, 55.8, 55.0, 42.3, 41.4, 31.9, 29.7, 27.8, 26.9, 25.6, 24.0, 22.7, 20.5, 16.0, 14.1, 13.6, 9.7.

[0300] ESI-MS *m/z:* 796.3 $(M-H_2O+H)^+$.

[0301] (+)-HR-ESI-TOF-MS *m/z*: 796.3062 $[M-H_2O+H]^+$ (Calcd. for $C_{42}H_{46}N_5O_9S$ 796.3011).

Example 13. Synthesis of allyl *N*-[(*R*)-2-amino-3-(5-methoxy-*1H*-indol-3-yl)propyl)]carbamate (24-R)

[0302]



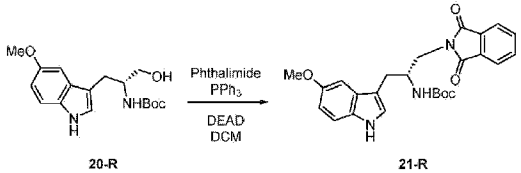
A)

[0303] To a solution of **17-R** (2.35 g, 10.7 mmol) in CH_3CN (43 mL, 4 mL/mmol) was added di-*tert*-butyl dicarbonate (4.67 g, 21.4 mmol). The reaction mixture was stirred at 23 °C for 2.5 h, concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) afforded **20-R** (1.7 g, 50%).

[0304] R_f= 0.6 (CH_2Cl_2:CH_3OH, 9:1).

[0305] ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (s, 1H), 7.25 (d, *J* = 8.9 Hz, 1H), 7.09 (d, *J* = 2.4 Hz, 1H), 7.02 (d, *J* = 2.4 Hz, 1H), 6.86 (dd, *J* = 8.8, 2.4 Hz, 1H), 4.83 (s, 1H), 3.98 (s, 1H), 3.87 (s, 3H), 3.69 (td, *J* = 9.2, 7.5, 5.3 Hz, 1H), 3.61 (dd, *J* = 10.9, 5.6 Hz, 1H), 2.95 (d, *J* = 6.8 Hz, 2H), 1.42 (s, 9H).

B)



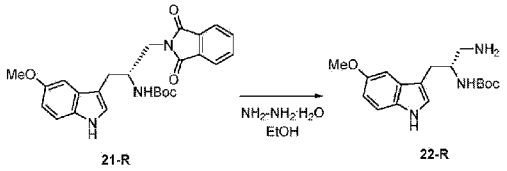
[0306] To a solution of **20-R** (1.7 g, 5.3 mmol) in CH_2Cl_2 (32 mL, 6 mL/mmol) was added phthalimide (1.72 g, 11.7 mmol), triphenylphosphine (3.06 g, 11.7 mmol) and the mixture was cooled at 0 °C. A solution of 40% of

diethyl azodicarboxylate in CH_2Cl_2 (4.0 mL, 13.2 mmol) was added for 15 min. The reaction was stirred at 23 °C for 16 h, concentrated under vacuum. The residue obtained was purified by flash chromatography (Hexane:EtOAc, from 99:1 to 85:15) to afford **21-R** (2.0 g, 84%).

[0307] R_f= 0.45 (Hexane:EtOAc, 1:1).

[0308] ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.31 (s, 1H), 7.80 (dd, *J* = 5.4, 3.0 Hz, 2H), 7.67 (dd, *J* = 5.4, 3.0 Hz, 2H), 7.30-7.12 (m, 2H), 7.08 (dd, *J* = 15.2, 2.4 Hz, 1H), 6.84 (dd, *J* = 8.8, 2.4 Hz, 1H), 4.85 (d, *J* = 9.2 Hz, 1H), 4.43 (q, *J* = 5.3 Hz, 1H), 3.86 (s, 3H), 3.83-3.68 (m, 2H), 3.01 (d, *J* = 5.4 Hz, 2H), 1.22 (s, 9H).

C)

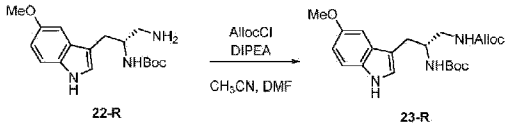


[0309] To a solution of **21-R** (2.0 g, 4.45 mmol) in ethanol (133 mL, 30 mL/mmol) was added hydrazine monohydrate (21.6 mL, 445 mmol). The reaction mixture was stirred at 80 °C in sealed tube for 2 h, concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 100:1 to 50:50) to afford **22-R** (1.15 g, 81%).

[0310] R_f= 0.1 (CH_2Cl_2:CH_3OH, 8:2).

[0311] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.21 (d, *J* = 8.8 Hz, 1H), 7.12 (s, 1H), 7.05 (s, 1H), 6.75 (dd, *J* = 8.8, 2.4 Hz, 1H), 3.95 (ddd, *J* = 10.7, 8.7, 5.4 Hz, 1H), 3.82 (s, 3H), 2.98-2.79 (m, 3H), 2.75 (dd, *J* = 13.1, 9.4 Hz, 1H), 1.37 (s, 9H).

D)

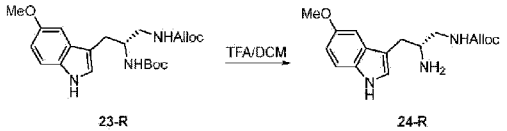


[0312] To a solution of **22-R** (1.1 g, 3.4 mmol) in CH_3CN (34 mL, 10 mL/mmol) and DMF (3.4 mL, 1 mL/mmol) was added *N,N*-diisopropylethylamine (0.5 mL, 2.7 mmol) and allyl chloroformate (3.7 mL, 34 mmol). The reaction was stirred at 23 °C for 19 h. The mixture was diluted with EtOAc and NH_4Cl was added and the mixture was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (Hexane:EtOAc, from 100:1 to 1:100) to afford **23-R** (0.95 g, 69%).

[0313] R_f= 0.5 (Hexane:EtOAc, 1:1).

[0314] ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.55 (s, 1H), 7.20 (d, *J* = 8.8 Hz, 1H), 7.05 (s, 1H), 6.98-6.87 (m, 1H), 6.82 (dt, *J* = 8.8, 1.8 Hz, 1H), 5.96-5.81 (m, 1H), 5.37-5.22 (m, 2H), 5.22-5.14 (m, 1H), 5.02-4.97 (m, 1H), 4.60-4.47 (m, 2H), 4.00 (s, 1H), 3.84 (s, 3H), 3.31 (s, 1H), 3.19 (s, 1H), 2.88 (td, *J* = 14.5, 13.3, 5.9 Hz, 2H), 1.40 (s, 9H).

E)



[0315] To a solution of **23-R** (0.94 g, 2.3 mmol) in CH_2Cl_2 (39 mL, 16.6 mL/mmol) was added trifluoroacetic acid

(19 mL, 8.3 mL/mmol). The reaction mixture was stirred at 23 °C for 1.5 h, concentrated under vacuum to afford **24-R** (0.72 g, 100%).

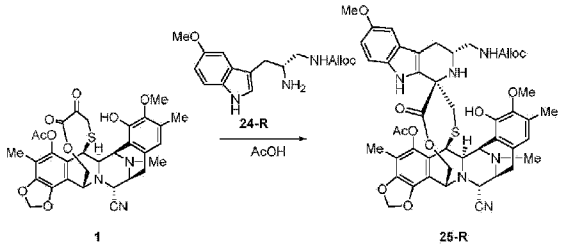
[0316] R_f= 0.1 (CH_2Cl_2:CH_3OH, 9:1).

[0317] ^{1}H NMR (400 MHz, CD_3OD): δ 7.27 (d, *J* = 8.8, 1H), 7.18 (s, 1H), 7.04 (d, *J* = 2.4 Hz, 1H), 6.80 (ddd, *J* = 8.8, 2.4, 0.9 Hz, 1H), 5.95 (ddt, *J* = 16.4, 10.8, 5.5 Hz, 1H), 5.32 (d, *J* = 17.1 Hz, 1H), 5.20 (d, *J* = 10.5 Hz, 1H), 4.60-4.53 (m, 2H), 3.83 (s, 3H), 3.59 (dt, *J* = 11.4, 5.5 Hz, 1H), 3.47-3.30 (m, 2H), 3.13-2.94 (m, 2H).

Example 14

[0318]

A)



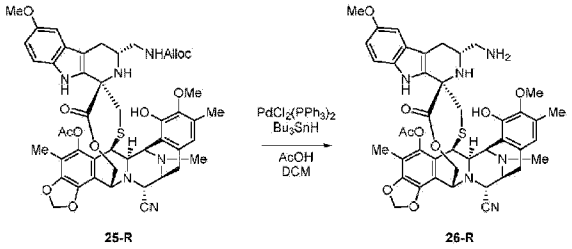
[0319] To a solution of **1** (0.71 g, 1.14 mmol) in acetic acid (45 mL, 0.08 M) was added **24-R** (0.54 mg, 1.8 mmol). The reaction mixture was stirred at 23 °C for 7 h and then acetic acid was evaporated. An aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, 1:1) gives compound **25-R** (670 mg, 65%).

[0320] R_f= 0.4 (Hexane: EtOAc, 1:1).

[0321] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 (s, 1H), 7.17 (d, *J* = 8.8 Hz, 1H), 6.83-6.73 (m, 2H), 6.61 (s, 1H), 6.23 (d, *J* = 1.0 Hz, 1H), 6.02 (d, *J* = 1.0 Hz, 1H), 6.05-5.89 (m, 1H), 5.75 (s, 1H), 5.44-5.30 (m, 1H), 5.25 (d, *J* = 10.4 Hz, 1H), 5.13-4.99 (m, 2H), 4.71-4.59 (m, 2H), 4.36 (s, 1H), 4.30-4.07 (m, 3H), 3.80 (s, 3H), 3.79 (s, 3H), 3.61-3.53 (m, 1H); 3.48-3.41 (m, 3H), 3.26 (dt, *J* = 13.3, 3.8 Hz, 1H), 3.04-2.88 (m, 2H), 2.52 (dd, *J* = 14.9, 3.7 Hz, 1H), 2.46-2.35 (m, 2H), 2.31 (s, 3H), 2.29 (s, 3H), 2.16 (s, 3H), 2.12-2.02 (m, 1H), 2.09 (s, 3H).

[0322] ESI-MS *m/z:* 907.3 $(M+H)^+$.

B)



[0323] To a solution of **25-R** (745 mg, 0.82 mmol) in CH_2Cl_2 (15 mL, 18 mL/mmol) was added bis(triphenylphosphine)palladium(II) dichloride (92 mg, 0.1 mmol) and acetic acid (0.47 mL, 8.2 mmol). Tributyltin hydride (1.33 mL, 4.9 mmol) was added at 0 °C, the reaction mixture was stirred at 0 °C for 0.75 h and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, from 100:1 to 1:100 and

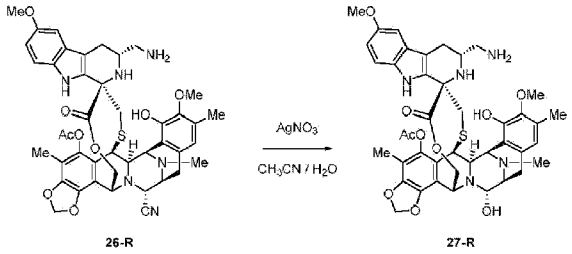
EtOAc:CH_3OH, from 100:1 to 1:100) to afford 26-R (680 mg, >100%).

[0324] R_f= 0.25 (CH_2Cl_2:CH_3OH, 1:1).

[0325] ^1H NMR (400 MHz, $CDCl_3$): δ 7.57 (s, 1H), 7.16 (d, *J* = 8.8 Hz, 1H), 6.85-6.72 (m, 2H), 6.57 (s, 1H), 6.21 (d, *J* = 1.4 Hz, 1H), 6.00 (d, *J* = 1.3 Hz, 1H), 5.05-4.97 (m, 1H), 4.63 (s, 1H), 4.35 (s, 1H), 4.31-4.09 (m, 4H), 3.80 (s, 3H), 3.78 (s, 3H), 3.50-3.40 (m, 3H), 3.24 (dq, *J* = 9.9, 5.3 Hz, 1H), 2.95 (s, 1H), 2.91-2.75 (m, 2H), 2.62 (dd, *J* = 14.8, 3.6 Hz, 1H), 2.43-2.28 (m, 2H), 2.36 (s, 3H), 2.25 (s, 3H), 2.22-2.14 (m, 1H), 2.15 (s, 3H), 2.08 (s, 3H).

[0326] ESI-MS *m/z:* 823.3 $(M+H)^+$.

C)



26-R **27-R**

[0327] To a solution of **26-R** (660 mg, 0.80 mmol) in CH_3CN:H_2O (1.39:1, 56 mL, 0.015 M) was added $AgNO_3$ (2.70 g, 16.0 mmol). After 16.5 h at 23 °C, the reaction was quenched with a mixture 1:1 of saturated aqueous solutions of NaCl and NaHCOs, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to give **27-R** (271 mg, 42%).

[0328] R_f= 0.1 (CH_2Cl_2:CH_3OH, 9:1).

[0329] ^1H NMR (400 MHz, $CDCl_3$): δ 7.46 (s, 1H), 7.16 (d, *J* = 8.9 Hz, 1H), 6.83 (s, 1H), 6.72 (d, *J* = 8.9 Hz, 1H), 6.58 (s, 1H), 6.20 (d, *J* = 1.8 Hz, 1H), 5.99 (d, *J* = 1.8 Hz, 1H), 5.76 (s, 1H), 5.15 (d, *J* = 11.4 Hz, 1H), 4.86 (s, 1H), 4.52 (m, 2H), 4.17 (d, *J* = 5.3 Hz, 1H), 4.07 (d, *J* = 11.4 Hz, 1H), 3.80 (s, 3H), 3.78 (s, 3H), 3.55-3.43 (m, 2H), 3.32-3.20 (m, 2H), 3.01-2.82 (m, 4H), 2.68-2.59 (m, 1H), 2.44-2.31 (m, 1H), 2.38 (s, 3H), 2.30-2.19 (m, 1H), 2.26 (s, 3H), 2.15 (s, 3H), 2.07 (s, 3H).

[0330] ^{13}C NMR (101 MHz, CD_3OD): δ 171.7, 171.3, 153.8, 153.3, 148.0, 147.6, 145.4, 145.4, 143.1, 141.3, 140.7, 131.6, 131.4, 131.2, 129.3, 126.8, 121.6, 120.9, 118.3, 115.6, 112.2, 111.8, 101.8, 100.2, 81.7, 63.5, 63.1, 61.7, 58.0, 57.8, 56.1, 55.8, 55.0, 42.2, 42.1, 41.4, 41.0, 25.1, 23.8, 20.5, 16.0, 9.7.

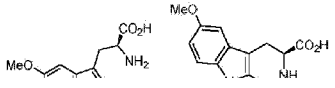
[0331] ESI-MS *m/z:* 796.3 $(M-H_2O+H)^+$.

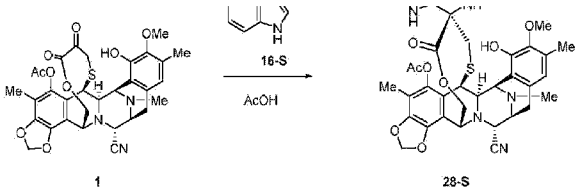
[0332] (+)-HR-ESI-TOF-MS *m/z:* 796.3045 $[M-H_2O+H]^+$ (Calcd. for $C_{42}H_{46}N_5O_9S$ 796.3011).

Reference Example 15. Synthesis of reference compounds 28-S and 29-S.

[0333]

A)





1 28-S

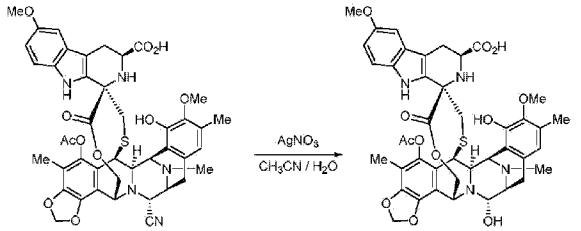
[0334] To a solution of **1** (450 mg, 0.72 mmol) in acetic acid (9 mL, 0.08 M) was added **16-S** (675 mg, 2.88 mmol). The reaction mixture was stirred a 52 °C for 3 h and then acetic acid was evaporated. An aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 80:20) gave compound **28-S** (400 mg, 66%).

[0335] R_f= 0.35 (CH_2Cl_2:CH_3OH, 10:1).

[0336] ^1H NMR (400 MHz, $CDCl_3$): δ 7.65 (s, 1H), 7.15 (d, *J* = 8.7 Hz, 1H), 6.85-6.76 (m, 2H), 6.57 (s, 1H), 6.25 (d, *J* = 1.4 Hz, 1H), 6.04 (d, *J* = 1.3 Hz, 1H), 5.16 (d, *J* = 11.7 Hz, 1H), 4.62 (s, 1H), 4.44 (s, 1H), 4.35 (dd, *J* = 11.7, 2.0 Hz, 1H), 4.29 (dd, *J* = 5.2, 1.6 Hz, 1H), 4.22 (d, *J* = 2.7 Hz, 1H), 3.80 (s, 3H), 3.79 (s, 3H), 3.52-3.43 (m, 3H), 3.02-2.81 (m, 4H), 2.41-2.31 (m, 2H), 2.36 (s, 3H), 2.29 (s, 3H), 2.15 (s, 3H), 2.05 (s, 3H).

[0337] ESI-MS *m/z:* 838.6 $(M+H)^+$.

B)



28-S 29-S

[0338] To a solution of **28-S** (400 mg, 0.48 mmol) in CH_3CN:H_2O (2:1, 33 mL, 0.015 M) was added $AgNO_3$ (1.20 g, 7.16 mmol). After 16 h at 23 °C, a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$ was added, stirred for 15 min, diluted with CH_2Cl_2, stirred for 30 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 70:30) to afford **29-S** (179 mg, 45%).

[0339] R_f= 0.25 (CH_2Cl_2:CH_3OH, 9:1).

[0340] ^1H NMR (500 MHz, CD_3OD): δ 7.17 (d, *J* = 8.9 Hz, 1H), 6.83 (d, *J* = 2.4 Hz, 1H), 6.70 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.66 (s, 1H), 6.29 (d, *J* = 1.3 Hz, 1H), 6.10 (d, *J* = 1.3 Hz, 1H), 5.32 (d, *J* = 11.6 Hz, 1H), 4.65 (s, 1H), 4.57 (s, 1H), 4.48 (s, 1H), 4.38 (dd, *J* = 11.7, 2.1 Hz, 1H), 3.75 (s, 3H), 3.73 (s, 3H), 3.41-3.35 (m, 1H), 3.16-2.91 (m, 5H), 2.71 (dd, *J* = 15.3, 11.4 Hz, 2H), 2.54 (s, 1H), 2.42-2.36 (m, 2H), 2.38 (s, 3H), 2.37 (s, 3H), 2.28 (s, 3H), 1.99 (s, 3H).

[0341] ^{13}C NMR (126 MHz, $CDCl_3$): d 171.3, 170.6, 155.2, 149.8, 147.5, 145.4, 142.8, 142.4, 133.0, 131.8, 130.0, 128.0, 122.2, 121.8, 115.5, 113.9, 113.3, 113.2, 111.4, 109.1, 103.8, 100.9, 91.6, 65.4, 61.9, 60.3, 59.4, 57.1, 56.4, 56.2, 55.2, 53.4, 43.7, 40.8, 38.3, 30.7, 26.4, 24.7, 20.4, 16.5, 9.6.

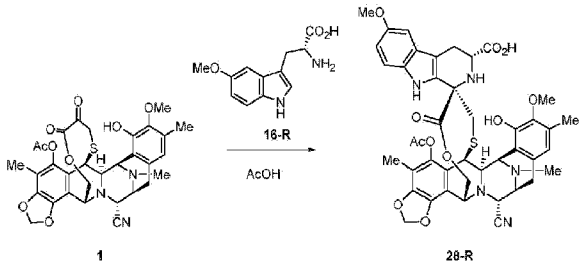
[0342] ESI-MS *m/z:* 811.3 $(M-H_2O+H)^+$.

[0343] (+)-HR-ESI-TOF-MS *m/z*: 811.2682 $[M-H_2O+H]^+$ (Calcd. for $C_{42}H_{43}N_4O_{11}S$ 811.2644).

Reference Example 16. Synthesis of Reference Compounds 28-R and 29-R

[0344]

A)



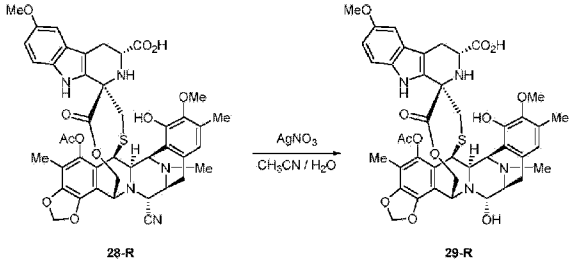
[0345] To a solution of 1 (50 mg, 0.08 mmol) in acetic acid (1 mL, 0.08 M) was added 16-R (66 mg, 0.3 mmol). The reaction mixture was stirred at 50 °C for 6 h and then acetic acid was evaporated. An aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 80:20) gave compound **28-R** (50 mg, 75%).

[0346] R_f= 0.20 (CH_2Cl_2:CH_3OH, 10:1).

[0347] ^1H NMR (400 MHz, $CDCl_3$): 7.63 (s, 1H), 7.16 (d, *J* = 8.8 Hz, 1H), 6.81 (d, *J* = 2.4 Hz, 1H), 6.77 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.56 (s, 1H), 6.21 (d, *J* = 1.2 Hz, 1H), 6.00 (d, *J* = 1.2 Hz, 1H), 5.77 (s, 1H), 5.00 (d, *J* = 11.8 Hz, 1H), 4.63 (s, 1H), 4.35 (s, 1H), 4.27 (d, *J* = 5.0 Hz, 1H), 4.22 - 4.04 (m, 3H), 3.79 (s, 3H), 3.77 (s, 3H), 3.48 - 3.40 (m, 2H), 3.00 (dd, *J* = 15.3, 4.8 Hz, 1H), 2.92 (d, *J* = 5.4 Hz, 2H), 2.71 (dd, *J* = 15.3, 10.1 Hz, 1H), 2.46 (d, *J* = 14.9 Hz, 1H), 2.34 (s, 3H), 2.26 (s, 3H), 2.21 (d, *J* = 15.0 Hz, 1H), 2.15 (s, 3H), 2.07 (s, 3H).

[0348] ESI-MS *m/z:* 838.8 $(M+H)^+$.

B)



[0349] To a solution of **28-R** (50 mg, 0.06 mmol) in CH_3CN:H_2O (2:1, 4.2 mL, 0.015M) was added $AgNO_3$ (304 mg, 1.80 mmol). After 3 h at 23 °C, a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$ was added, stirred for 15 min, diluted with CH_2Cl_2, stirred for 30 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH from 99:1 to 70:30) to afford **29-R** (30 mg, 60%).

[0350] R_f= 0.15 (CH_2Cl_2:CH_3OH, 9:1).

[0351] ^{1}H NMR (400 MHz, $CDCl_3$): 7.68 (s, 1H), 7.14 (d, *J* = 8.8 Hz, 1H), 6.80 (d, *J* = 2.4 Hz, 1H), 6.76 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.57 (s, 1H), 6.17 (d, *J* = 1.3 Hz, 1H), 5.95 (d, *J* = 1.3 Hz, 1H), 5.75 (s, 1H), 5.12 (d, *J* = 11.5 Hz, 1H), 4.85 (s, 1H), 4.56 - 4.46 (m, 2H), 4.17 (s, 1H), 4.10 (dd, *J* = 9.9, 4.9 Hz, 1H), 4.05 (dd, *J* = 11.4, 2.0 Hz, 1H), 3.78 (s, 3H), 3.76 (s, 3H), 3.51 (s, 1H), 3.48 - 3.42 (m, 2H), 3.23 (s, 1H), 3.00 (dd, *J* = 15.3, 4.9 Hz, 1H), 2.90 - 2.77 (m, 2H), 2.71 (dd, *J* = 15.2, 9.9 Hz, 1H), 2.48 (d, *J* = 14.6 Hz, 1H), 2.34 (s, 3H), 2.25 (s, 3H), 2.20 (d, *J* = 14.6 Hz, 1H), 2.14 (s, 3H), 2.05 (s, 3H).

[0352] ^{13}C NMR (101 MHz, $CDCl_3$): 175.6, 171.0, 168.7, 154.1, 147.3, 145.6, 143.1, 141.3, 140.8, 131.1, 130.4, 126.5, 121.9, 121.5, 121.3, 115.5, 112.9, 112.7, 112.0, 109.1, 101.9, 100.2, 81.5, 62.8, 61.7, 60.4, 57.9, 57.8, 56.0, 55.8, 54.8, 53.4, 42.5, 41.2, 40.3, 29.7, 24.6, 23.8, 20.5, 15.9, 9.8.

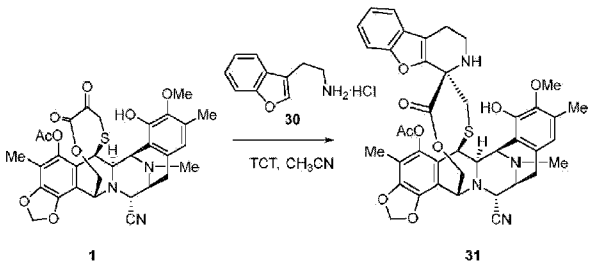
[0353] ESI-MS *m/z:* 811.6 $(M\text{-}H_2O+H)^+$.

[0354] (+)-HR-ESI-TOF-MS *m/z*: 811.2687 $[M\text{-}H_2O+H]^+$ (Calcd. for $C_{42}H_{43}N_4O_{11}S$ 811.2644).

Reference Example 17.

[0355]

A)



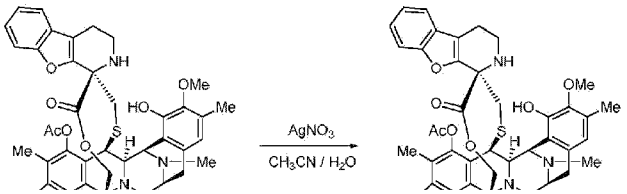
[0356] To a solution of compound **1** (2.0 g, 3.21 mmol) in acetonitrile (200 mL, 0.01 M) was added 2-benzofuran-3-yl-ethylamine hydrochloride (**30**) (1.90 g, 9.65 mmol, Sigma Aldrich) and cyanuric chloride (TCT) (200 mg, 10%). The reaction mixture was stirred at 85 °C for 24 h and then aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, from 9:1 to 1:9) gives compound **31** (1.95 g, 79%).

[0357] R_f= 0.5 (Hexane:EtOAc, 1:1).

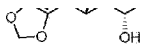
[0358] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38-7.36 (m, 2H), 7.19-7.10 (m, 2H), 6.64 (s, 1H), 6.20 (d, *J* = 1.5 Hz, 1H), 6.05 (d, *J* = 1.5 Hz, 1H), 5.76 (s, 1H), 5.05 (d, *J* = 11.7 Hz, 1H), 4.54 (s, 1H), 4.33-4.24 (m, 2H), 4.23-4.16 (m, 2H), 3.81 (s, 3H), 3.49-3.38 (m, 2H), 3.28-3.21 (m, 1H), 3.06-2.78 (m, 5H), 2.57-2.50 (m, 2H), 2.37 (s, 3H), 2.27 (s, 3H), 2.21 (m, 3H), 2.08 (s, 3H).

[0359] ESI-MS *m/z:* 765.3 $(M+H)^+$.

B)



31



32

[0360] To a solution of compound **31** (380 mg, 0.49 mmol) in CH_3CN:H_2O (1.39:1, 25 mL, 0.015 M) was added $AgNO_3$ (1.30 g, 7.45 mmol). After 5 h at 23 °C, a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$ was added, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to afford compound **32** (175 mg, 47%).

[0361] R_f= 0.40 (CH_2Cl_2:CH_3OH, 9:1).

[0362] ^1H NMR (400 MHz, $CDCl_3$): δ 7.35 (ddd, *J* = 10.7, 7.6, 1.1 Hz, 2H), 7.14 (dtd, *J* = 19.7, 7.3, 1.3 Hz, 2H), 6.65 (s, 1H), 6.16 (d, *J*= 1.5 Hz, 1H), 6.01 (d, *J* = 1.5 Hz, 1H), 5.75 (s, 1H), 5.15 (dd, *J* = 11.5, 1.2 Hz, 1H), 4.80 (s, 1H), 4.48 (d, *J* = 3.2 Hz, 1H), 4.44 (s, 1H), 4.20-4.06 (m, 2H), 3.81 (s, 1H), 3.50 (d, *J* = 18.8 Hz, 1H), 3.30 (ddd, *J* = 12.6, 7.9, 5.1 Hz, 1H), 3.22 (d, *J* = 9.1 Hz, 1H), 2.99 (d, *J* = 17.9 Hz, 1H), 2.84 (dd, *J* = 19.2, 12.0 Hz, 3H), 2.59-2.49 (m, 2H), 2.36 (s, 3H), 2.27 (s, 3H), 2.21-2.14 (m, 1H), 2.18 (s, 3H), 2.06 (s, 3H).

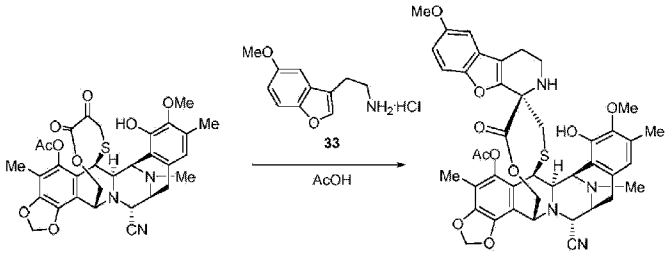
[0363] ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.2, 168.7, 154.4, 150.0, 147.9, 145.5, 142.9, 140.9, 140.8, 131.3, 129.0, 127.7, 123.7, 122.2, 121.2, 120.8, 118.9, 118.3, 115.5, 113.5, 111.7, 101.7, 82.1, 62.7, 61.7, 60.3, 57.8, 57.4, 55.9, 55.0, 42.2, 41.3, 39.7, 38.2, 29.7, 23.7, 21.3, 20.6, 15.9, 9.7. ESI-MS *m/z:* 738.6 $(M-H_2O+H)^+$.

[0364] (+)-HR-ESI-TOF-MS *m/z*: 756.2654 $[M+H]^+$ (Calcd. for $C_{40}H_{42}N_3O_{10}S$ 756.2585).

Reference Example 18.

[0365]

A)



1 34

[0366] To a solution of 1 (500 mg, 0.80 mmol) in acetic acid (10 mL, 0.08 M) was added 2-(5-methoxybenzofuran-3-yl)-ethylamine hydrochloride (**33**) (Diverchim, ref: DW04590) (**444** mg, 1.60 mmol). The reaction mixture was stirred at 50 °C for 6 days and then acetic acid was evaporated. An aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, 1:1) affords 34 (270 mg, 43%).

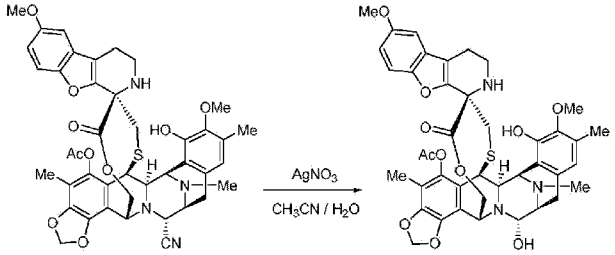
[0367] R_f= 0.3 (Hexane:EtOAc, 1:1).

[0368] ^1H NMR (400 MHz, $CDCl_3$): δ 7.25 (d, *J* = 9.1 Hz, 1H), 6.80-6.73 (m, 2H), 6.63 (s, 1H), 6.18 (d, *J* = 1.4

Hz, 1H), 6.03 (d, *J* = 1.4 Hz, 1H), 5.78 (s, 1H), 5.03 (dd, *J* = 11.5, 1.3 Hz, 1H), 4.52 (s, 1H), 4.29 (s, 1H), 4.26 (dd, *J* = 4.7, 1.5 Hz, 1H), 4.23-4.16 (m, 2H), 3.80 (s, 3H), 3.78 (s, 3H), 3.46-3.43 (m, 1H), 3.43-3.37 (m, 1H), 3.24 (s, 1H), 3.03 (d, *J* = 18.0 Hz, 1H), 2.91 (dd, *J* = 17.9, 9.2 Hz, 1H), 2.87-2.72 (m, 2H), 2.53-2.47 (m, 2H), 2.36 (s, 3H), 2.27 (s, 3H), 2.20 (s, 3H), 2.06 (s, 3H).

[0369] ESI-MS *m/z:* 795.8 $(M+H)^+$.

B)



34 35

[0370] To a solution of **34** (345 mg, 0.43 mmol) in CH_3CN:H_2O (1.39: 1, 30 mL, 0.015 M) was added $AgNO_3$ (2.20 g, 13.0 mmol). After 3 h at 23 °C, a mixture 1: 1 of saturated aqueous solutions of NaCl and $NaHCO_3$ was added, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to obtain **35** (175 mg, 51%).

[0371] R_f= 0.35 (CH_2Cl_2:CH_3OH, 9:1).

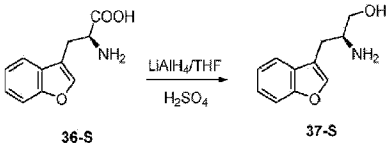
[0372] ^1H NMR (500 MHz, CD_3OD): δ 7.27 (d, *J* = 9.0 Hz, 1H), 6.90 (d, *J* = 2.6 Hz, 1H), 6.80 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.57 (s, 1H), 6.23 (d, *J* = 1.2 Hz, 1H), 6.05 (d, *J* = 1.2 Hz, 1H), 5.23 (d, *J* = 11.5 Hz, 1H), 4.27-4.08 (m, 4H), 3.77 (s, 3H), 3.75 (s, 3H), 3.63 (d, *J* = 14.1 Hz, 2H), 3.40-3.34 (m, 2H), 2.93-2.87 (m, 5H), 2.80 (d, *J* = 15.5 Hz, 1H), 2.57-2.54 (m, 2H), 2.34 (s, 3H), 2.30 (s, 3H), 2.14 (s, 3H), 2.05 (s, 3H).

[0373] ^{13}C NMR (126 MHz, CD_3OD): δ 171.9, 170.6, 157.5, 147.0, 145.0, 142.3, 141.0, 132.2, 131.1, 129.1, 122.2, 120.9, 120.2, 116.3, 115.1, 114.0, 112.7, 111.4, 103.5, 102.7, 92.9, 62.0, 60.3, 59.8, 59.4, 56.5, 56.2, 56.0, 54.0, 43.8, 41.2, 40.7, 30.8, 30.3, 28.7, 24.5, 21.6, 20.6, 16.2, 9.6. ESI-MS *m/z:* 768.6 $(M-H_2O+H)^+$.

[0374] (+)-HR-ESI-TOF-MS *m/z*: 768.2630 $[M-H_2O+H]^+$ (Calcd. for $C_{41}H_{42}N_3O_{10}S$ 768.2585).

Example 19

[0375]



36-S 37-S

[0376] To a solution of $LiAlH_4$ (148 mL, 1.0 M in THF, 148 mmol) at -40 °C was added carefully H_2SO_4 (7.14 mL, 72.9 mmol) and a suspension of (S)-2-amino-3-(benzofuran-3-yl)propanoic acid **(36-S)** (prepared as described in Tetrahedron Asymmetry 2008, 19, 500-511) (5.54 g, 26.9 mmol) in THF (85 mL, 0.003 M). The reaction mixture was left evolution at 23 °C, heated at 80 °C for 3 h and 18 h at 23 °C. Cool at -21 °C the reaction

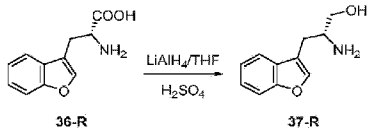
mixture was quenched carefully with NaOH 2N until basic pH. EtOAc was added and the mixture filtered through Celite® and washed with CH_3OH. The crude was concentrated under vacuum to afford compound **37-S** (3.93 g, >100%).

[0377] R_f= 0.1 (CH_2Cl_2:CH_3OH, 4:1).

[0378] ^1H NMR (400 MHz, CD_3OD): δ 7.67 - 7.62 (m, 1H), 7.61 (s, 1H), 7.51 - 7.41 (m, 1H), 7.34 - 7.18 (m, 2H), 3.69 - 3.48 (m, 1H), 3.44 (dd, *J* = 10.8, 6.6 Hz, 1H), 3.18 (dtd, *J* = 7.4, 6.4, 4.6 Hz, 1H), 2.88 (ddd, *J* = 14.4, 6.1, 1.0 Hz, 1H), 2.68 (ddd, *J* = 14.4, 7.5, 0.9 Hz, 1H).

Example 20

[0379]



[0380] To a solution of $LiAlH_4$ (118 mL, 1.0 M in THF, 118 mmol) at -40 °C was added carefully H_2SO_4 (3.1 mL, 57.8 mmol) and a suspension of (*R*)-2-amino-3-(benzofuran-3-yl)propanoic acid (**36-R**) (prepared as described in Tetrahedron Asymmetry 2008, 19, 500-511) (4.4 g, 21.4 mmol) in THF (67.4 mL, 0.003 M). The reaction mixture was left evolution at 23 °C, heated at 80 °C for 3 h and 18 h at 23 °C. Cool at -21 °C the reaction mixture was quenched carefully with NaOH 2N until basic pH. EtOAc was added and the mixture filtered through Celite® and washed with CH_3OH. The crude was concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15, Silice amine) to afford compound **37-R** (2.77 g, 68%).

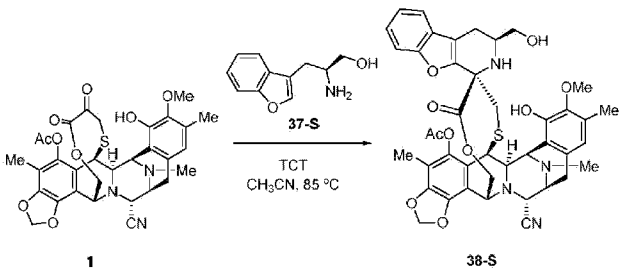
[0381] R_f= 0.1 (CH_2Cl_2:CH_3OH, 4:1).

[0382] ^1H NMR (400 MHz, CD_3OD): δ 7.63 - 7.52 (m, 1H), 7.56 (s, 1H), 7.46 - 7.33 (m, 1H), 7.21 (dtd, *J* = 19.9, 7.3, 1.3 Hz, 2H), 3.57 (dd, *J* = 10.7, 4.6 Hz, 1H), 3.42 (dd, *J* = 10.8, 6.6 Hz, 1H), 3.15 (dtd, *J* = 7.6, 6.3, 4.6 Hz, 1H), 2.84 (ddd, *J* = 14.4, 6.0, 1.0 Hz, 1H), 2.64 (ddd, *J* = 14.4, 7.5, 0.9 Hz, 1H).

Reference Example 21

[0383]

A)



[0384] To a solution of compound **1** (850 mg, 1.36 mmol) in CH_3CN (136 mL, 0.01 M) was added (*S*)-2-amino-3-(benzofuran-3-yl)propan-1-ol (**37-S**) (1.30 g, 6.83 mmol and cyanuric chloride (TCT) (170 mg, 20%). The

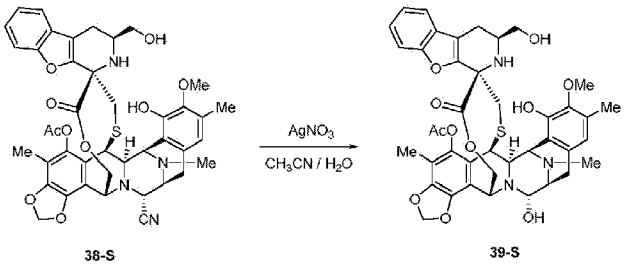
reaction mixture was stirred at 85 °C for 24 h and then aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, from 9:1 to 1:9) gives compound **38-S** (750 mg, 69%).

[0385] R_f= 0.25 (Hexane:EtOAc, 1:1).

[0386] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39 - 7.33 (m, 1H), 7.33 - 7.29 (m, 1H), 7.20 (ddd, *J* = 8.3, 7.2, 1.4 Hz, 1H), 7.14 (td, *J* = 7.4, 1.0 Hz, 1H), 6.61 (s, 1H), 6.21 (d, *J* = 1.4 Hz, 1H), 6.06 (d, *J* = 1.4 Hz, 1H), 5.74 (s, 1H), 5.08 (d, *J* = 11.2 Hz, 1H), 4.58 (s, 1H), 4.37 (s, 1H), 4.32 - 4.23 (m, 2H), 4.19 (d, *J* = 2.7 Hz, 1H), 3.81 (s, 3H), 3.52 - 3.41 (m, 3H), 3.36 - 3.29 (m, 1H), 3.13 (d, *J* = 9.8 Hz, 1H), 3.00 - 2.81 (m, 3H), 2.57 (dd, *J* = 15.7, 4.9 Hz, 1H), 2.50 (d, *J* = 15.2 Hz, 1H), 2.37 (s, 3H), 2.31 - 2.25 (m, 1H), 2.29 (s, 3H), 2.16 (s, 3H), 2.10 (d, *J* = 7.2 Hz, 1H), 2.05 (s, 3H).

[0387] ESI-MS *m/z:* 795.2 $(M)^+$.

B)



38-S 39-S

[0388] To a solution of compound **38-S** (890 mg, 1.12 mmol) in CH_3CN:H_2O (1.39:1, 75 mL, 0.015 M) was added $AgNO_3$ (4.70 g, 28.0 mmol). After 18 h at 23 °C, a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$ was added, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to afford compound **39-S** (500 mg, 57%).

[0389] R_f= 0.30 (CH_2Cl_2:CH_3OH, 9:1).

[0390] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38 - 7.33 (m, 1H), 7.33 - 7.28 (m, 1H), 7.23 - 7.16 (m, 1H), 7.16 - 7.09 (m, 1H), 6.62 (s, 1H), 6.18 (d, *J* = 1.4 Hz, 1H), 6.03 (d, *J* = 1.4 Hz, 1H), 5.71 (s, 1H), 5.19 (d, *J* = 11.2 Hz, 1H), 4.85 (s, 1H), 4.49 (s, 2H), 4.24 - 4.10 (m, 3H), 3.81 (s, 3H), 3.54 (d, *J* = 4.9 Hz, 1H), 3.49 (d, *J* = 2.3 Hz, 3H), 3.33 (t, *J* = 10.1 Hz, 2H), 3.22 (s, 1H), 2.98 (s, 1H), 2.84 (d, *J* = 7.6 Hz, 2H), 2.62 - 2.53 (m, 2H), 2.37 (s, 3H), 2.30 - 2.24 (m, 1H), 2.28 (s, 3H), 2.14 (s, 3H), 2.04 (s, 3H).

[0391] ^{13}C NMR (126 MHz, $CDCl_3$): δ 172.0, 170.7, 156.1, 150.6, 149.9, 147.1, 145.0, 142.4, 142.2, 132.0, 131.4, 128.7, 125.5, 123.8, 122.6, 121.6, 120.1, 116.5, 114.4, 112.3, 103.5, 92.6, 66.0, 65.1, 62.2, 60.4, 59.7, 56.6, 56.1, 54.8, 54.1, 51.6, 44.0, 41.3, 38.3, 30.8, 24.8, 20.6, 16.3, 9.6.

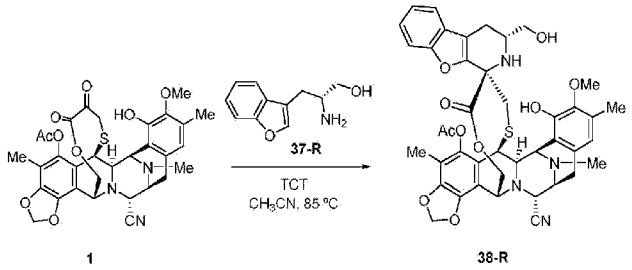
[0392] ESI-MS *m/z:* 768.2 $(M-H_2O+H)^+$.

[0393] (+)-HR-ESI-TOF-MS *m/z*: 768.2652 $[M-H_2O+H]^+$ (Calcd. for $C_{41}H_{42}N_3O_{10}S$ 768.2585)

Reference Example 22.

[0394]

A)



1 38-R

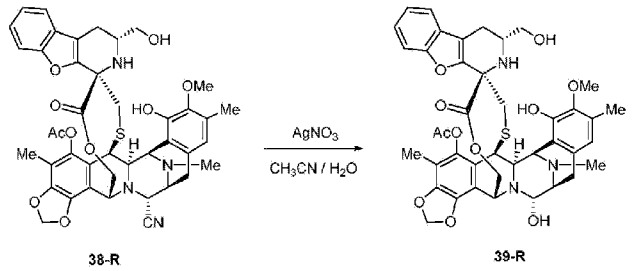
[0395] To a solution of compound 1 (100 mg, 0.16 mmol) in CH_3CN (16 mL, 0.01 M) was added (*R*)-2-amino-3-(benzofuran-3-yl)propan-1-ol (**37-R**) (307 mg, 1.6 mmol) and cyanuric chloride (TCT) (40 mg, 40%). The reaction mixture was stirred at 85 °C for **44** h and then aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, from 9:1 to 1:9) gives compound **38-R** (95 mg, 75%).

[0396] R_f= 0.3 (Hexane:EtOAc, 1:1).

[0397] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42 - 7.27 (m, 2H), 7.28 -7.09 (m, 2H), 6.58 (s, 1H), 6.20 (d, *J* = 1.4 Hz, 1H), 6.05 (d, *J* = 1.4 Hz, 1H), 5.79 (s, 1H), 5.00 (d , *J* = 11.4 Hz, 1H), 4.59 (s, 1H), 4.34 (s, 1H), 4.31 - 4.16 (m, 4H), 3.80 (s, 3H), 3.79 - 3.76 (m, 1H), 3.63 (s, 1H), 3.54 - 3.40 (m, 4H), 2.99 - 2.87 (m, 2H), 2.68 (d, *J* = 15.0 Hz, 1H), 2.56 - 2.47 (m, 1H), 2.38 (s, 3H), 2.27 (s, 3H), 2.17 (s, 3H), 2.07 (s, 3H).

[0398] ESI-MS *m/z:* 795.2 $(M+H)^+$.

B)



38-R 39-R

[0399] To a solution of compound **38-R** (95 mg, 0.11 mmol) in CH_3CN:H_2O (1.39:1, 11 mL, 0.015 M) was added $AgNO_3$ (601 mg, 3.58 mmol). After 18 h at 23 °C, a mixture 1:1 of saturated aqueous solutions of NaCl and $NaHCO_3$ was added, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to afford compound **39-R** (66 mg, 70%).

[0400] R_f= 0.3 (CH_2Cl_2:CH_3OH, 9:1).

[0401] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39 - 7.31 (m, 2H), 7.23 - 7.07 (m, 2H), 6.59 (s, 1H), 6.17 (d, *J* = 1.4 Hz, 1H), 6.01 (d, *J* = 1.4 Hz, 1H), 5.75 (s, 1H), 5.12 (dd, *J* = 11.3, 1.2 Hz, 1H), 4.84 (s, 1H), 4.56 - 4.43 (m, 2H), 4.19 - 4.07 (m, 3H), 3.79 (s, 3H), 3.83 - 3.74 (m, 1H), 3.66 - 3.51 (m, 3H), 3.24 (s, 1H), 2.99 - 2.79 (m, 2H), 2.75 - 2.64 (m, 1H), 2.59 - 2.43 (m, 2H), 2.38 (s, 3H), 2.27 (s, 3H), 2.16 (s, 3H), 2.07 (s, 3H).

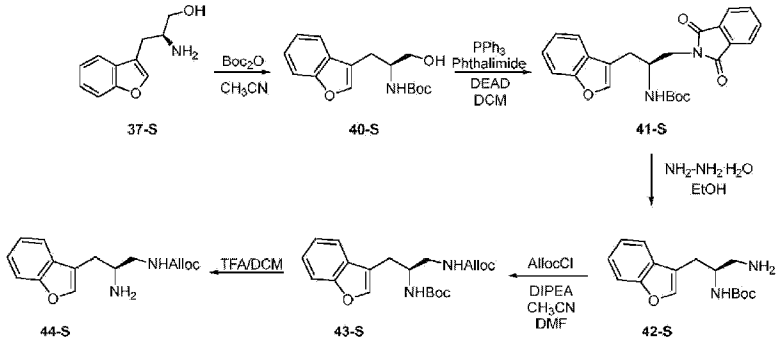
[0402] ^{13}C NMR (101 MHz, CD_3OD): δ 170.5, 169.1, 154.9, 148.9, 148.5, 145.7, 143.6, 141.1, 140.8, 130.6, 129.9, 127.1, 124.1, 122.4, 122.4, 121.2, 120.3, 118.7, 118.2, 115.1, 113.6, 110.9, 102.1, 91.1, 65.0, 63.3, 60.2, 59.0, 58.4, 55.4, 54.5, 52.7, 52.3, 42.5, 38.7, 29.4, 23.5, 23.2, 19.1, 14.8, 8.3.

[0403] ESI-MS *m/z:* 768.2 $(M-H_2O+H)^+$.

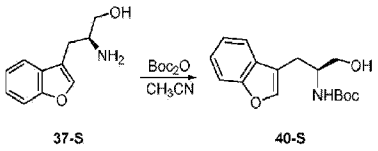
[0404] (+)-HR-ESI-TOF-MS *m/z*: 767.2628 $[M-H_2O+H]^+$ (Calcd. for $C_{41}H_{42}N_3O_{10}S$ 768.2585).

Example 23. Synthesis of allyl-*N*-[(*S*)-2-amino-3-(benzofuran-3-yl)propyl]carbamate (44-S).

[0405]



A)

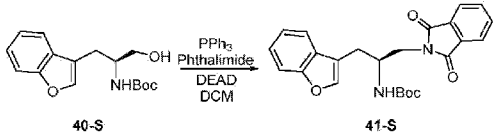


[0406] To a solution of compound **37-S** (1.0 g, 5.22 mmol) in CH_3CN (21 mL, 4 mL/mmol) was added di-*tert*-butyl dicarbonate (2.28 g, 10.4 mmol). The reaction mixture was stirred at 23 °C for 2 h, concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to afford compound **40-S** (0.5 g, 33%).

[0407] R_f= 0.7 (CH_2Cl_2:CH_3OH, 9:1).

[0408] ^1H NMR (400 MHz, $CDCl_3$): δ 7.64 (d, *J* = 7.6 Hz, 1H), 7.49 (s, 1H), 7.46 (d, *J* = 7.6 Hz, 1H), 7.36 - 7.19 (m, 2H), 4.94 (s, 1H), 3.98 (s, 1H), 3.71 - 3.56 (m, 2H), 2.93 (d, *J* = 6.9 Hz, 2H), 1.41 (s, 9H).

B)

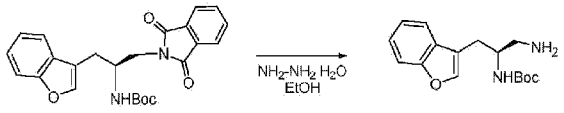


[0409] To a solution of compound **40-S** (0.5 g, 1.71 mmol) in CH_2Cl_2 (11 mL, 6 mL/mmol) was added phthalimide (0.55 g, 3.77 mmol), Triphenylphosphine (0.99 g, 3.77 mmol) and the mixture was cooled at 0 °C. A solution of 40% of Diethyl azodicarboxylate in CH_2Cl_2 (1.26 mL, 4.29 mmol) was added for 15 min. The reaction was stirred at 23 °C for 18 h, concentrated under vacuum. The residue obtained was purified by flash chromatography (Hexane:EtOAc, from 99:1 to 40:60) to afford compound **41-S** (0.68 g, 94%).

[0410] R_f= 0.8 (CH_2Cl_2:CH_3OH, 9:1).

[0411] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89 - 7.79 (m, 2H), 7.83 - 7.62 (m, 2H), 7.65 - 7.55 (m, 2H), 7.49 - 7.42 (m, 1H), 7.33 - 7.20 (m, 2H), 4.83 (d, *J* = 9.0 Hz, 1H), 4.39 (ddt, *J* = 12.1, 6.3, 2.9 Hz, 1H), 3.88 - 3.70 (m, 2H), 2.96 (d, *J* = 6.4 Hz, 2H), 1.24 (s, 9H).

C)



41-S 42-S

[0412] To a solution of compound **41-S** (345 mg, 0.82 mmol) in ethanol (25 mL, 30 mL/mmol) was added hydrazine monohydrate (3.6 mL, 73.8 mmol). The reaction mixture was stirred at 80 °C in sealed tube for 2 h, concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 100:1 to 50:50) to afford compound **42-S** (233 mg, 98%).

[0413] R_f= 0.1 (CH_2Cl_2:CH_3OH, 8:2).

[0414] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62 (d, *J* = 7.5 Hz, 1H), 7.49 - 7.42 (m, 2H), 7.33 - 7.18 (m, 2H), 4.85 (d, *J* = 8.8 Hz, 1H), 3.91 (s, 1H), 2.91 - 2.76 (m, 3H), 2.67 (dd, *J* = 13.1, 6.8 Hz, 1H), 1.25 (s, 9H).

D)

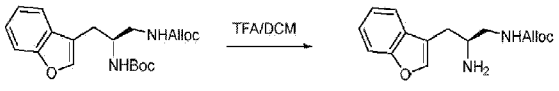
42-S 43-S

[0415] To a solution of compound **42-S** (280 mg, 0.96 mmol) in CH_3CN (10 mL, 10 mL/mmol) and DMF (16 mL, 1 mL/mmol) was added *N,N*-diisopropylethylamine (0.14 mL, 0.77 mmol) and allyl chloroformate (1.02 mL, 9.64 mmol). The reaction was stirred at 23 °C for 2 h. The mixture was diluted with EtOAc and NH_4Cl was added and the mixture was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (Hexane:EtOAc, from 100:1 to 1:100) to afford compound **43-S** (445 mg, >100%).

[0416] R_f= 0.5 (Hexane:EtOAc, 1:1).

[0417] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 (d, *J* = 7.6 Hz, 1H), 7.52 - 7.43 (m, 2H), 7.34 - 7.20 (m, 2H), 5.90 (ddt, *J* = 16.4, 10.8, 5.6 Hz, 1H), 5.32 - 5.17 (m, 2H), 4.93 - 4.86 (m, 1H), 4.56 (d, *J* = 5.6 Hz, 2H), 4.08 - 3.98 (m, 1H), 3.40 - 3.21 (m, 2H), 2.88 (m, 2H), 1.25 (s, 9H).

E)



43-S 44-S

[0418] To a solution of compound **43-S** (160 mg, 0.43 mmol) in CH_2Cl_2 (8 mL, 16.6 mL/mmol) was added trifluoroacetic acid (4 mL, 8.3 mL/mmol). The reaction mixture was stirred at 23 °C for 1.5 h, concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 100:1 to 50:50) to afford compound **44-S** (175 mg, >100%).

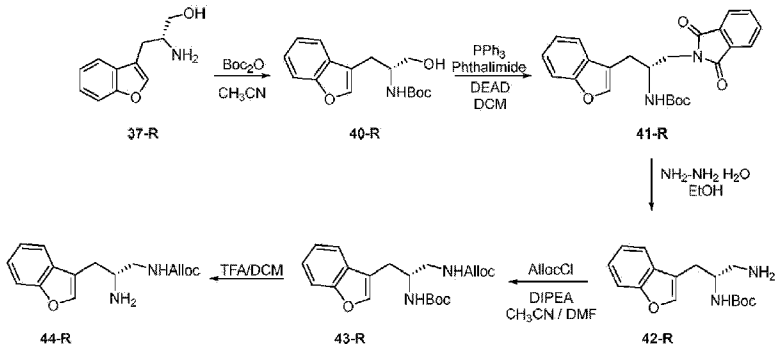
[0419] R_f= 0.2 (CH_2Cl_2:CH_3OH, 9:1).

[0420] ^{1}H NMR (400 MHz, CD_3OD): δ 7.72 (s, 1H), 7.64 (dt, *J* = 8.4, 0.9 Hz, 1H), 7.49 (dt, *J* = 8.4, 0.9 Hz, 1H),

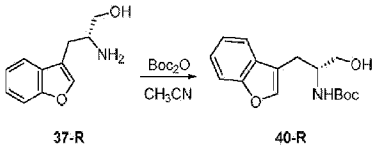
7.37 - 7.22 (m, 2H), 5.94 (ddt, *J* = 16.3, 10.7, 5.5 Hz, 1H), 5.32 (dq, *J* = 17.3, 1.7 Hz, 1H), 5.19 (dq, *J* = 10.6, 1.5 Hz, 1H), 4.56 (dt, *J* = 5.7, 1.5 Hz, 2H), 3.56 (qd, *J* = 7.0, 4.4 Hz, 1H), 3.46 - 3.32 (m, 1H), 3.32 - 3.24 (m, 1H), 3.03 (dd, *J* = 14.8, 6.9 Hz, 1H), 2.91 (ddd, *J* = 14.8, 7.1, 0.9 Hz, 1H).

Example 24. Synthesis of allyl-*N*-[(*R*)-2-amino-3-(benzofuran-3-yl)propyl]carbamate (44-R).

[0421]



A)

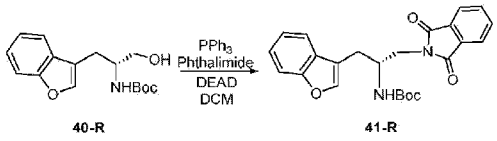


[0422] To a solution of compound **37-R** (2.75 g, 14.4 mmol) in CH_3CN (58 mL, 4 mL/mmol) was added di-*tert*-butyl dicarbonate (6.27 g, 28.76 mmol). The reaction mixture was stirred at 23 °C for 2.5 h, concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to afford compound **40-R** (3.7 g, 88%).

[0423] R_f= 0.6 (CH_2Cl_2:CH_3OH, 9:1).

[0424] ^1H NMR (400 MHz, $CDCl_3$): δ 7.64 (d, *J* = 7.6 Hz, 1H), 7.52 - 7.43 (m, 2H), 7.35 - 7.20 (m, 2H), 4.85 (d, *J* = 8.2 Hz, 1H), 4.00 (bs, 1H), 3.69 (dd, *J* = 11.0, 4.0 Hz, 1H), 3.62 (dd, *J* = 10.9, 5.1 Hz, 1H), 2.94 (d, *J* = 6.9 Hz, 2H), 1.42 (s, 9H).

B)



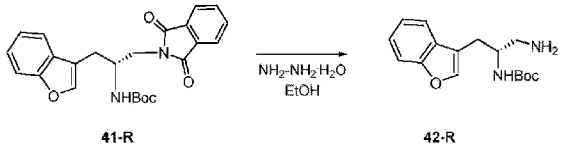
[0425] To a solution of compound **40-R** (3.7 g, 12.7 mmol) in CH_2Cl_2 (76 mL, 6 mL/mmol) was added phthalimide (4.1 g, 28 mmol), triphenylphosphine (7.3 g, 28 mmol) and the mixture was cooled at 0 °C. A solution of 40% of diethyl azodicarboxylate in CH_2Cl_2 (9.4 mL, 31.7 mmol) was added for 15 min. The reaction was stirred at 23 °C for 16 h, concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to afford compound **41-R** (4.05 g, 76%).

[0426] R_f= 0.8 (CH_2Cl_2:CH_3OH, 9:1).

[0427] ^1H NMR (400 MHz, $CDCl_3$): δ 7.67 - 7.68 (m, 4H), 7.61 (d, *J* = 7.5 Hz, 1H), 7.58 (s, 1H), 7.46 (d, *J* = 7.5 Hz, 1H), 7.27 (dtd, *J* = 17.2, 7.3, 1.4 Hz, 2H), 4.84 (d, *J* = 9.0 Hz, 1H), 4.46 - 4.30 (m, 1H), 3.89 - 3.66 (m, 2H),

2.97 (d, *J* = 6.4 Hz, 2H), 1.24 (s, 9H).

C)



41-R 42-R

[0428] To a solution of compound **41-R** (4.0 g, 9.5 mmol) in ethanol (285 mL, 30 mL/mmol) was added hydrazine monohydrate (41.5 mL, 856 mmol). The reaction mixture was stirred at 80 °C in sealed tube for 2 h, concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 100:1 to 50:50) to afford compound **42-R** (2.2 g, 80%).

[0429] R_f= 0.1 (CH_2Cl_2:CH_3OH, 8:2).

[0430] ^1H NMR (400 MHz, $CDCl_3$): δ 7.60 (d, *J* = 7.5 Hz, 1H), 7.45 (s, 1H), 7.44 (d, *J* = 7.1 Hz, 1H), 7.25 (dtd, *J* = 18.8, 7.3, 1.3 Hz, 2H), 4.94 (d, *J* = 8.8 Hz, 1H), 3.98 - 3.78 (m, 1H), 2.90 - 2.77 (m, 2H), 2.65 (dd, *J* = 13.1, 7.0 Hz, 1H), 1.40 (s, 9H).

D)

42-R 43-R

[0431] To a solution of compound **42-R** (2.2 g, 7.6 mmol) in CH_3CN (76 mL, 10 mL/mmol) and DMF (7.6 mL, 1 mL/mmol) was added *N,N*-diisopropylethylamine (1.1 mL, 6.08 mmol) and allyl chloroformate (8.05 mL, 76 mmol). The reaction was stirred at 23 °C for 7 h. The mixture was diluted with EtOAc and NH_4Cl was added and the mixture was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (Hexane:EtOAc, from 100:1 to 1:100) to afford compound **43-R** (2.3 g, 81%).

[0432] R_f= 0.7 (Hexane:EtOAc, 1:1).

[0433] ^1H NMR (400 MHz, $CDCl_3$): δ 7.60 (d, *J* = 7.5 Hz, 1H), 7.52 - 7.43 (m, 2H), 7.34 - 7.20 (m, 2H), 5.90 (ddt, *J* = 17.3, 10.8, 5.6 Hz, 1H), 5.29 (d, *J* = 17.2, 1H), 5.20 (d, *J* = 10.4, 1H), 5.10 (t, *J* = 6.2 Hz, 1H), 4.86 (d, *J* = 8.4 Hz, 1H), 4.56 (d, *J* = 5.4, 2H), 4.08 - 3.97 (m, 1H), 3.36 (dt, *J* = 10.7, 4.7 Hz, 1H), 3.30 - 3.23 (m, 1H), 2.87 (td, *J* = 14.8, 6.5 Hz, 2H), 1.41 (s, 9H).

E)

43-R 44-R

[0434] To a solution of compound **43-R** (1.32 g, 3.52 mmol) in CH_2Cl_2 (60 mL, 16.6 mL/mmol) was added Trifluoroacetic acid (30 mL, 8.3 mL/mmol). The reaction mixture was stirred at 23 °C for 1.5 h, concentrated under vacuum. Flash chromatography (CH_2Cl_2:CH_3OH, from 100:1 to 50:50) to afford compound **44-R** (0.90 g, 94%).

[0435] R_f= 0.2 (CH_2Cl_2:CH_3OH, 9:1).

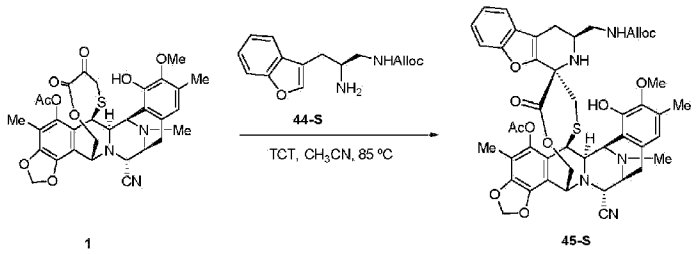
[0436] ^1H NMR (400 MHz, $CDCl_3$): δ 7.75 (s, 1H), 7.69 - 7.61 (m, 1H), 7.54 - 7.46 (m, 1H), 7.39 - 7.24 (m, 2H),

5.95 (ddt, *J* = 16.3, 10.8, 5.5 Hz, 1H), 5.32 (dd, *J* = 17.3, 1.8 Hz, 1H), 5.24 - 5.16 (m, 1H), 4.57 (dt, *J* = 5.7, 1.5 Hz, 2H), 3.68 (qd, *J* = 7.1, 4.2 Hz, 1H), 3.48 (dd, *J* = 14.8, 4.2 Hz, 1H), 3.42 - 3.30 (m, 1H), 3.14 - 2.95 (m, 2H).

Example 25

[0437]

A)



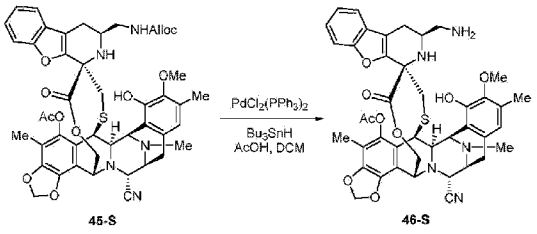
[0438] To a solution of compound **1** (750 mg, 1.2 mmol) in CH_3CN (120 mL, 0.01 M) was added compound **44-S** (1370 mg, 6 mmol) and cyanuric chloride (TCT) (184 mg, 20%). The reaction mixture was stirred at 85 °C for 23 h and then aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, from 9:1 to 1:9) gives compound **45-S** (755 mg, 72%).

[0439] R_f= 0.36 (Hexane:EtOAc, 1:1).

[0440] ^1H NMR (400 MHz, $CDCl_3$): δ 7.38 - 7.28 (m, 2H), 7.23 - 7.08 (m, 2H), 6.67 (s, 1H), 6.19 (d, *J* = 1.4 Hz, 1H), 6.09 - 5.95 (m, 1H), 6.04 (d, *J* = 1.4 Hz, 1H), 5.92 (s, 1H), 5.80 (s, 1H), 5.44 - 5.34 (m, 1H), 5.26 (dq, *J* = 10.4, 1.3 Hz, 1H), 5.08 (dd, *J* = 11.4, 1.1 Hz, 1H), 4.70 - 4.63 (m, 2H), 4.56 (s, 1H), 4.34 (s, 1H), 4.31 - 4.18 (m, 3H), 3.80 (s, 3H), 3.50 - 3.39 (m, 2H), 3.24 - 3.15 (m, 1H), 3.00 (dt, *J* = 12.2, 6.0 Hz, 2H), 2.95 (d, *J* = 5.2 Hz, 2H), 2.60 (dd, *J* = 15.4, 4.5 Hz, 2H), 2.44 (dd, *J* = 15.6, 5.2 Hz, 1H), 2.29 (s, 3H), 2.27 (s, 3H), 2.25 - 2.20 (m, 1H), 2.18 (s, 3H), 2.12 (s, 1H), 2.04 (s, 3H).

[0441] ESI-MS *m/z:* 878.2 $(M+H)^+$.

B)



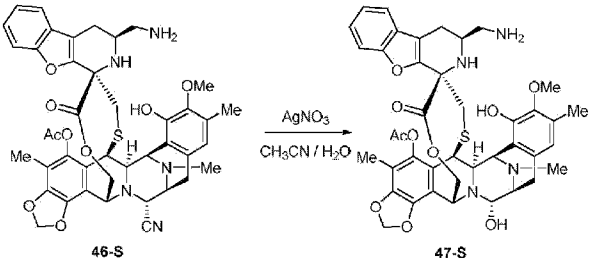
[0442] To a solution of compound **45-S** (750 mg, 0.85 mmol) in CH_2Cl_2 (15.3 mL, 18 mL/mmol) was added bis(triphenylphosphine)palladium(II) dichloride (96 mg, 0.14 mmol) and acetic acid (0.5 mL, 8.5 mmol). Tributyltin hydride (1.4 mL, 5.1 mmol) was added at 0 °C, and the reaction mixture was stirred at 0 °C for 30 minutes, and was concentrated under vacuum. Flash chromatography (Hexane:EtOAc, from 100:1 to 1:100 and CH_2Cl_2:CH_3OH, from 100:1 to 1:100) to afford compound **46-S** (430 mg, 64%).

[0443] R_f= 0.3 (CH_2Cl_2:CH_3OH, 1:1).

[0444] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37 - 7.29 (m, 2H), 7.22 - 7.11 (m, 2H), 6.57 (s, 1H), 6.21 (d, *J* = 1.5 Hz, 1H), 6.06 (d, *J* = 1.5 Hz, 1H), 5.07 (d, *J* = 11.5 Hz, 1H), 4.57 (s, 1H), 4.37 (s, 1H), 4.29 - 4.23 (m, 2H), 4.14 (s, 1H), 3.79 (s, 3H), 3.50 - 3.47 (m, 2H), 3.38 (d, *J* = 8.7 Hz, 1H), 2.95 -2.71 (m, 4H), 2.68 - 2.52 (m, 2H), 2.51 - 2.38 (m, 1H), 2.35 (s, 3H), 2.33 - 2.26 (m, 1H), 2.29 (s, 3H), 2.17 - 2.08 (m, 1H), 2.10 (s, 3H), 2.04 (s, 3H).

[0445] ESI-MS *m/z:* 794.3 $(M+H)^+$.

C)



46-S 47-S

[0446] To a solution of compound **46-S** (550 mg, 0.7 mmol) in CH_3CN:H_2O (1.39:1, 49 mL, 0.015 M) was added $AgNO_3$ (2.4 g, 14 mmol). After 16 h at 23 °C, the reaction was quenched with a mixture 1:1 of saturated aqueous solutions of NaCl and NaHCOs, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to give compound **47-S** (53 mg, 10%).

[0447] R_f= 0.1 (CH_2Cl_2:CH_3OH, 9:1).

[0448] ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.36 (d, 7.9 Hz, 1H), 7.33 (d, 7.4 Hz, 1H), 7.23 (t, *J* = 7.4 Hz, 1H), 7.16 (t, *J* = 7.4 Hz, 1H), 6.77 (s, 1H), 6.20 (s, 1H), 6.04 (s, 1H), 5.92 (s, 1H), 5.20 (d, *J* = 11.1 Hz, 1H), 4.90 (s, 1H), 4.50 (s, 1H), 4.46 - 4.39 (m, 1H), 4.25 (d, *J* = 11.1 Hz, 1H), 420 (s, 1H), 3.84 (s, 3H), 3.81 (d, *J* = 4.2 Hz, 1H), 3.58 (s, 1H), 3.40 - 3.14 (m, 3H), 2.90 (t, *J* = 13.0 Hz, 1H), 2.76 (m, 3H), 2.50 (s, 3H), 2.46 - 2.37 (m, 1H), 2.32 - 2.26 (m, 2H), 2.30 (s, 3H), 2.15 (s, 3H), 2.04 (s, 3H).

[0449] ^{13}C NMR (126 MHz, CD_3OD): δ 170.5, 169.2, 154.6, 149.1, 148.7, 145.7, 143.5, 141.0, 140.9, 131.2, 129.6, 126.9, 124.4, 122.5, 121.4, 119.7, 118.7, 115.0, 112.7, 111.0, 110.7, 102.1, 91.2, 63.5, 61.2, 59.2, 58.5, 55.3, 54.7, 53.4, 52.7, 43.3, 42.5, 39.9, 36.9, 29.3, 24.1, 23.6, 19.1, 15.0, 8.2.

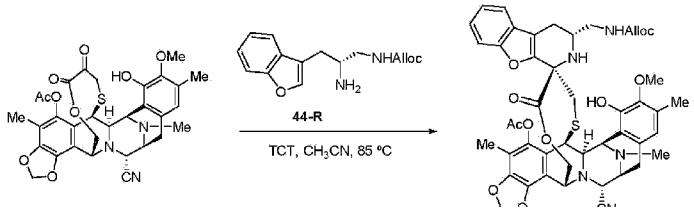
[0450] ESI-MS *m/z:* 767.2 $(M-H_2O+H)^+$.

[0451] (+)-HR-ESI-TOF-MS *m/z*: 767.2794 $[M-H_2O+H]^+$ (Calcd. for $C_{41}H_{43}N_4O_9S$ 767.2745).

Example 26.

[0452]

A)



1 45-R

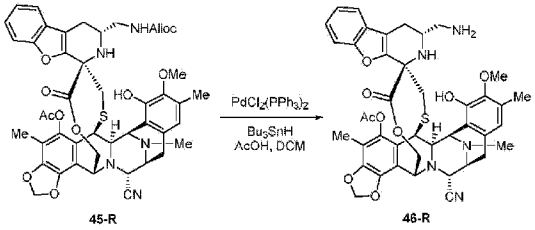
[0453] To a solution of compound **1** (621 mg, 1 mmol) in CH_3CN (100 mL, 0.01 M) was added compound **44-R** (825 mg, 3 mmol) and cyanuric chloride (TCT) (248 mg, 40%). The reaction mixture was stirred at 85 °C for 66 h and then aqueous saturated solution of $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, from 9:1 to 1:9) gives compound **45-R** (530 mg, 58%).

[0454] R_f= 0.4 (Hexane:EtOAc, 1:1).

[0455] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42 - 7.28 (m, 2H), 7.23 - 7.08 (m, 2H), 6.60 (s, 1H), 6.20 (d, *J* = 1.4 Hz, 1H), 6.04 (d, *J* = 1.4 Hz, 1H), 6.01 - 5.92 (m, 1H), 5.77 (s, 1H), 5.44 - 5.20 (m, 2H), 5.09 (s, 1H), 5.04 - 4.96 (m, 1H), 4.71 - 4.55 (m, 2H), 4.34 (s, 1H), 4.30 - 4.18 (m, 3H), 3.79 (s, 3H), 3.53 (dd, *J* = 10.2, 4.4 Hz, 1H), 3.46 (m, 2H), 3.50 - 3.40 (m, 1H), 3.03 - 2.87 (m, 2H), 2.67 (d, *J* = 15.0 Hz, 1H), 2.47 (dd, *J* = 15.6, 3.7 Hz, 1H), 2.40 - 2.32 (m, 2H), 2.30 (s, 3H), 2.29 (s, 3H), 2.19 - 2.12 (m, 2H), 2.16 (s, 3H), 2.09 (s, 3H).

[0456] ESI-MS *m/z:* 878.3 $(M+H)^+$.

B)



45-R 46-R

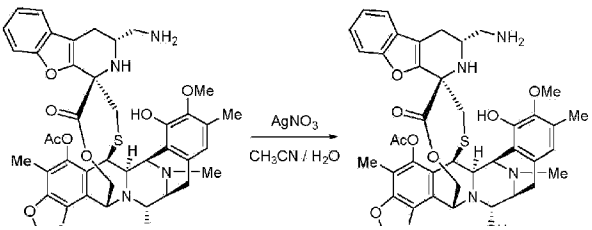
[0457] To a solution of compound **45-R** (552 mg, 0.63 mmol) in CH_2Cl_2 (11.3 mL, 18 mL/mmol) was added bis(triphenylphosphine)palladium(II) dichloride (70.7 mg, 0.1 mmol) and acetic acid (0.36 mL, 6.3 mmol). Tributyltin hydride (1.02 mL, 3.8 mmol) was added at 0 °C and the reaction mixture was stirred at 0 °C for 0.5 h, and concentrated under vacuum The crude obtained was diluted with EtOAc, saturated aqueous solution of NH_4Cl was added and the mixture was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash chromatography (Hexane:EtOAc, from 100:1 to 1:100 and EtOAc:CH_3OH, from 100:1 to 1:100) to afford compound **46-R** (423 mg, 85%).

[0458] R_f= 0.3 (CH_2Cl_2:CH_3OH, 1:1).

[0459] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45 - 7.28 (m, 2H), 7.23 - 7.08 (m, 2H), 6.56 (s, 1H), 6.19 (d, *J* = 1.4 Hz, 1H), 6.05 (d, *J* = 1.4 Hz, 1H), 4.98 (d, *J* = 11.5 Hz, 1H), 4.59 (s, 1H), 4.34 (s, 1H), 4.27 (dd, *J* = 5.1, 1.7 Hz, 1H), 4.22 - 4.16 (m, 2H), 3.80 (s, 3H), 3.49 - 3.39 (m, 2H), 3.31 (dq, *J* = 9.8, 5.5, 4.5 Hz, 2H), 2.95 (s, 1H), 2.83 (d, *J* = 5.6 Hz, 2H), 2.74 - 2.51 (m, 3H), 2.35 (s, 3H), 2.32 - 2.21 (m, 2H), 2.26 (s, 3H); 2.16 (s, 3H), 2.06 (s, 3H).

[0460] ESI-MS *m/z:* 794.3 $(M+H)^+$.

C)



46-R 47-R

[0461] To a solution of compound **46-R** (412 mg, 0.52 mmol) in CH_3CN:H_2O (1.39:1, 36 mL, 0.015 M) was added $AgNO_3$ (1.76 g, 10.4 mmol). After 22 h at 23 °C, the reaction was quenched with a mixture 1:1 of saturated aqueous solutions of NaCl and NaHCOs, stirred for 15 min, diluted with CH_2Cl_2, stirred for 5 min, and extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained was purified by flash chromatography (CH_2Cl_2:CH_3OH, from 99:1 to 85:15) to give compound **47-R** (175 mg, 43%).

[0462] R_f= 0.1 (CH_2Cl_2:CH_3OH, 9:1).

[0463] ^1H NMR (500 MHz, $CDCl_3$): δ 7.34 (dd, *J* = 11.1, 7.9 Hz, 2H), 7.22 - 7.07 (m, 2H), 6.57 (s, 1H), 6.17 (d, *J* = 1.2 Hz, 1H), 6.01 (d, *J* = 1.2 Hz, 1H), 5.11 (d, *J* = 11.2 Hz, 1H), 4.84 (s, 1H), 4.53 - 4.47 (m, 2H), 4.21 - 4.07 (m, 2H), 3.80 (s, 3H), 3.56 (d, *J* = 5.1 Hz, 1H), 3.43 (s, 1H), 3.24 (d, *J* = 9.1 Hz, 1H), 2.98 - 2.78 (m, 4H), 2.72 - 2.58 (m, 2H), 2.38 (s, 3H), 2.35 - 2.27 (m, 2H), 2.28 (s, 3H), 2.14 (s, 3H), 2.08 (s, 3H).

[0464] ^{13}C NMR (101 MHz, CD_3OD): δ 170.6, 169.1, 155.0, 148.8, 145.6, 143.7, 141.1, 140.8, 130.9, 129.7, 126.9, 124.2, 122.4, 121.1, 119.6, 118.9, 118.7, 115.0, 113.2, 112.5, 111.0, 102.1, 91.3, 63.3, 60.4, 59.0, 58.4, 55.3, 54.6, 52.6, 51.1, 44.9, 42.4, 39.8, 38.7, 29.4, 24.0, 23.2, 19.1, 15.0, 8.3.

[0465] ESI-MS *m/z:* 767.2 $(M-H_2O+H)^+$.

[0466] (+)-HR-ESI-TOF-MS m/z: 767.2806 $[M-H_2O+H]^+$ (Calcd. for $C_{41}H_{43}N_4O_9S$ 767.2745).

Example 27. *In vitro* bioassays for the detection of antitumor activity

[0467] The aim of this assay is to evaluate the *in vitro* cytostatic (ability to delay or arrest tumor cell growth) or cytotoxic (ability to kill tumor cells) activity of the samples being tested.

CELL LINES

[0468]

Name	N° ATCC	Species	Tissue	Characteristics
A549	CCL-185	human	lung	lung carcinoma (NSCLC)
HT29	HTB-38	human	colon	colorectal adenocarcinoma
MDA-MB-231	HTB-26	human	breast	breast adenocarcinoma
PSN1	CRM-CRL-3211	human	pancreas	pancreas adenocarcinoma
PC-3	CRL-1435	human	prostate	prostate adenocarcinoma
22Rv1	CRL-2505	human	prostate	prostate carcinoma

EVALUATION OF CYTOTOXIC ACTIVITY USING THE SBR AND THE MTT COLORIMETRIC ASSAYS

[0469] A colorimetric assay, using sulforhodamine B (SRB) reaction has been adapted to provide a quantitative measurement of cell growth and viability (following the technique described by Skehan et al. J. Natl. Cancer Inst. 1990, 82, 1107-1112). Another colorimetric assay based on 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction to a purple formazan has been also used to assess the antiproliferative activity (following the technique described by Mosmann et al. J. Immunol. Meth. 1983, 65, 55-63).

[0470] These forms of assays employ 96-well cell culture microplates following the standards of the American National Standards Institute and the Society for Laboratory Automation and Screening (ANSI SLAS 1-2004 (R2012) 10/12/2011. All the cell lines used in this study were obtained from the American Type Culture Collection (ATCC) and derive from different types of human cancer.

[0471] A549, HT29, MDA-MB-231 and PSN1 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) while PC-3 and 22Rv1 cells were maintained in Roswell Park Memorial Institute Medium (RPMI). All cell lines were supplemented with 10% Fetal Bovine Serum (FBS), 2mM L-glutamine, 100 U/mL penicillin, and 100 U/mL streptomycin at 37 °C, 5% CO_2 and 98% humidity. For the experiments, cells were harvested from subconfluent cultures using trypsinization and resuspended in fresh medium before counting and plating.

[0472] A549, HT29, MDA-MB-231 and PSN1 cells were seeded in 96 well microtiter plates, at 5000 cells per well in aliquots of 150 µL, and allowed to attach to the plate surface for 18 hours (overnight) in drug free medium. After that, one control (untreated) plate of each cell line was fixed (as described below) and used for time zero reference value. Culture plates were then treated with test compounds (50 µL aliquots of 4X stock solutions in complete culture medium plus 4% DMSO) using ten 2/5 serial dilutions (concentrations ranging from 10 to 0.003 µg/mL) and triplicate cultures (1% final concentration in DMSO). After 72 hours treatment, the antitumor effect was measured by using the SRB methodology: Briefly, cells were washed twice with PBS, fixed for 15 min in 1% glutaraldehyde solution at room temperature, rinsed twice in PBS, and stained in 0.4% SRB solution for 30 min at room temperature. Cells were then rinsed several times with 1% acetic acid solution and air-dried at room temperature. SRB was then extracted in 10 mM trizma base solution and the absorbance measured in an automated spectrophotometric plate reader at 490 nm.

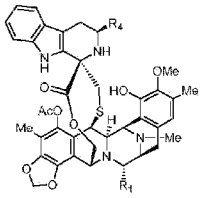
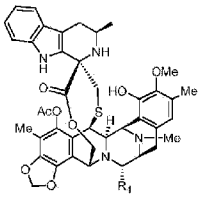
[0473] An appropriate number of PC-3 and 22Rv1 cells, to reach a final cell density in the assay ranging from 5,000 to 15,000 cells per well depending on the cell line, were seeded in 96-well plates and allowed to stand in culture medium for 24 h at 37°C under 5% CO2 and 98% humidity. Then, compounds or DMSO in culture medium were added to reach a final volume of 200 µL and the intended compound concentration in a range covering ten serial 2/5 dilutions starting from 0.1 µg/mL in 1% (v/v) DMSO. At this point a set of "time zero control plates" treated with 1% (v/v) DMSO were processed with MTT as described below. The rest of the plates were incubated during 72 h under the aforementioned environmental conditions. Afterwards 50 µL of a 1 mg/mL MTT solution in culture medium were added to the wells and incubated for 6-8 hours at 37°C to allow formazan crystals generation. Culture medium was then removed and 100 µL of neat DMSO added to each well to dissolve the formazan product into a coloured solution whose absorbance at 540 nm was finally measured in a PolarStar Omega microplate multilabel reader (BMG Labtech, Ortenberg, Germany).

[0474] Effects on cell growth and survival were estimated by applying the NCI algorithm (Boyd MR and Paull KD. Drug Dev. Res. 1995, 34, 91-104).The values obtained in triplicate cultures were fitted by nonlinear regression to a four-parameters logistic curve by nonlinear regression analysis. Three reference parameters were calculated (according to the aforementioned NCI algorithm) by automatic interpolation of the curves obtained by such fitting: GI_{50} = compound concentration that produces 50% cell growth inhibition, as compared to control cultures; TGI = total cell growth inhibition (cytostatic effect), as compared to control cultures, and LC_{50} = compound concentration that produces 50% net cell killing cytotoxic effect).

[0475] Tables 1-7 illustrate data on the biological activity of compounds of the present invention together with biological activity of the reference compounds. Tables 8-9 provide data on the biological activity of several compounds of the invention compared to their analogues with a carboxylic acid group. Compounds **10-S, 10-R,**

11-S, 11-R, 12-S, 12-R, 13-S, 13-R, 25-S, 25-R, 26-S, 26-R, 27-S, 27-R, 45-S, 45-R, 46-S, 46-R, 47-S, 47-R are compounds according to the present invention in tables 1-9; other compounds such as **A, B, E, F, ET-736, PM01183**, **14-S, 15-S, 28-S, 28-R, 29-S**, and **29-R**, are not part of the present invention.

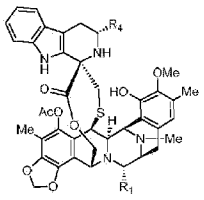
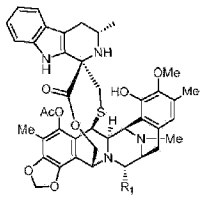
Table 1. Biological activity (Molar)

	Compound	Reference compound
		
	3-S R_1 = CN, R_4 = $-CH_2OH$	
	3a-S R_1 = CN, R_4 = $-CH_2OAc$	
	10-S R_1 = CN, R_4 = $-CH_2NHAlloc$	
	11-S R_1 = CN, R_4 = $-CH_2NH_2$	**A** R_1 = CN
	4-S R_1 = OH, R_4 = $-CH_2OH$	**C** R_1 = OH
	4a-S R_1 = OH, R_4 = $-CH_2OAc$	
	12-S R_1 = OH, R_4 = $-CH_2NH_2$	
	13-S R_1 = OH, R_4 = $-CH_2NHAlloc$	

		A549	HT29	MDA-MB-231	PSN1	PC-3	22Rv1		A549	HT29	MDA-MB-231	PSN1
GI_{50}	**3-S**	4.03E-10	2.77E-10	4.91E-10	9.95E-10			**A**	8.36E-09	7.71E-09	7.07E-09	1.29E-08
TGI		6.17E-10	>1.26E-07	5.29E-10	1.64E-09				8.87E-09	8.36E-09	9.38E-09	1.54E-08
LC_{50}		>1.26E-07	>1.26E-07	6.17E-10	>1.26E-07				>1.29E-07	>1.29E-07	1.41E-08	1.93E-08
GI_{50}	**3a-S**	3.11E-09	2.99E-09	2.87E-09	2.15E-09							
TGI		3.23E-09	3.23E-09	3.59E-09	3.59E-09							
LC_{50}		>1.20E-07	>1.20E-07	4.90E-09	1.20E-08							
GI_{50}	**10-S**	2.05E-08	1.14E-08	4.79E-09	7.64E-09							
TGI		3.08E-08	1.25E-08	8.44E-09	1.25·E-08							
LC_{50}		7.53E-08	>1.14E-06	1.60E-08	2.39E-08							
GI_{50}	**11-S**	8.45E-09	3.41E-09	2.27E-09	3.28E-09							
TGI		2.65E-08	>1.26E-07	3.41E-09	4.54E-09							
LC_{50}		>1.26E-07	>1.26E-07	6.43E-09	8.07E-09							

		A549	HT29	MDA-MB-231	PSN1	PC-3	22Rv1		A549	HT29	MDA-MB-231	PSN1
GI_{50}		1.27E-09	1.27E-09	1.22E-09	1.78E-09	8.08E-10	3.58E-10		2.73E-08	2.08E-08	2.60E-08	3.64E-08
TGI	4-S	1.40E-09	1.40E-09	2.55E-09	2.29E-09			C	6.63E-08	2.34E-08	5.46E-08	4.42E-08
LC_{50}		>1.27E-07	>1.27E-07	6.50E-09	3.44E-09				>1.30E-07	>1.30E-07	>1.30E-07	6.50E-08
GI_{50}	4a-S	3.99E-09	3.14E-09	3.39E-09	3.02E-09							
TGI		6.17E-09	3.39E-09	5.44E-09	3.27E-09							
LC_{50}		>1.21E-07	>1.21E-07	1.00E-08	3.51E-09							
GI_{50}	12-S	2.04E-08	4.85E-09	5.23E-09	3.44E-09							
TGI		5.61E-08	8.42E-09	8.42E-09	5.49E-09							
LC_{50}		>1.28E-07	>1.28E-07	1.53E-08	1.21E-08							
GI_{50}	13-S	1.15E-08	1.15E-08	1.15E-08	1.96E-08							
TGI		1.61E-08	1.27E-08	1.27E-08	2.88E-08							
LC_{50}		2.42E-08	>1.15E-06	1.38E-08	4.61E-08							

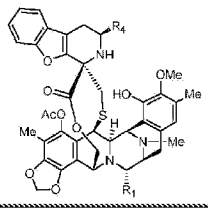
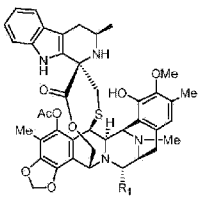
Table 2. Biological activity (Molar)

	Compound	Reference compound
		
	3-R R_1 = CN, R_4 = $-CH_2OH$	
	10-R R_1 = CN, R_4 = $-CH_2NHAlloc$	
	11-R R_1 = CN, R_4 = $-CH_2NH_2$	B R_1 = CN
	4-R R_1 = OH, R_4 = $-CH_2OH$	D R_1 = OH
	12-R R_1 = OH, R_4 = $-CH_2NH_2$	
	13-R R_1 = OH, R_4 = $-CH_2NHAlloc$	

		A549	HT29	MDA-MB-231	PSN1		A549	HT29	MDA-MB-231	PSN1
GI_{50}	3-R	4.03E-10	2.77E-10	2.77E-10	3.90E-10	B	2.06E-08	8.48E-09	9.00E-09	1.93E-08
TGI		5.79E-10	>1.26E-07	5.04E-10	6.05E-10		2.19E-08	9.13E-09	1.67E-08	2.06E-08

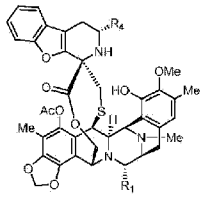
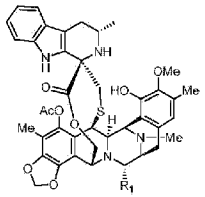
		A549	HT29	MDA-MB-231	PSN1		A549	HT29	MDA-MB-231	PSN1
LC_{50}		>1.26E-07	>1.26E-07	1.25E-09	>1.26E-07		>1.29E-07	>1.29E-07	3.47E-08	2.31E-08
GI_{50}	**10-R**	3.76E-09	3.08E-09	2.85E-09	2.62E-09					
TGI		5.93E-09	>1.14E-07	4.33E-09	3.88E-09					
LC_{50}		>1.14E-07	>1.14E-07	7.18E-09	6.61E-09					
GI_{50}	**11-R**	1.77E-09	1.39E-09	1.01E-09	1.39E-09					
TGI		4.54E-09	>1.26E-07	1.51E-09	1.89E-09					
LC_{50}		>1.26E-07	>1.26E-07	2.65E-09	>1.26E-07					
GI_{50}	**4-R**	1.27E-09	1.26E-09	1.27E-09	4.59E-10	**D**	1.25E-08	1.03E-08	9.88E-09	2.08E-08
TGI		1.40E-09	1.40E-09	1.40E-09	8.54E-10		2.86E-08	2.34E-08	1.95E-08	2.21E-08
LC_{50}		> 1.27E-07	>1.27E-07	1.53E-09	2.55E-09		>1.30E-07	>1.30E-07	5.33E-08	2.47E-08
GI_{50}	**12-R**	1.40E-09	5.74E-10	3.19E-10	4.98E-10					
TGI		2.93E-09	1.10E-09	6.76E-10	1.22E-09					
LC_{50}		1.22E-08	2.93E-09	1.40E-09	>1.28E-07					
GI_{50}	**13-R**	7.26E-09	6.91E-09	4.95E-09	2.88E-09					
TGI		7.72E-09	7.60E-09	7.95E-09	3.11E-09					
LC_{50}		>1.15E-07	>1.15E-07	1.38E-08	3.46E-09					

Table 3. Biological activity (Molar)

	Compound	Reference compound
		
	38-S R_1 = CN, R_4 = $-CH_2OH$	
	45-S R_1 = CN, R_4 = $-CH_2NHAlloc$	
	46-S R_1 = CN, R_4 = $-CH_2NH_2$	**A** R_1 = CN
	39-S R_1 = OH, R_4 = $-CH_2OH$	**C** R_1 = OH
	47-S R_1 = OH, R_4 = $-CH_2NH_2$	

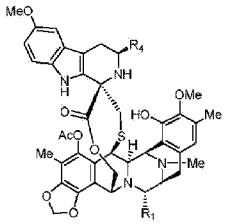
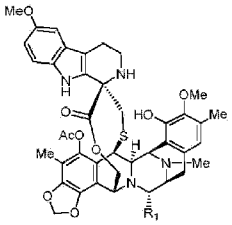
		A549	HT29	MDA-MB-231	PSN1	PC-3	22Rv1		A549	HT29	MDA-MB-231	PSN1
GI_{50}	**38-S**	8.05E-09	4.53E-09	2.52E-09	5.03E-09			**A**	8.36E-09	7.71E-09	7.07E-09	1.29E-08
TGI		8.55E-09	7.05E-09	4.28E-09	8.18E-09				8.87E-09	8.36E-09	9.38E-09	1.54E-08
LC_{50}		9.44E-09	>1.26E-07	7.80E-09	1.51E-08				>1.29E-07	>1.29E-07	1.41E-08	1.93E-08
GI_{50}	**45-S**	1.82E-08	1.82E-08	1.71E-08	1.94E-08							
TGI		1.94E-08	1.94E-08	2.16E-08	2.62E-08							
LC_{50}		2.16E-08	>1.14E-07	2.96E-08	3.64E-08							
GI_{50}	**46-S**	8.19E-09	2.77E-09	3.65E-09	3.15E-09							
TGI		2.14E-08	6.17E-09	6.80E-09	4.79E-09							
LC_{50}		>1.26E-07	>1.26E-07	1.26E-08	9.20E-09							
GI_{50}	**39-S**	4.84E-09	3.94E-09	3.44E-09	8.02E-09	2.78E-09	4.81E-10	**C**	2.73E-08	2.08E-08	2.60E-08	3.64E-08
TGI		8.27E-09	6.74E-09	7.13E-09	1.02E-08				6.63E-08	2.34E-08	5.46E-08	4.42E-08
LC_{50}		1.65E-08	>1.27E-07	1.78E-08	1.27E-08				>1.30E-07	>1.30E-07	>1.30E-07	6.50E-08
GI_{50}	**47-S**	1.40E-08	4.33E-09	6.24E-09	5.99E-09							
TGI		2.80E-08	6.75E-09	9.68E-09	8.54E-09							
LC_{50}		>1.27E-07	>1.27E-07	1.66E-08	1.27E-08							

Table 4. Biological activity (Molar)

	Compound	Reference compound
		
	38-R R_1 = CN, R_4 = $-CH_2OH$	
	45-R R_1 = CN, R_4 = $-CH_2NHAlloc$	
	46-R R_1 = CN, R_4 = $-CH_2NH_2$	**B** R_1 = CN
	39-R R_1 = OH, R_4 = $-CH_2OH$	**D** R_1 = OH
	47-R R_1 = OH, R_4 = $-CH_2NH_2$	

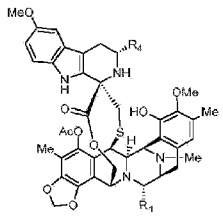
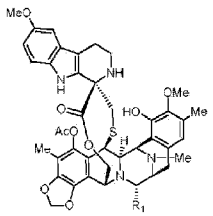
		A549	HT29	MDA-MB-231	PSN1		A549	HT29	MDA-MB-231	PSN1
GI_{50}	**38-R**	6.54E-10	5.41E-10	4.53E-10	6.54E-10	**B**	2.06E-08	8.48E-09	9.00E-09	1.93E-08
TGI		1.04E-09	5.91E-10	8.43E-10	9.94E-10		2.19E-08	9.13E-09	1.67E-08	2.06E-08
LC_{50}		>1.26E-07	>1.26E-07	2.01E-09	1.76E-09		>1.29E-07	>1.29E-07	3.47E-08	2.31E-08
GI_{50}	**45-R**	1.82E-08	1.25E-08	9.57E-09	1.06E-08					
TGI		1.94E-08	2.28E-08	1.94E-08	1.94E-08					
LC_{50}		2.39E-08	>1.14E-07	4.33E-08	3.76E-08					
GI_{50}	**46-R**	1.51E-09	1.21E-09	1.23E-09	9.95E-10					
TGI		2.77E-09	1.39E-09	1.39E-09	1.51E-09					
LC_{50}		>1.26E-07	>1.26E-07	1.51E-09	2.65E-09					
GI_{50}	**39-R**	2.67E-10	2.93E-10	2.04E-10	3.65E-10	**D**	1.25E-08	1.03E-08	9.88E-09	2.08E-08
TGI		4.33E-10	6.24E-10	5.98E-10	5.73E-10		2.86E-08	2.34E-08	1.95E-08	2.21E-08
LC_{50}		>1.27E-07	>1.27E-07	2.80E-09	1.06E-09		>1.30E-07	>1.30E-07	5.33E-08	2.47E-08
GI_{50}	**47-R**	2.04E-09	8.03E-10	5.99E-10	1.40E-09					
TGI		3.82E-09	1.40E-09	1.17E-09	2.04E-09					
LC_{50}		1.40E-08	>1.27E-07	2.55E-09	3.31E-09					

Table 5. Biological activity (Molar)

	Compound	Reference Compound
		
	18-S R_1 = CN, R_4 = $-CH_2OH$	
	25-S R_1 = CN, R_4 = $-CH_2NHAlloc$	**E** R_1 = CN
	26-S R_1 = CN, R_4 = $-CH_2NH_2$	**PM01183** R_1 = OH
	19-S R_1 = OH, R_4 = $-CH_2OH$	
	27-S R_1 = OH, R_4 = $-CH_2NH_2$	

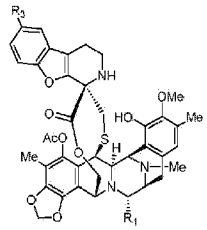
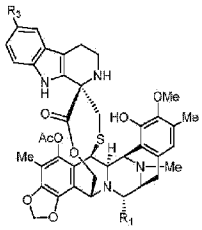
		A549	HT29	MDA-MB-231	PSN1		A549	HT29	MDA-MB-231	PSN1
GI_{50}	**18-S**	1.70E-09	1.21E-09	1.21E-09	9.59E-10	**E**	3.28E-09	3.15E-09	2.27E-09	2.77E-09
TGI		3.03E-09	1.34E-09	1.34E-09	1.34E-09		3.40E-09	3.40E-09	3.78E-09	4.53E-09
LC_{50}		>1.21E-07	>1.21E-07	1.58E-09	>1.21E-07		4.41E-09	>1.26E-07	7.43E-09	8.94E-09
GI_{50}	**25-S**	7.17E-09	7.17E-09	5.84E-09	6.84E-09					
TGI		7.61E-09	7.72E-09	9.04E-09	9.26E-09					
LC_{50}		>1.10E-07	>1.10E-07	1.54E-08	1.43E-08					
GI_{50}	**26-S**	1.12E-08	2.79E-09	1.34E-09	3.04E-09					
TGI		2.19E-08	3.16E-09	1.94E-09	3.28E-09					
LC_{50}		>1.22E-07	>1.22E-07	3.89E-09	3.52E-09					
GI_{50}	**19-S**	3.07E-09	1.35E-09	1.96E-09	2.95E-09	**PM 01183**	3.31E-09	1.91E-09	2.29E-09	3.19E-09
TGI		3.31E-09	1.60E-09	3.31E-09	3.19E-09		3.57E-09	4.46E-09	3.95E-09	3.95E-09
LC_{50}		>1.23E-07	>1.23E-07	1.10E-08	>1.23E-07		>1.27E-07	>1.27E-07	1.02E-08	5.73E-09
GI_{50}	**27-S**	6.02E-09	1.23E-09	1.19E-09	1.97E-09					
TGI		1.12E-08	1.35E-09	1.23E-09	2.83E-09					
LC_{50}		>1.23E-07	>1.23E-07	1.35E-09	4.55E-09					

Table 6. Biological activity (Molar)

	Compound	Reference Compound
		
	18-R R_1 = CN, R_4 = $-CH_2OH$	
	25-R R_1 = CN, R_4 = $-CH_2NHAlloc$	**E** R_1 = CN
	26-R R_1 = CN, R_4 = $-CH_2NH_2$	**PM01183** R_1 = OH
	19-R R_1 = OH, R_4 = $-CH_2OH$	
	27-R R_1 = OH, R_4 = $-CH_2NH_2$	

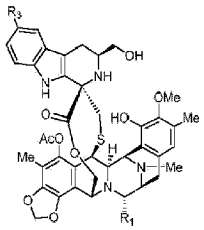
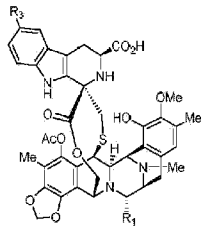
		A549	HT29	MDA-MB-231	PSN1		A549	HT29	MDA-MB-231	PSN1
GI_{50}	**18-R**	1.21E-09	1.21E-09	1.21E-09	5.70E-10	**E**	3.28E-09	3.15E-09	2.27E-09	2.77E-09
TGI		1.34E-09	1.34E-09	1.34E-09	1.06E-09		3.40E-09	3.40E-09	3.78E-09	4.53E-09
LC_{50}		>1.21E-07	>1.21E-07	1.46E-09	>1.21E-07		4.41E-09	>1.26E-07	7.43E-09	8.94E-09
GI_{50}	**25-R**	1.32E-09	1.54E-09	1.21E-09	1.21E-09					
TGI		2.43E-09	2.76E-09	2.54E-09	2.32E-09					
LC_{50}		9.92E-09	>1.10E-07	8.38E-09	6.73E-09					
GI_{50}	**26-R**	1.94E-09	7.29E-10	1.17E-09	9.72E-10					
TGI		3.40E-09	1.58E-09	1.22E-09	1.70E-09					
LC_{50}		>1.22E-07	>1.22E-07	1.46E-09	3.52E-09					
GI_{50}	**19-R**	1.47E-09	1.72E-09	1.23E-09	1.23E-09	**PM01183**	3.31E-09	1.91E-09	2.29E-09	3.19E-09
TGI		3.56E-09	1.72E-09	1.35E-09	1.35E-09		3.57E-09	4.46E-09	3.95E-09	3.95E-09
LC_{50}		>1.23E-07	>1.23E-07	>1.23E-07	1.47E-09		>1.27E-07	>1.27E-07	1.02E-08	5.73E-09
GI_{50}	**27-R**	2.09E-09	5.04E-10	3.07E-10	6.39E-10					
TGI		3.93E-09	5.53E-10	5.41E-10	1.17E-09					
LC_{50}		1.01E-08	>1.23E-07	8.60E-10	2.46E-09					

Table 7. Biological activity (Molar)

	Compound	Reference compound
		
	31 R_1 = CN, R_3 = H	**F** R_1 = CN, R_3 = H
	32 R_1 = OH, R_3 = H	**ET-736** R_1 = OH, R_3 = H
	34 R_1 = CN, R_3 = OMe	**E** R_1 = CN, R_3 = OMe
	35 R_1 = OH, R_3 = OMe	**PM01183** R_1 = OH, R_3 = OMe

		A549	HT29	MDA-MB-231	PSN1		A549	HT29	MDA-MB-231	PSN1
GI_{50}	**31**	1.96E-08	1.05E-08	8.89E-09	6.80E-09	**F**	3.80E-08	2.09E-08	1.96E-08	3.27E-08
TGI		2.09E-08	1.57E-08	1.70E-08	1.57E-08		7.20E-08	2.36E-08	3.40E-08	6.02E-08
LC_{50}		2.35E-08	> 1.31E-07	3.53E-08	4.31E-08		> 1.31E-07	> 1.31E-07	7.33E-08	1.07E-07
GI_{50}	**32**	6.88E-09	6.88E-09	4.76E-09	6.09E-09	**ET-736**	2.25E-08	2.12E-08	2.12E-08	3.97E-08
TGI		>1.32E-08	>1.32E-08	1.05E-08	8.34E-09		4.77E-08	2.25E-08	2.52E-08	5.96E-08
LC_{50}		>1.32E-08	>1.32E-08	>1.32E-08	1.20E-08		>1.32E-07	>1.32E-07	4.77E-08	1.02E-07
GI_{50}	**34**	5.91E-08	5.41E-08	4.53E-08	5.41E-08	**E**	3.28E-09	3.15E-09	2.27E-09	2.77E-09
TGI		8.05E-08	8.55E-08	7.67E-08	5.91E-08		3.40E-09	3.40E-09	3.78E-09	4.53E-09
LC_{50}		>1.26E-07	1.25E-07	1.12E-07	>1.26E-07		4.41E-09	>1.26E-07	7.43E-09	8.94E-09
GI_{50}	**35**	8.14E-09	7.89E-09	4.58E-09	6.24E-09	**PM01183**	3.31E-09	1.91E-09	2.29E-09	3.19E-09
TGI		8.78E-09	8.65E-09	8.27E-09	9.03E-09		3.57E-09	4.46E-09	3.95E-09	3.95E-09
LC_{50}		>1.27E-07	>1.27E-07	1.65E-08	1.40E-08		>1.27E-07	>1.27E-07	1.02E-08	5.73E-09

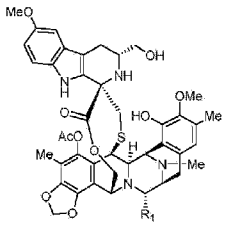
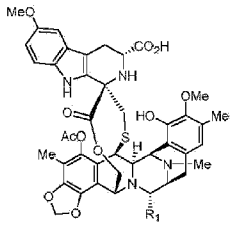
Table 8. Biological activity (Molar)

	Compound	**Reference compound**
		
	3-S R_1 = CN, R_3 = H	**14-S** R_1 = CN, R_3 = H
	4-S R_1 = OH, R_3 = H	**15-S** R_1 = OH, R_3 = H
	18-S R_1 = CN, R_3 = OMe	**28-S** R_1 = CN, R_3 = OMe
	19-S R_1 = OH, R_3 = OMe	**29-S** R_1 = OH, R_3 = OMe

		A549	HT29	MDA-MB-231	PSN1		A549	HT29	MDA-MB-231	PSN1
GI_{50}	**3-S**	4.03E-10	2.77E-10	4.91E-10	9.95E-10	**14-S**	>1.24E-07	1.21E-07	5.45E-08	>1.24E-07
TGI		6.17E-10	>1.26E-07	5.29E-10	1.64E-09		>1.24E-07	>1.24E-07	1.13E-07	>1.24E-07
LC_{50}		>1.26E-07	>1.26E-07	6.17E-10	>1.26E-07		>1.24E-07	>1.24E-07	>1.24E-07	>1.24E-07

		A549	HT29	MDA-MB-231	PSN1		A549	HT29	MDA-MB-231	PSN1
GI_{50}	4-S	1.27E-09	1.27E-09	1.22E-09	1.78E-09	15-S	>1.25E-06	3.00E-07	1.63E-07	2.38E-07
TGI		1.40E-09	1.40E-09	2.55E-09	2.29E-09		>1.25E-06	5.13E-07	2.13E-07	4.63E-07
LC_{50}		>1.27E-07	>1.27E-07	6.50E-09	3.44E-09		>1.25E-06	9.14E-07	2.75E-07	8.39E-07
GI_{50}	18-S	1.70E-09	1.21E-09	1.21E-09	9.59E-10	28-S	4.89E-07	2.51E-07	1.67E-07	2.51E-07
TGI		3.03E-09	1.34E-09	1.34E-09	1.34E-09		>1.19E-06	3.46E-07	2.51E-07	3.94E-07
LC_{50}		>1.21E-07	>1.21E-07	1.58E-09	>1.21E-07		>1.19E-06	6.33E-07	3.94E-07	6.92E-07
GI_{50}	19-S	3.07E-09	1.35E-09	1.96E-09	2.95E-09	29-S	6.15E-07	3.62E-07	2.17E-07	3.86E-07
TGI		3.31E-09	1.60E-09	3.31E-09	3.19E-09		>1.21E-06	5.31E-07	3.74E-07	5.07E-07
LC_{50}		>1.23E-07	>1.23E-07	1.10E-08	>1.23E-07		>1.21E-06	8.32E-07	6.88E-07	6.88E-07

Table 9. Biological activity (Molar)

	Compound	Reference Compound
		
	18-R R_1 = CN	**28-R** R_1 = CN
	19-R R_1 = OH	**29-R** R_1 = OH

		A549	HT29	MDA-MB-231	PSN1		A549	HT29	MDA-MB-231	PSN1
GI_{50}	18-R	1.21E-09	1.21E-09	1.21E-09	5.71E-10	28-R	1.67E-07	3.10E-08	1.91E-08	2.15E-08
TGI		1.34E-09	1.34E-09	1.34E-09	1.06E-09		3.58E-07	3.34E-08	3.22E-08	3.58E-08
LC_{50}		>1.21E-07	>1.21E-07	1.46E-09	>1.21E-07		>1.19E-06	>1.19E-06	9.19E-08	6.68E-08
GI_{50}	19-R	1.47E-09	1.72E-09	1.23E-09	1.23E-09	29-R	9.05E-08	3.02E-08	1.69E-08	3.02E-08
TGI		3.56E-09	1.72E-09	1.35E-09	1.35E-09		1.93E-07	3.26E-08	2.77E-08	3.14E-08
LC_{50}		>1.23E-07	>1.23E-07	>1.23E-07	1.47E-09		>1.21E-06	>1.21E-06	1.57E-07	3.50E-08

[0476] The compounds of the present invention are shown to have high potency *in vitro,* when compared against reference compounds. This demonsrates that the compounds according to the present invention exhibit

high cytoxiticy towards cancer cells and are useful in the treatment of cancer.

Example 28. MTD and MTMD determination

[0477] Female CD-1 or Athymic Nude-Fox1 nu/nu mice (Envigo) were utilized for all experiments. Animals (N=10/cage) were housed in individually ventilated cages (Sealsafe Plus®, Techniplast S.P.A.), on a 12-hour light-dark cycle at 21-23 °C and 40-60% humidity. Mice were allowed free access to irradiated standard rodent diet (Tecklad 2914C) and sterilized water. Animals were acclimated for five days prior to being individually tattoo-identified. Animal protocols were reviewed and approved according to the regional Institutional Animal Care and Use Committees.

[0478] Mice were randomly allocated into experimental groups and intravenously administered, once for the MTD (Maximum Tolerated Dose) determination or one administration a week during three consecutive weeks, for the MTMD (Maximum Tolerated Multiple Dose) determination study. The animals were administered with white formulation or with compound dissolved in the experimental formulation at different concentrations. The volume administered was always 10 mL/kg. Once administered, animals were monitored for clinical signs of systemic toxicity, changes in body weight and mortality up to 14 days after the administration.

[0479] MTD results are summarized in **Table 10**

Table 10

Compound	Route / Schedule	Doses (mg/Kg)	MTD (mg/kg)
4-S (reference)	iv / SD	0.00, 0.25, 0.50, 1.00, 1.50, 2.00, 2.50, 5.00	1.0
4-R (reference)			0.25
19-S (reference)			0.5
19-R (reference)		0.00, 0.10, 0.15, 0.25, 0.50, 1.00, 1.50, 2.00, 2.50, 5.00	0.15
Comp C (reference)		0.00, 0.25, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 4.00, 5.00	3.0
Comp D (reference)		0.00, 0.25, 0.50, 1.00, 2.00, 4.00, 6.00, 8.00	0.5
32 (reference)		0.00, 0.25, 0.50, 1.00, 1.50, 2.00, 2.50, 5.00	0.5

[0480] **MTMD** results are summarized in **Table 11**

Table 11

Compound	Route / Schedule	Doses (mg/Kg)	MTMD (mg/kg)
4-S (reference)	iv / Q7dx3	0.00, 0.50, 0.75, 1.00, 1.25	1.25
4-R (reference)		0.00, 0.15, 0.20, 0.25, 0.30	0.30
12-S		0.00, 0.10, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 2.00, 2.50, 5.00	0.25
12-R		0.00, 0.010, 0.025, 0.050, 0.075, 0.10, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 2.00, 2.50, 5.00	0.05
19-S (reference)		0.00, 0.10, 0.25, 0.50, 0.75	0.75
19-R (reference)		0.00, 0.025, 0.075, 0.10, 0.15	0.15

Compound	Route / Schedule	Doses (mg/Kg)	MTMD (mg/kg)
Comp C (reference)		0.0, 1.0, 1.5, 2.0, 3.0, 4.0	3.0
Comp D (reference)		0.00, 0.10, 0.25, 0.50, 0.75	0.5
32 (reference)		0.00, 0.10, 0.25, 0.50, 0.75	0.5
35 (reference)		0.00, 0.10, 0.25, 0.50, 0.75	0.25
39-S (reference)		0.00, 0.01, 0.025, 0.05, 0.075, 0.10, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 2.00, 2.50, 5.00	1.25
47-R		0.00, 0.01, 0.025, 0.05, 0.075, 0.10, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 2.00, 2.50, 5.00	0.1
ET-736 (reference)		0.00, 0.10, 0.25, 0.50, 0.75	0.5
PM01183 (reference)		0.00, 0.14, 0.18	0.18
iv, intravenously			
Q7dx3, three cumulated doses administered in a weekly basis.			

Examples 29-40. *In vivo* xenografts

[0481] Female athymic nu/nu mice (Harlan Laboratories Models, S.L. Barcelona, Spain or Envigo, Spain) were utilized for all experiments. Animal were housed in individually ventilated cages Sealsafe® Plus, Techniplast S.P.A.), up to ten per cage on a 12-hour light-dark cycle at 21-23 °C and 40-60 % humidity. Mice were allowed free access to irradiated standard rodent diet (Tecklad 2914C) and sterilized water. Animals were acclimated for at least 5 days prior to tumor implantation with a tumor cell suspension.

CELL LINES

[0482]

Name	N° ATCC	N° ECCC*	Species	Tissue	Characteristics
HT1080	CCL-121	-	human	connective	Fibrosarcoma
MDA-MB-231	HTB-26	-	human	breast	Breast adenocarcinoma
H460	HTB-177	-	human	lung, pleural effusion	NSCLC
A2780	-	93112519	human	ovarian	Ovarian carcinoma
HGC27	-	94042256	human	gastric	Gastric carcinoma
H526	CRL-5811	-	human	lung	SCLC
H82	HTB-175	-	human	lung	SCLC
PC3	CLR-1435	-	human	prostate; derived from metastatic site: bone	Prostatic adenocarcinoma
DU145	HTB-81		human	prostate; derived from metastatic site: brain	Prostatic carcinoma
22Rv1	CRL-2505		human	prostate	Prostatic carcinoma

* European Collection of Cell Cultures

[0483] HT1080 cells were maintained *in vitro* at 37 °C with 5% CO_2 in Minimum Essential Medium Eagle (MEME) (Sigma-Aldrich, Co). Each animal was orthotopically implanted into gastroecnemius muscle by an intramuscular injection using a 26G needle and a 1 cc syringe at 4-6 weeks of age, with 10×10^6 HT1080 cells, suspended in serum free medium, without antibiotics.

[0484] MDA-MB-231 cells were maintained *in vitro* at 37° C with 5% CO_2 in Dulbecco's Modified Eagle's Medium (Sigma-Aldrich, Co). Culture cells were passaged every 3 to 5 days upon reaching confluence. Each animal was subcutaneously implanted (on the right flank using 26G needle and a 1 cc syringe) at 4-6 weeks of age with 7.5×10^6 MDA-MB-231 cells suspended in 0.05 mL of a solution consisting of 50% Matrigel® (Corning Incorporated Life Sciences) and 50% medium without serum or antibiotics.

[0485] H460 cells were maintained *in vitro* at 37 °C with 5% CO_2 in Dulbecco's Modified Eagle's Medium (Sigma-Aldrich, Co). Culture cells were passaged every 3 to 5 days upon reaching confluence. Each animal was subcutaneously implanted (on the right flank using 26G needle and a 1 cc syringe) at 4-6 weeks of age with 5×10^6 H460 cells suspended in 0.05 mL of a solution consisting of 50% Matrigel® (Corning Incorporated Life Sciences) and 50% medium without serum or antibiotics.

[0486] A2780 cells were maintained *in vitro* at 37 °C with 5% CO_2 in RPMI-1640 (Sigma-Aldrich, Co). Culture cells were passaged every 3 to 5 days upon reaching confluence. Each animal was subcutaneously implanted (on the right flank using 26G needle and a 1 cc syringe) at 4-6 weeks of age with 10×10^6 A2780 cells suspended in 0.05 mL of a solution consisting of 50% Matrigel® (Corning Incorporated Life Sciences) and 50% medium without serum or antibiotics.

[0487] HGC27 cells were maintained *in vitro* at 37 °C with 5% CO_2 in Iscove's Modified Dulbecco's Medium (Sigma Aldrich, Co). Culture cells were passage every 3 to 5 days on reaching confluence. Each animal was subcutaneously implanted (on the right flank using 26G needle and a 1 cc syringe) at 4-6 weeks of age with 5×10^6 HGC-27 cells suspended in 0.05 mL of a solution consisting of 50% Matrigel® (Corning Incorporated Life Sciences), 50% medium without serum or antibiotics.

[0488] H526 cells were maintained *in vitro* at 37 °C with 5% CO_2 in RPMI-1640 Medium (Sigma-Aldrich, Co). H526 cells were grown as a suspension and maintained by addition of fresh medium, as the cell density increases, every 2 to 3 days. Every week, culture was reestablished by centrifugation of the suspension with subsequent resuspension in fresh medium at a concentration of 1×10^5 cell/mL. Each animal was subcutaneously implanted (on the right flank using 26G needle and a 1 cc syringe) at 4-6 weeks of age with 5×10^6 H526 cells suspended in 0.05 mL of a solution consisting of 50% Matrigel® (Corning Incorporated Life Sciences) and 50% medium without serum or antibiotics.

[0489] H82 cells were maintained *in vitro* at 37 °C with 5% CO_2 in RPMI-1640 Medium (Sigma-Aldrich, Co). H82 cells were grown as a suspension and maintained by addition of fresh medium, as the cell density increases, every 2 to 3 days. Every week, culture was reestablished by centrifugation of the suspension with subsequent resuspension in fresh medium at a concentration of 1×10^5 cell/ml. Animals were subcutaneously implanted (on the right flank using 26G needle and a 1 cc syringe) at 4-6 weeks of age with 5×10^6 H82 cells, suspended in 0.05 mL of a solution consisting of 50% Matrigel® (Corning Incorporated Life Sciences) and 50% medium without serum or antibiotics.

[0490] PC3 cells were maintained *in vitro* at 37 °C with 5 % CO2 in RPMI-1640 Medium (Sigma-Aldrich, Co). Culture cells were passaged every 3 to 5 days upon reaching confluence. Each female athymic mice was subcutaneously implanted (on the right flank using a 26G needle and a 1 cc syringe) at 4-6 weeks of age with 3×10^6 PC3 cells suspended in 0.05 mL of a solution consisting of 50% Matrigel® Matrix (Corning Incorporated Life Sciences) and 50% medium without serum or antibiotics. In this model, instead of male, female animals were used because PC-3 growth is not hormone dependant.

[0491] DU-145 cells were maintained *in vitro* at 37 °C with 5 % CO2 in RPMI-1640 Medium (Sigma-Aldrich, Co). Culture cells were passaged every 3 to 5 days upon reaching confluence Each male athymic mice was subcutaneously implanted (on the right flank using a 26G needle and a 1 cc syringe) at 4-6 weeks of age with 5×10^6 DU-145 cells suspended in 0.05 mL of a solution consisting of 50% Matrigel® Matrix (Corning Incorporated Life Sciences) and 50% medium without serum or antibiotics.

[0492] 22Rv1 cells were maintained *in vitro* at 37 °C with 5 % CO_2 in RPMI-1640 Medium (Sigma-Aldrich, Co). Culture cells were passage every 3 to 5 days upon reaching confluence. Each male athymic mice was subcutaneously implanted (on the right flank using 26G needle and a 1 cc syringe) at 4-6 weeks of age with 5×10^6 22Rv1 cells suspended in 0.05 mL of a solution consisting of 50% Matrigel® Matrix (Corning Incorporated Life Sciences) and 50% medium without serum or antibiotics.

[0493] Treatment tolerability was assessed by monitoring body weight evolution, clinical signs of systemic toxicity, as well as evidences of local damage in the injection site.

[0494] In xenograft studies with HT1080 cell line:

- Total diameter (tumor + leg) measurements were determined by using digital caliper (Fowler Sylvac, S235PAT). This total diameter and animal body weights were measured 2-3 times per week starting from the first day of treatment (day 0).
- When total diameter reached a length of about 7.0-8.0 mm, mice were randomly allocated into the treatments and control groups (N = 8-10/group) based on body weight and tumor measurements by using NewLab Oncology Software (version 2.25.06.00).
- Comparison of the median total diameter (tumor + leg) in the treatment groups to the median total diameter (tumor + leg) in the control group was used for evaluation of the antitumoral efficacy.
- Animals were euthanized when their total leg diameter reached *ca.* 18 mm.

[0495] In xenograft studies with other cell lines:

- Tumor volume was calculated using the equation $(a\cdot b^2)/2$, where a: length (longest diameter) and b: width (shortest diameter) were measured in mm by using digital caliper (Fowler Sylvac, S235PAT). Tumor dimensions and body weights were recorded 2-3 times per week starting from the first day of treatment.
- When tumors reached ca. 150-250 mm^3, tumor bearing animals (N = 8-10/group) were randomly allocated into the treatment groups, based on body weight and tumor measurements by using NewLab Oncology Software (version 2.25.06.00).
- Comparison between median tumor volume of treated groups and control group was used for evaluation of the antitumoral efficacy.
- Animals were euthanized when their tumors reached ca. 2000 mm^3 and/or severe necrosis was seen.

[0496] Treatments producing >20 % lethality and/or 20% net body weight loss were considered toxic.

[0497] Tables and figures summarize the data obtained from complete experimental groups, i.e. those groups keeping the initial number of animals, n = 8-10. However, once the first animal is sacrificed due to a tumor length > 18 mm or a tumor size > 2000 mm^3, the experimental group will be considered incomplete. Therefore, data generated subsequently to the sacrifice day and onwards will not be presented (i.e. neither in tables nor in the figures).

Example 29. *In vivo* studies to determine the effect of 4-S (reference) and 12-S in several xenograft models

[0498] **4-S** (reference), **12-S** and compound **C** (reference) were provided in the form of freeze-dried vials of lyophilized product. Each vial was reconstituted with water for infusion to a concentration of 0.5 mg/mL. Further dilutions were made with 5% dextrose solution for injection to the dosing formulation concentration. The administered doses of **4-S** (reference), **12-S** and compound **C** (reference) were 1.25 mg/kg, 0.25 mg/kg and 3.0 mg/kg, respectively.

[0499] Placebo was provided in the form of lyophilised cake containing 100 mg Sucrose + Potassium dihydrogen phosphate 6.8 mg + Phosphoric acid q.s. pH 3.8-4.5 which was reconstituted with water for infusion.

[0500] In these experiments, **4-S** (reference), **12-S** and Compound **C** (reference), as well as placebo, were intravenously administered once per week for 3 consecutive weeks, on Days 0, 7 and 14, whenever it was possible.

[0501] Example 29a. *In vivo* studies to determine the effect of **4-S** (reference) and **12-S** in human fibrosarcoma xenografts.

[0502] The aim of this study was to compare the antitumoral activity of **4-S** (reference) and **12-S** with the antitumoral activity of compound **C** (reference) by using a xenograft model of human sarcoma.

[0503] The tumor model used in this study was HT1080 cell line.

[0504] Table 12 reports the total diameter (tumor + leg) evaluation of HT1080 tumors in mice treated with placebo, compound **C** (reference), **4-S** (reference), and **12-S.** These results are also showed in Figure 1.

Table 12

Days	Total diameter (tumor + leg) (mm)			
	Control	Compound C	4-S	12-S
0.0	7.5	7.5	7.5	7.5
2.0	9.4	8.8	7.7	8.2
5.0	11.4	9.0	8.3	8.6
7.0	12.1	9.6	8.8	9.5
9.0	13.2	10.2	8.4	100
12.0	14.5	10.2	8.4	11.2
14.0	15.2	11.2	9.6	11.7
16.0	15.9	12.4	10.0	12.7
19.0	18.0	13.3	10.4	13.5
21.0		15.2	12.1	14.4
23.0		18.0	12.7	16.5
27.0			13.5	15.2

Days	Total diameter (tumor + leg) (mm)			
	Control	Compound C	4-S	12-S
30.0			15.6	16.4
33.0			18.0	

Example 29b. *In vivo* studies to determine the effect of 4-S (reference) and 12-S in human breast xenografts.

[0505] The aim of this study was to compare the antitumoral activity of **4-S** (reference) and **12-S** with the antitumoral activity of compound **C** (reference) by using a xenograft model of human breast cancer.

[0506] The tumor model used in this study was MDA-MB-231 cell line.

[0507] Table 13 reports the median tumor volume evaluation of MDA-MB-231 tumors in mice treated with placebo, compound **C** (reference), **4-S** (reference), and **12-S.** These results are also showed in Figure 2.

Table 13

Days	Median Tumor Volume (mm^3)			
	Control	Compound C	4-S	12-S
0.0	149.4	149.4	150.6	150.2
2.0	240.0	217.1	197.3	229.9
5.0	325.1	281.3	250.9	290.5
7.0	407.8	338.6	265.0	398.2
9.0	514.8	385.1	272.5	508.9
12.0	648.1	400.4	270.6	602.5
14.0	799.0	436.9	281.3	751.0
16.0	1002.5	585.7	293.6	977.7
19.0	1233.9	774.7	322.1	1252.6
21.0	1539.1	965.9	324.4	1560.7
23.0	2006.5	1215.2	326.6	2005.9
26.0	2027.7	1503.2	398.8	2066.2
28.0		1785.3	501.8	
30.0		2037.1	654.8	
33.0			856.7	
35.0			1147.1	
37.0			1635.9	

Example 29c. *In vivo* studies to determine the effect of 4-S (reference) and 12-S in human lung tumor xenografts.

[0508] The aim of this study was to compare the antitumoral activity of **4-S** (reference) and **12-S** with the antitumoral activity of compound **C** (reference) by using three different xenograft models of human lung cancer. These models correspond to non-small cell lung cancer (H-460 cell line) and to small cell lung cancer (H526 and

H82 cell lines).

[0509] Table 14 reports the median tumor volume evaluation of H460 tumors in mice treated with placebo, compound **C** (reference), **4-S** (reference), and **12-S.** These results are also showed in Figure 3.

Table 14

Days	Median Tumor Volume (mm^3)			
	Control	**Compound C**	**4-S**	**12-S**
0.0	187.4	186.1	185.9	186.0
2.0	577.5	395.4	310.9	460.5
5.0	1352.0	665.9	634.6	922.4
7.0	1642.9	929.5	959.1	1252.1
9.0	2025.0	1063.7	1064.9	1409.4
12.0		1436.5	1421.0	1531.7
14.0		2025.0	1845.5	2025.0
16.0		2025.0	2025.0	

[0510] Table 15 reports the median tumor volume evaluation of H526 tumors in mice treated with placebo, compound **C** (reference), **4-S** (reference) and **12-S.** These results are also showed in Figure 4.

Table 15

Days	Median Tumor Volume (mm^3)			
	Control	**Compound C**	**4-S**	**12-S**
0.0	217.2	217.9	211.8	212.7
2.0	410.7	262.4	279.0	412.7
4.0	778.5	108.3	98.8	637.9
7.0	1083.2	129.8	56.7	968.5
9.0	1371.0	85.9	62.5	1250.3
11.0	1782.0	52.3	32.0	1568.0
14.0	2025.0	54.1	18.0	2025.0
16.0		47.3	32.0	
21.0		4.0	4.0	
28.0		4.0	4.0	
35.0		4.0	4.0	
42.0		62.5	4.0	
49.0		53.5	4.0	

[0511] Table 16 reports the median tumor volume evaluation of H82 tumors in mice treated with placebo, compound **C** (reference), **4-S** (reference) and **12-S.** These results are also showed in Figure 5.

Table 16

Days	Median Tumor Volume (mm^3)			
	Control	**Compound C**	**4-S**	**12-S**
0.0	171.6	170.5	168.3	174.0
2.0	439.4	265.3	215.2	360.1

Days	Median Tumor Volume (mm^3)			
	Control	**Compound C**	**4-S**	**12-S**
5.0	1024.7	488.7	253.6	899.7
7.0	1422.0	760.0	341.4	1398.6
9.0	1923.8	899.5	349.4	1847.6
12.0	2025.0	1038.5	436.4	2089.7
14.0		1213.4	516.0	
16.0		1256.4	521.8	
19.0		1741.5	560.9	
21.0		1878.8	627.7	
23.0		2057.0	690.9	
26.0			953.4	
28.0			847.1	
30.0			1067.5	
33.0			1200.6	
35.0			1257.7	
37.0			1497.7	
41.0			2014.2	

Example 29d. *In vivo* studies to determine the effect of 4-S (reference) and 12-S in human ovarian tumor xenografts.

[0512] The aim of this study was to compare the antitumoral activity of **4-S** (reference) and **12-S** with the antitumoral activity of compound **C** (reference) by using a xenograft model of human ovarian cancer.

[0513] The tumor model used in this study was A2780.

[0514] Table 17 reports the volume evaluation of A2780 tumors in mice treated with placebo, compound **C** (reference), **4-S** (reference), and **12-S.** These results are also showed in Figure 6.

Table 17

Days	Median Tumor Volume (mm^3)			
	Control	**Compound C**	**4-S**	**12-S**
0.0	169.5	169.6	168.3	168.5
2.0	317.5	206.3	150.6	262.1
5.0	758.9	372.7	175.9	628.6
7.0	1351.9	607.6	317.7	976.3
9.0	1675.8	696.2	281.9	1387.5
12.0	2025.0	855.6	372.1	1666.0
14.0		1293.9	709.2	2025.0
16.0		1683.5	870.9	
19.0		2137.5	1235.4	
21.0			1453.3	

Days	Median Tumor Volume (mm^3)			
	Control	Compound C	4-S	12-S
23.0			1666.0	
26.0			2025.0	

Example 29e. *In vivo* studies to determine the effect of 4-S (reference) and 12-S in human gastric tumor xenografts.

[0515] The aim of this study was to compare the antitumoral activity of **4-S** (reference) and **12-S** with the antitumoral activity of Compound **C** (reference) by using a xenograft model of human gastric cancer.

[0516] The tumor model used in this study was HGC27.

[0517] Table 18 reports tumor volume growth of HGC27 tumors in mice treated with placebo, compound **C** (reference), **4-S** (reference), and **12-S.** These results are also showed in Figure 7.

Table 18

Days	Median Tumor Volume (mm^3)			
	Control	Compound C	4-S	12-S
0.0	200.7	195.0	194.8	196.6
2.0	429.0	391.0	358.6	411.9
5.0	835.5	578.6	515.3	834.1
7.0	1256.5	708.2	589.2	1176.6
9.0	1602.2	937.7	779.4	1531.6
12.0	2040.7	1169.5	980.8	2030.2
14.0		1496.8	1153.3	
16.0		1690.6	1346.2	
19.0		2004.0	1643.4	
21.0			2004.7	

Reference Example 30. *In vivo* studies to determine the effect of 4-R (reference) in several xenograft models

[0518] 4-R (reference) was provided in the form of freeze dried vials. **4-R** (reference) cake was reconstituted with water for infusion to a concentration of 0.5 mg/mL. The **4-R** (reference) stock solution was further diluted in 5% dextrose solution for injection to the dosing formulation concentration. The **4-R** (reference) administered dose was 0.30 mg/kg.

[0519] Compound **D** (reference) was provided in the form of drug substance vials. Each vial was reconstituted first by total dissolution in DMSO and then adding Kolliphor ELP (BASF) / ethanol absolute (1:1, v/v) to a concentration of 0.8 mg/mL. Further dilutions were made with a lactate buffer solution (pH = 4.0) to the dosing formulation concentration. The Compound **D** (reference) administered dose was 0.5 mg/kg.

[0520] PM01183 (reference) was provided in the form of vials of lyophilized product. Each vial was reconstituted

with water for infusion to a concentration of 0.2 mg/mL. Further dilutions were made with 5% glucose or 0.9% sodium chloride solution for injection to the dosing formulation concentrations. The administered dose was 0.18 mg/kg.

[0521] Placebo was provided in the form of lyophilised cake containing 100 mg Sucrose + Potassium dihydrogen phosphate 6.8 mg + Phosphoric acid q.s. pH 3.8-4.5 which was reconstituted with water for infusion.

[0522] In these experiments, **4-R** (reference), Compound **D** (reference) and **PM01183** (reference), as well as placebo, were intravenously administered once per week for 3 consecutive weeks, on Days 0, 7 and 14, whenever it was possible.

Reference Example 30a. *In vivo* studies to determine the effect of 4-R (reference) in human fibrosarcoma xenografts.

[0523] The aim of this study was to compare the antitumoral activity of **4-R** (reference) and Compound **D** (reference) with the antitumoral activity of **PM01183** (reference) by using a xenograft model of human sarcoma.

[0524] The tumor model used in this study was HT1080 cell line.

[0525] Table 19 reports the total diameter (tumor + leg) evaluation of HT1080 tumors in mice treated with placebo, **PM01183** (reference) and **4-R** (reference). These results are also showed in Figure 8.

Table 19

Days	Total diameter (tumor + leg) (mm)		
	Control	**PM01183**	**4-R**
0	8.1	8.1	8.1
2	11.2	9.7	8.6
7	13.6	11.2	8.7
9	15.2	12.3	9.0
14	16.9	14.6	9.3
18	18.1	15.6	10.3
21		15.1	11.5
23		16.3	13.3
25		18.0	15.8
28			18.0

[0526] Table 20 reports the total diameter (tumor + leg) evaluation of HT1080 tumors in mice treated with placebo, **PM01183** (reference) and Compound **D** (reference). These results are also showed in Figure 9.

Table 20

Days	Total diameter (tumor + leg) (mm)		
	Control	**PM01183**	Compound **D**
0	7.8	7.7	7.7
2	11.0	9.2	9.5
5	14.0	9.8	8.8
7	15.0	12.2	8.7
9	18.0	12.6	9.4

Days	Total diameter (tumor + leg) (mm)		
	Control	**PM01183**	Compound **D**
12		13.1	9.4
14		14.6	10.1
16		14.5	10.9
19		15.0	11.2
21		18.0	12.1
23			13.0
26			15.0
28			18.0

Reference Example 30b. *In vivo* studies to determine the effect of 4-R (reference) in human breast xenografts.

[0527] The aim of this study was to compare the antitumoral activity of **4-R** (reference) and Compound **D** (reference) with the antitumoral activity of **PM01183** (reference) by using a xenograft model of human breast cancer.

[0528] The tumor model used in this study was MDA-MB-231 cell line.

[0529] Table 21 reports the median tumor volume evaluation of MDA-MB-231 tumors in mice treated with placebo, **PM01183** (reference) and **4-R** (reference). These results are also showed in Figure 10.

Table 21

Days	Median Tumor Volume (mm^3)		
	Control	**PM01183**	**4-R**
0	130.6	129.3	129.3
7	230.7	189.0	151.9
14	422.2	230.1	164.1
21	687.7	305.9	136.8
28	1114.9	535.8	195.9
35	1555.3	819.7	294.2
42	2138.5	962.7	494.4
49		1301.3	843.8
52		2199.4	1042.5

[0530] Table 22 reports the volume evaluation of MDA-MB-231 tumors in mice treated with placebo, **PM01183** (reference) and Compound **D** (reference). These results are also showed in Figure 11.

Table 22

Days	Median Tumor Volume (mm^3)		
	Control	**PM01183**	Compound **D**
0	129.2	129.6	129.5
7	284.0	185.9	147.9

Days	Median Tumor Volume (mm^3)		
	Control	**PM01183**	Compound **D**
14	564.3	290.8	186.4
21	686.0	337.9	136.5
28	1068.6	507.4	290.7
35	1359.4	796.1	431.7
42	1533.7	1062.5	770.1
49	1653.1	1416.3	970.0
56	2029.3	1673.3	1461.9
63	2060.8	1811.9	1526.4

Reference Example 30c. *In vivo* studies to determine the effect of 4-R (reference) in human lung tumor xenografts.

[0531] The aim of this study was to compare the antitumoral activity of **4-R** (reference) and Compound **D** (reference) with the antitumoral activity of **PM01183** (reference) by using a xenograft model of human lung cancer.

[0532] The tumor model used in this study was H-460 cell line.

[0533] Table 23 reports the volume evaluation of H460 tumors in mice treated with placebo, **PM01183** (reference) and **4-R** (reference). These results are also showed in Figure 12.

Table 23

Days	Median Tumor Volume (mm^3)		
	Control	**PM01183**	**4-R**
0	156.2	156.7	155.5
2	290.9	227.3	223.3
7	1323.8	940.4	737.8
9	1816.9	1210.3	861.0
11	2120.9	1433.8	1102.9
14		1529.5	1638.0
16			2028.6

[0534] Table 24 reports the volume evaluation of H460 tumors in mice treated with placebo, **PM01183** (reference) and Compound **D** (reference). These results are also showed in Figure 13.

Table 24

Days	Median Tumor Volume (mm^3)		
	Control	**PM01183**	Compound **D**
0	205.2	204.5	203.4
2	508.0	418.1	367.3
7	1355.8	1004.0	792.0
9	1682.1	1211.3	854.6

Days	Median Tumor Volume (mm^3)		
	Control	**PM01183**	Compound **D**
12	1938.6	1515.4	1026.7
14	2275.9	1633.3	1175.8
16		1723.9	1322.1
19		2112.3	1581.1
21		2409.4	1789.3
23			1966.5
26			2080.7

Reference Example 30d. *In vivo* studies to determine the effect of 4-R (reference) in human ovarian tumor xenografts.

[0535] The aim of this study was to compare the antitumoral activity of **4-R** (reference) and Compound **D** (reference) with the antitumoral activity of **PM01183** (reference) by using a xenograft model of human ovarian cancer.

[0536] The tumor model used in this study was A2780.

[0537] Table 25 reports the volume evaluation of A2780 tumors in mice treated with placebo, **PM01183** (reference) and **4-R** (reference). These results are also showed in Figure 14.

Table 25

Days	Median Tumor Volume (mm^3)		
	Control	**PM01183**	**4-R**
0	172.8	175.5	175.2
5	896.6	671.2	611.4
7	1415.3	1048.9	1036.5
12	2205.3	2020.3	1992.0
14			2165.3

[0538] Table 26 reports the volume evaluation of A2780 tumors in mice treated with placebo, **PM01183** (reference) and Compound **D** (reference). These results are also showed in Figure 15.

Table 26

Days	Median Tumor Volume (mm^3)		
	Control	**PM01183**	Compound **D**
0	189.4	191.2	190.1
3	588.5	454.5	319.6
5	1086.0	772.1	514.4
7	1428.6	1161.5	897.4
10	2077.1	1615.6	1239.8
12	2163.1	1703.0	1656.2
14		2029.3	1951.7

Days	Median Tumor Volume (mm^3)		
	Control	**PM01183**	Compound **D**
17			2121.7
19			2068.6

Reference Example 30e. *In vivo* studies to determine the effect of 4-R (reference) in human gastric tumor xenografts.

[0539] The aim of this study was to compare the antitumoral activity of **4-R** (reference) and Compound **D** (reference) with the antitumoral activity of **PM01183** (reference) by using a xenograft model of human gastric cancer.

[0540] The tumor model used in this study was HGC27.

[0541] Table 27 reports tumor volume growth of HGC27 tumors in mice treated with placebo, **PM01183** (reference) and **4-R** (reference). These results are also showed in Figure 16.

Table 27

Days	Median Tumor Volume (mm^3)		
	Control	**PM01183**	**4-R**
0	174.6	171.6	173.0
2	319.1	317.5	266.8
5	632.5	404.0	370.7
7	1046.0	485.7	418.5
9	1359.1	604.6	627.8
12	1863.8	760.8	713.5
14	2115.0	789.6	837.0
16		719.5	867.1
19		895.9	1040.2
21		1051.3	1229.8
26		1901.2	1784.5
28		2028.9	2073.6

[0542] Table 28 reports tumor volume growth of HGC27 tumors in mice treated with placebo, **PM01183** (reference) and Compound **D** (reference). These results are also showed in Figure 17.

Table 28

Days	Median Tumor Volume (mm^3)		
	Control	**PM01183**	Compound **D**
0	142.3	169.5	157.4
2	286.5	372.4	327.6
5	527.7	474.1	439.6
7	821.4	571.8	418.7
9	1130.9	787.9	567.9

Days	Median Tumor Volume (mm^3)		
	Control	**PM01183**	Compound **D**
12	1547.8	951.1	537.0
14	1868.5	1064.4	654.6
16	1887.0	1346.1	672.4
19	2162.3	1691.8	843.0
21		1920.0	842.7
23		2011.4	963.7
26		2102.2	1203.3
28			1589.7
30			1777.6
33			2146.2

Example 31. *In vivo* studies to determine the effect of 12-R in several xenograft models.

[0543] **12-R** was provided in the form of freeze dries vials. **12-R** cake was reconstituted with water for infusion to a concentration of 0.5 mg/mL. The **12-R** stock solution was further diluted in 5% dextrose solution for injection to the dosing formulation concentration. The **12-R** administered dose was 0.05 mg/kg.

[0544] Compound **D** (reference) was provided in the form of drug substance vials. Each vial was reconstituted first by total dissolution in DMSO and then adding Kolliphor ELP (BASF) / ethanol absolute (1:1, v/v) to a concentration of 0.8 mg/mL. Further dilutions were made with a lactate buffer solution (pH = 4.0) to the dosing formulation concentration. The Compound **D** (reference) administered dose was 0.5 mg/kg.

[0545] Placebo was provided in the form of lyophilised cake containing 100 mg Sucrose + Potassium dihydrogen phosphate 6.8 mg + Phosphoric acid q.s. pH 3.8-4.5 which was reconstituted with water for infusion.

[0546] In these experiments, **12-R,** Compound **D** (reference), as well as placebo, were intravenously administered once per week for 3 consecutive weeks, on Days 0, 7 and 14, whenever it was possible.

Example 31a. *In vivo* studies to determine the effect of 12-R in human fibrosarcoma xenografts.

[0547] The aim of this study was to compare the antitumoral activity of **12-R** with the antitumoral activity of Compound **D** (reference) by using a xenograft model of human sarcoma.

[0548] The tumor model used in this study was HT1080 cell line.

[0549] **Table 29** reports the total diameter (tumor + leg) evaluation of HT1080 tumors in mice treated with placebo, Compound **D** (reference) and **12-R.** These results are also showed in Figure 18.

Table 29

Days	Total diameter (tumor + leg) (mm)		
	Control	**Compound D**	**12-R**
0.0	7.5	7.5	7.5
2.0	9.4	8.2	8.9

Days	Total diameter (tumor + leg) (mm)		
	Control	Compound D	12-R
5.0	11.4	7.5	8.8
7.0	12.1	7.4	9.5
9.0	13.2	8.1	9.5
12.0	14.5	7.9	11.0
14.0	15.2	7.7	11.7
16.0	15.9	8.8	12.9
19.0	18.0	10.2	13.5
21.0		11.2	15.5
23.0		12.2	18.0
27.0		13.2	
30.0		14.6	
33.0		16.3	
35.0		18.0	

Example 31b. ***In vivo*** **studies to determine the effect of 12-R in human breast xenografts.**

[0550] The aim of this study was to compare the antitumoral activity of **12-R** with the antitumoral activity of Compound **D** (reference) by using a xenograft model of human breast cancer.

[0551] The tumor model used in this study was MDA-MB-231 cell line.

[0552] Table 30 reports the median tumor volume evaluation of MDA-MB-231 tumors in mice treated with placebo, Compound **D** (reference) and **12-R.** These results are also showed in Figure 19.

Table 30

Days	Median Tumor Volume (mm^3)		
	Control	Compound D	12-R
0.0	149.4	149.6	149.8
2.0	240.0	217.2	223.0
5.0	325.1	284.5	296.1
7.0	407.8	310.0	378.3
9.0	514.8	325.5	472.7
12.0	648.1	268.4	609.9
14.0	799.0	237.7	782.5
16.0	1002.5	261.2	972.4
19.0	1233.9	251.3	1211.0
21.0	1539.1	219.9	1463.4
23.0	2006.5	221.8	1756.5
26.0	2027.7	245.5	2028.6
28.0		320.3	
30.0		401.6	

Days	Median Tumor Volume (mm^3)		
	Control	Compound D	12-R
33.0		545.8	
35.0		629.2	
37.0		670.7	
40.0		669.9	
42.0		696.3	
44.0		798.1	
47.0		857.7	

Example 31c. *In vivo* studies to determine the effect of 12-R in human lung tumor xenografts.

[0553] The aim of this study was to compare the antitumoral activity of **12-R** with the antitumoral activity of Compound **D** (reference) by using three different xenograft models of human lung cancer. These models correspond to non-small cell lung cancer (H460 cell line and to small cell lung cancer (H526 and H82 cell lines).

[0554] Table 31 reports the volume evaluation of H460 tumors in mice treated with placebo, Compound **D** (reference) and **12-R.** These results are also showed in Figure 20.

Table 31

Days	Median Tumor Volume (mm^3)		
	Control	Compound D	12-R
0.0	187.4	187.2	187.0
2.0	577.5	329.7	410.7
5.0	1352.0	559.4	796.7
7.0	1642.9	756.5	1167.9
9.0	2025.0	971.9	1360.3
12.0		1370.9	1666.0
14.0		1626.8	2025.0
16.0		2025.0	

[0555] Table 32 reports the median tumor volume evaluation of H526 tumors in mice treated with placebo, compound **D** (reference) and **12-R.** The results are also shown in Figure 21.

Table 32

Days	Median Tumor Volume (mm^3)		
	Control	Compound D	12-R
0.0	217.20	216.1	214.20
2.0	410.70	240.9	404.50
4.0	778.50	99.3	680.50
7.0	1083.20	56.7	995.20
9.0	1371.00	62.5	1290.50
11.0	1782.00	62.5	1568.00
14.0	2025.00	32.0	2025.00

Days	Median Tumor Volume (mm^3)		
	Control	Compound D	12-R
16.0		4.0	
21.0		4.0	
28.0		4.0	
35.0		4.0	
42.0		4.0	
49.0		4.0	

[0556] Table 33 reports the median tumor volume evaluation of H82 tumors in mice treated with placebo, compound **D** (reference) and **12-R.** The results are also shown in Figure 22.

Table 33

Days	Median Tumor Volume (mm^3)		
	Control	Compound D	12-R
0.0	171.60	169.4	170.50
2.0	439.40	340.6	381.40
5.0	1024.70	443.3	793.20
7.0	1422.00	496.2	1187.20
9.0	1923.80	614.1	1699.30
12.0	2025.00	665.5	2125.60
14.0		1041.6	
16.0		1151.2	
19.0		1516.7	
21.0		1748.0	

Example 31d. *In vivo* studies to determine the effect of 12-R in human ovarian tumor xenografts.

[0557] The aim of this study was to compare the antitumoral activity of **12-R** with the antitumoral activity of Compound **D** (reference) by using a xenograft model of human ovarian cancer.

[0558] The tumor model used in this study was A2780.

[0559] Table 34 reports the volume evaluation of A2780 tumors in mice treated with placebo, Compound **D** (reference) and **12-R.** These results are also showed in Figure 23.

Table 34

Days	Median Tumor Volume (mm^3)		
	Control	Compound D	12-R
0.0	169.5	168.8	169.6
2.0	317.5	225.7	302.8
5.0	758.9	256.6	786.5
7.0	1351.9	473.8	1113.3
9.0	1675.8	633.6	1490.6

Days	Median Tumor Volume (mm^3)		
	Control	**Compound D**	**12-R**
12.0	2025.0	822.8	2025.00
14.0		1129.3	2025.00
16.0		1198.6	
19.0		1649.6	
21.0		2025.0	

Example 31e. *In vivo* studies to determine the effect of 12-R in human gastric tumor xenografts.

[0560] The aim of this study was to compare the antitumoral activity of **12-R** with the antitumoral activity of Compound **D** (reference) by using a xenograft model of human gastric cancer.

[0561] The tumor model used in this study was HGC27.

[0562] Table 35 reports tumor volume growth of HGC27 tumors in mice treated with placebo, Compound **D** (reference) and **12-R.** These results are also showed in Figure 24.

Table 35

Days	Median Tumor Volume (mm^3)		
	Control	**Compound D**	**12-R**
0.0	200.7	194.0	193.3
2.0	429.0	324.2	413.3
5.0	835.5	561.6	809.1
7.0	1256.5	504.2	1261.5
9.0	1602.2	584.2	1589.5
12.0	2040.7	767.7	2017.9
14.0		1056.8	2034.9
16.0		1440.2	
19.0		1717.9	
21.0		2043.4	

Reference Example 32. *In vivo* studies to determine the effect of 19-S (reference) in several xenograft models

[0563] 19-S (reference) was provided in the form of freeze dried vials. **19-S** (reference) cake was reconstituted with water for infusion to a concentration of 0.5 mg/mL. The **19-S** (reference) stock solution was further diluted in 5 % dextrose solution for injection to the dosing formulation concentration. The **19-S** (reference) administered dose was 0.75 mg/kg.

[0564] PM01183 (reference) was provided in the form of vials of lyophilized product. Each vial was reconstituted with water for infusion to a concentration of 0.2 mg/mL. The **PM01183** (reference) stock solution was further diluted in 5% glucose solution for injection to the dosing formulation concentrations. The administered dose was

0.18 mg/kg.

[0565] Placebo was provided in the form of lyophilised cake containing 100 mg Sucrose + Potassium dihydrogen phosphate 6.8 mg + Phosphoric acid q.s. pH 3.8-4.5 which was reconstituted with water for infusion.

[0566] In these experiments, **19-S** (reference) and **PM01183** (reference), as well as placebo, were intravenously administered once per week for 3 consecutive weeks, on Days 0, 7 and 14, whenever it was possible.

Reference Example 32a. *In vivo* studies to determine the effect of 19-S (reference) in human fibrosarcoma xenografts.

[0567] The aim of this study was to compare the antitumoral activities of **19-S** (reference) and **PM01183** (reference) by using a xenograft model of human sarcoma.

[0568] The tumor model used in this study was HT1080 cell line.

[0569] Table 36 reports the total diameter (tumor + leg) evaluation of HT1080 tumors in mice treated with placebo, **PM01183** (reference) and **19-S** (reference). These results are also showed in Figure 25.

Table 36

Days	Total diameter (tumor + leg) (mm)		
	Control	**PM01183**	**19-S**
0	8.4	8.4	8.2
2	10.9	9.8	8.4
5	14.8	9.7	7.8
7	15.9	11.4	9.5
9	18.0	12.7	9.9
12		13.7	10.7
14		14.6	11.3
16		15.5	11.9
19		15.6	13.4
21		18.0	14.4
23			18.0

Reference Example 32b. *In vivo* studies to determine the effect of 19-S (reference) in human breast adenocarcinoma xenografts.

[0570] The aim of this study was to compare the antitumoral activities of **19-S** (reference) and **PM01183** (reference) by using a xenograft model of human breast cancer.

[0571] The tumor model used in this study was MDA-MB-231 cell line.

[0572] Table 37 reports the median tumor volume evaluation of MDA-MB-231 tumors in mice treated with placebo, **PM01183** (reference) and **19-S** (reference). These results are also showed in Figure 26.

Table 37

Days	Median Tumor Volume (mm^3)		
	Control	**PM01183**	**19-S**
0	132.6	134.3	133.6
4	194.1	177.2	157.2
7	248.2	186.3	142.6
11	377.6	250.7	133.9
14	461.3	266.1	117.3
18	679.2	327.7	79.3
21	753.2	391.0	89.2
25	909.2	493.1	120.6
28	1090.7	627.3	144.4
32	1433.4	789.0	246.1
36	1887.5	1022.0	419.3
39	1785.2	1294.2	593.7
42	2081.5	1643.3	945.9
46	2137.5	1658.9	985.3
49		1938.0	1211.5
53			1324.3
56			1703.9
60			1793.3
63			1603.0
70			2324.2

Reference Example 32c. *In vivo* studies to determine the effect of 19-S (reference) in human lung cancer xenografts.

[0573] The aim of this study was to compare the antitumoral activities of **19-S** (reference) and **PM01183** (reference) by using a xenograft model of human lung cancer.

[0574] The tumor model used in this study was H-460 cell line.

[0575] Table 38 reports the median tumor volume evaluation of H-460 tumors in mice treated with placebo, **PM01183** (reference) and **19-S** (reference). These results are also showed in Figure 27.

Table 38

Days	Median Tumor Volume (mm^3)		
	Control	**PM01183**	**19-S**
0	197.0	196.3	196.9
2	529.5	457.0	364.0
4	1057.4	861.5	624.9
7	1582.5	1280.2	966.5
9	2094.8	1424.9	1078.2
11		1969.9	1449.0

Days	Median Tumor Volume (mm^3)		
	Control	**PM01183**	**19-S**
14			1761.5

Reference Example 32d. *In vivo* studies to determine the effect of 19-S (reference) in human ovarian tumor xenografts.

[0576] The aim of this study was to compare the antitumoral activities of **19-S** (reference) and **PM01183** (reference) by using a xenograft model of human ovarian cancer.

[0577] The tumor model used in this study was A2780.

[0578] Table 39 reports the median tumor volume evaluation of A2780 tumors in mice treated with placebo, **PM01183** (reference) and **19-S** (reference). These results are also showed in Figure 28.

Table 39

Days	Median Tumor Volume (mm^3)		
	Control	**PM01183**	**19-S**
0	163.4	163.6	164.4
2	287.1	235.5	187.9
4	568.7	463.2	205.4
7	1211.3	986.3	513.6
9	1633.7	1451.4	650.6
11	2047.8	2062	659.8
14			1236.2
18			1575.9
23			1895.7
25			2177.0

Reference Example 32e. *In vivo* studies to determine the effect of 19-S (reference) in human gastric tumor xenografts.

[0579] The aim of this study was to compare the antitumoral activities of **19-S** (reference) and **PM01183** (reference) by using a xenograft model of human gastric cancer.

[0580] The tumor model used in this study was HGC27.

[0581] Table 40 reports the median tumor volume evaluation of HGC27 tumors in mice treated with placebo, **PM01183** (reference) and **19-S** (reference). These results are also showed in Figure 29.

Table 40

Days	Median Tumor Volume (mm^3)		
	Control	**PM01183**	**19-S**
0	178.3	177.6	181.5

Days	Median Tumor Volume (mm^3)		
	Control	**PM01183**	**19-S**
2	409	395.6	404.6
5	907.4	572.4	600.3
7	1283.6	766.6	660.3
9	1664	950.7	787.5
14	2102.8	1199.4	864.4
16		1353.1	882.4
19		1294.3	925.2
21		1335.1	893.6
23		1320.3	874.4
26		1364.5	932.1
30		1671.9	1547.8
33		2009.2	2020.4

Reference Example 33. *In vivo* studies to determine the effect of 19-R (reference) in several xenograft models

[0582] **19-R** (reference) was provided in the form of freeze dried vials. **19-R** (reference) cake was reconstituted with water for infusion to a concentration of 0.5 mg/mL. The **19-R** (reference) stock solution was further diluted in 5 % dextrose solution for injection to the dosing formulation concentration. The **19-R** (reference) administered dose was 0.15 mg/kg.

[0583] **PM01183** (reference) was provided in the form of vials of lyophilized product. Each vial was reconstituted with water for infusion to a concentration of 0.2 mg/mL. The **PM01183** (reference) stock solution was further diluted in 5% glucose solution for injection to the dosing formulation concentrations. The administered dose was 0.18 mg/kg.

[0584] Placebo was provided in the form of lyophilised cake containing 100 mg Sucrose + Potassium dihydrogen phosphate 6.8 mg + Phosphoric acid q.s. pH 3.8-4.5 which was reconstituted with water for infusion.

[0585] In these experiments, **19-R** (reference) and **PM01183** (reference), as well as placebo, were intravenously administered once per week for 3 consecutive weeks, on Days 0, 7 and 14, whenever it was possible.

Reference Example 33a. *In vivo* studies to determine the effect of 19-R (reference) in human fibrosarcoma xenografts.

[0586] The aim of this study was to compare the antitumoral activity of **19-R** (reference) with the antitumoral activity of **PM01183** (reference) by using a xenograft model of human sarcoma.

[0587] The tumor model used in this study was HT1080 cell line.

[0588] **Table 41** reports the total diameter (tumor + leg) evaluation of HT-1080 tumors in mice treated with placebo, **PM01183** (reference) and **19-R** (reference). These results are also showed in Figure 30.

Table 41

Days	Total diameter (tumor + leg) (mm)		
	Control	**PM01183**	**19-R**
0	8.4	8.4	8.3
2	10.9	9.8	9.4
5	14.8	9.7	8.0
7	15.9	11.4	7.2
9	18.0	12.7	7.8
12		13.7	7.8
14		14.6	8.4
16		15.5	8.2
19		15.6	11.3
21		18.0	12.2
23			13.3
26			15.2
28			18.0

Reference Example 33b. *In vivo* studies to determine the effect of 19-R (reference) in human breast adenocarcinoma xenografts.

[0589] The aim of this study was to compare the antitumoral activity of **19-R** (reference) with the antitumoral activity of **PM01183** (reference) by using a xenograft model of human breast cancer.

[0590] The tumor model used in this study was MDA-MB-231 cell line.

[0591] Table 42 reports the median tumor volume evaluation of MDA-MB-231 tumors in mice treated with placebo, **PM01183** (reference) and **19-R** (reference). These results are also showed in Figure 31.

Table 42

Days	Median Tumor Volume (mm^3)		
	Control	**PM01183**	**19-R**
0	132.6	134.3	132.5
4	194.1	177.2	189.3
7	248.2	186.3	151.9
11	377.6	250.7	167.5
14	461.3	266.1	152.6
18	679.2	327.7	162.2
21	753.2	391.0	201.2
25	909.2	493.1	208.5
28	1090.7	627.3	274.8
32	1433.4	789.0	355.8
36	1887.5	1022.0	513.8
39	1785.2	1294.2	793.7
42	2081.5	1643.3	1012.2

Days	Median Tumor Volume (mm^3)		
	Control	**PM01183**	**19-R**
46	2137.5	1658.9	1188.5
49		1938.0	1380.7
53			1568.0
56			1862.6
60			2129.4

Reference Example 33c. *In vivo* studies to determine the effect of 19-R (reference) in human lung tumor xenografts.

[0592] The aim of this study was to compare the antitumoral activity of **19-R** (reference) with the antitumoral activity of **PM01183** (reference) by using a xenograft model of human lung cancer.

[0593] The tumor model used in this study was H-460 cell line.

[0594] Table 43 reports the median tumor volume evaluation of H460 tumors in mice treated with placebo, **PM01183** (reference) and **19-R** (reference). These results are also showed in Figure 32.

Table 43

Days	Median Tumor Volume (mm^3)		
	Control	**PM01183**	**19-R**
0	197.0	196.3	196.8
2	529.5	457.0	418.7
4	1057.4	861.5	697.2
7	1582.5	1280.2	911.7
9	2094.8	1424.9	1111.5
11		1969.9	1281.3
14			1478.7
16			1594.0

Reference Example 33d. *In vivo* studies to determine the effect of 19-R (reference) in human ovarian tumor xenografts.

[0595] The aim of this study was to compare the antitumoral activity of **19-R** (reference) with the antitumoral activity of **PM01183** (reference) by using a xenograft model of human ovarian cancer.

[0596] The tumor model used in this study was A2780.

[0597] Table 44 reports the median tumor volume evaluation of A2780 tumors in mice treated with placebo, **PM01183** (reference) and **19-R** (reference). These results are also showed in Figure 33.

Table 44

Days	Median Tumor Volume (mm^3)		
	Control	**PM01183**	**19-R**
0	163.4	163.6	162.8
2	287.1	236.5	212.9
4	568.7	463.2	368.5
7	1211.3	986.3	841.3
9	1633.7	1451.4	1138.9
11	2047.8	2062.0	1519.9
14			2056.0

Reference Example 33e. *In vivo* studies to determine the effect of 19-R (reference) in human gastric tumor xenografts.

[0598] The aim of this study was to compare the antitumoral activity of **19-R** (reference) with the antitumoral activity of **PM01183** (reference) by using a xenograft model of human gastric cancer.

[0599] The tumor model used in this study was HGC27.

[0600] Table 45 reports the median tumor volume evaluation of HGC-27 tumors in mice treated with placebo, **PM01183** (reference) and **19-R** (reference). These results are also showed in Figure 34.

Table 45

Days	Median Tumor Volume (mm^3)		
	Control	**PM01183**	**19-R**
0	178.3	177.6	182.0
2	409.0	395.6	414.9
5	907.4	572.4	735.0
7	1283.6	766.6	901.2
9	1664.0	950.7	1048.1
14	2102.8	1199.4	1293.9
16		1353.1	1488.8
19		1294.3	1668.3
21		1335.1	1845.0
23		1320.3	2025.0
26		1364.5	
30		1671.9	
33		2009.2	

Reference Example 34. *In vivo* studies to determine the effect of 39-S (reference) in several xenograft models.

[0601] Compound **39-S** (reference) and **C** (reference) were provided in the form of freeze-dried vials of

lyophilized product. Each vial was reconstituted with sterile water for injection to a concentration of 0.5 mg/mL. Further dilutions were made with 5% dextrose solution for injection to the dosing formulation concentration. The administered doses of **39-S** (reference) and **C** (reference) were 1.25 and 3 mg/Kg, respectively.

[0602] Placebo was provided in the forms of vials of lyophilised product. Each vial (sucrose 200 mg + potassium dihydrogen phosphate 13.6 mg + phosphoric acid q.s. pH 3.8-4.5) was reconstituted with sterile water for injection (2 mL). Further dilutions were made with 5% dextrose solution for injection.

[0603] In these experiments, **39-S** (reference) and compound C (reference), as well as placebo, were intravenously administered on a weekly schedule at a volume of 10 mL/Kg.

Reference Example 34a. *In vivo* studies to determine the effect of 39-S (reference) in human fibrosarcoma xenografts.

[0604] The aim of this study was to evaluate the antitumoral activity of compound **39-S** (reference) by comparison with the antitumoral activity of compound C (reference) by using a xenograft model of human sarcoma.

[0605] The tumor model used in this study was HT1080 cell line.

[0606] Table 46 reports the total diameter (tumor + leg) evaluation of HT1080 tumors in mice treated with placebo, compound **C** (reference) and **39-S** (reference). These results are also showed in **Figure 35.**

Table 46

Days	Total diameter (tumor + leg) (mm)		
	Control	39-S	Compound C
0	7.5	7.5	7.5
2	9.4	7.9	8.8
5	11.4	6.4	9.0
7	12.1	6.8	9.6
9	13.2	6.9	10.2
12	14.5	6.6	10.2
14	15.2	6.4	11.2
16	15.9	6.8	12.4
19	18.0	7.0	13.3
21		7.0	15.2
23		8.5	18.0
27		10.8	
30		12.5	
33		14.3	
35		15.3	
37		18.0	

Reference Example 34b. *In vivo* studies to determine the effect of 39-S (reference) in human breast adenocarcinoma xenografts.

[0607] The aim of this study was to compare the antitumoral activities of **39-S** (reference) and compound **C** (reference) by using a xenograft model of human breast cancer.

[0608] The tumor model used in this study was MDA-MB-231 cell line.

[0609] **Table 47** reports the median tumor volume evaluation of MDA-MB-231 tumors in mice treated with placebo, compound **C** (reference) and **39-S** (reference). These results are also showed in **Figure 36.**

Table 47.

Days	Median Tumor Volume (mm^3)		
	Control	39-S	Compound C
0	149.4	151.0	149.4
2	240.0	209.3	217.1
5	325.1	290.9	281.3
7	407.8	301.8	338.6
9	514.8	300.8	385.1
12	648.1	278.7	400.4
14	799.0	249.7	436.9
16	1002.5	243.6	585.7
19	1233.9	248.3	774.7
21	1539.1	250.0	965.9
23	2006.5	260.3	1215.2
26	2027.7	304.9	1503.2
28		337.1	1785.3
30		451.3	2037.1
33		584.1	
35		683.4	
37		784.7	
40		937.4	
42		1060.5	
44		1170.5	
47		1112.9	
49		1138.6	
51		1283.2	
54		1415.1	
56		1518.7	
58		1728.5	
61		2017.9	

Reference Example 34c. *In vivo* studies to determine the effect of 39-S (reference) in human lung cancer xenografts.

[0610] The aim of this study was to compare the antitumoral activity of **39-S** (reference) with the antitumoral

activity of compound **C** (reference) by using three different xenograft models of human lung cancer. These models correspond to non-small cell lung cancer (H-460 cell line) and to small cell lung cancer (H526 and H82 cell lines).

[0611] Table 48 reports the median tumor volume evaluation of H460 tumors in mice treated with placebo, compound **C** (reference) and **39-S** (reference). These results are also showed in **Figure 37.**

Table 48

Days	Median Tumor Volume (mm^3)		
	Control	39-S	Compound C
0	187.4	187.8	186.1
2	577.5	314.4	395.4
5	1352.0	584.1	665.9
7	1642.9	831.2	929.5
9	2025.0	841.0	1063.7
12		1008.0	1436.5
14		1309.8	2025.0
16		1470.0	2025.0
19		2025.0	

[0612] Table 49 reports the median tumor volume evaluation of H526 tumors in mice treated with placebo, compound **C** (reference) and **39-S** (reference). These results are also showed in **Figure 38.**

Table 49

Days	Median Tumor Volume (mm^3)		
	Control	39-S	Compound C
0	217.2	214.5	217.9
2	410.7	260.3	262.4
4	778.5	80.0	108.3
7	1083.2	46.2	129.8
9	1371.0	32.0	85.9
11	1782.0	32.0	52.3
14	2025.0	4.0	54.1
16		4.0	47.3
21		4.0	4.0
28		4.0	4.0
35		4.0	4.0
42		4.0	62.5
49		4.0	53.5
56		4.0	70.0
63		4.0	132.3
70		4.0	368.5
77		4.0	465.8
84		4.0	107.4
91		4.0	130.0

Days	Median Tumor Volume (mm^3)		
	Control	39-S	Compound C
98		4.0	4.0
105		4.0	4.0
112		4.0	4.0
119		4.0	4.0
126		4.0	4.0
133		4.0	4.0
140		4.0	4.0
147		4.0	4.0
165		4.0	4.0
175		4.0	4.0
191		4.0	4.0
205		4.0	4.0

[0613] Table 50 reports the median tumor volume evaluation of H82 tumors in mice treated with placebo, compound **C** (reference) and **39-S** (reference). These results are also showed in **Figure 39.**

Table 50.

Days	Median Tumor Volume (mm^3)		
	Control	39-S	Compound C
0	171.6	170.3	170.5
2	439.4	325.2	265.3
5	1024.7	430.8	488.7
7	1422.0	466.2	760.0
9	1923.8	544.3	899.5
12	2025.0	640.3	1038.5
14		711.2	1213.4
16		802.7	1256.4
19		916.0	1741.5
21		1047.2	1878.8
23		1189.1	2057.0
26		1497.2	
28		1741.8	
30		1731.7	
33		2029.4	

Reference Example 34d. *In vivo* studies to determine the effect of 39-S (reference) in human ovarian tumor xenografts.

[0614] The aim of this study was to compare the antitumoral activity of **39-S** (reference) with the antitumoral activity of compound **C** (reference) by using a xenograft model of human ovarian cancer.

[0615] The tumor model used in this study was A2780.

[0616] **Table 51** reports the volume evaluation of A2780 tumors in mice treated with placebo, compound **C** (reference) and **39-S** (reference). These results are also showed in **Figure 40.**

Table 51

Day	Median Tumor Volume (mm^3)		
	Control	39-S	Compound C
0	169.5	170.5	169.6
2	317.5	206.5	206.3
5	758.9	163.4	372.7
7	1351.9	298.6	607.6
9	1675.8	317.4	696.2
12	2025.0	378.2	855.6
14		668.5	1293.9
16		853.5	1683.5
19		1415.5	2137.5
21		1519.2	
23		1666.0	
30		2025.0	

Reference Example 34e. *In vivo* studies to determine the effect of 39-S (reference) in human gastric tumor xenografts.

[0617] The aim of this study was to compare the antitumoral activity of **39-S** (reference) with the antitumoral activity of compound **C** (reference) by using a xenograft model of human gastric cancer.

[0618] The tumor model used in this study was HGC27.

[0619] **Table 52** reports tumor volume growth of HGC27 tumors in mice treated with placebo, compound **C** (reference), and **39-S** (reference). These results are also showed in **Figure 41.**

Table 52

Days	Median Tumor Volume (mm^3)		
	Control	39-S	Compound C
0	200.7	195.6	195.0
2	429.0	356.3	391.0
5	835.5	469.7	578.6
7	1256.5	467.8	708.2
9	1602.2	575.2	937.7
12	2040.7	611.1	1169.5
14		637.3	1496.8
16		690.4	1690.6
19		701.8	2004.0
21		697.4	1741.4

Days	Median Tumor Volume (mm^3)		
	Control	39-S	Compound C
23		715.5	2056.4
26		898.1	
28		1163.4	
30		1409.3	
33		1450.5	
35		1708.5	
37		1804.4	
40		2075.2	

Example 35. *In vivo* studies to determine the effect of 47-R in several xenograft models.

[0620] Compound **47-R** was provided in the form of freeze-dried vials of lyophilized product. Each vial was reconstituted with sterile water for injection to a concentration of 0.5 mg/mL. Further dilutions were made with 5% dextrose solution for injection to the dosing formulation concentration. **47-R** administered dose was 0.1 mg/Kg.

[0621] Compound **D** (reference) was provided in the form of powder drug substance. Each vial was reconstituted first by total dissolution in DMSO (Fisher) and then adding Kolliphor ELP (Basf)/ethanol absolute (Merk) (1:1, v/v) to a concentration of 0.8 mg/mL. Further dilutions were made with a lactate buffer solution (pH = 4.0) to the dosing formulation concentration. Compound **D** (reference) administered dose was 0.5 mg/Kg.

[0622] Placebo was provided in the form of vials of lyophilised product. Each vial (sucrose 200 mg + potassium dihydrogen phosphate 13.6 mg + phosphoric acid q.s. pH 3.8-4.5) was reconstituted with sterile water for injection (2 mL). Further dilutions were made with 5% dextrose solution for injection.

[0623] In these experiments, **47-R** and compound **D** (reference), as well as placebo, were intravenously administered on a weekly schedule at a volume of 10 mL/Kg.

Example 35a. *In vivo* studies to determine the effect of 47-R in human fibrosarcoma xenografts.

[0624] The aim of this study was to evaluate the antitumoral activity of compound **47-R** by comparison with the antitumoral activity of compound **D** (reference) by using a xenograft model of human sarcoma.

[0625] The tumor model used in this study was HT1080 cell line.

[0626] Table 53 reports the total diameter (tumor + leg) evaluation of HT1080 tumors in mice treated with placebo, compound **D** (reference) and **47-R.** These results are also showed in **Figure 42.**

Table 53

Days	Total diameter (tumor + leg) (mm)		
	Control	47-R	Compound D
0	7.5	7.5	7.5
2	9.4	8.9	8.2

Days	Total diameter (tumor + leg) (mm)		
	Control	47-R	Compound D
5	11.4	10.1	7.5
7	12.1	10.5	7.4
9	13.2	11.5	8.1
12	14.5	13.5	7.9
14	15.2	13.9	7.7
16	15.9	14.6	8.8
19	18.0	18.0	10.2
21			11.2
23			12.2
27			13.2
30			14.6
33			16.3
35			18.0

Example 35b. ***In vivo*** **studies to determine the effect of 47-R in human breast adenocarcinoma xenografts.**

[0627] The aim of this study was to compare the antitumoral activities of **47-R** and compound **D** (reference) by using a xenograft model of human breast cancer.

[0628] The tumor model used in this study was MDA-MB-231 cell line.

[0629] Table 54 reports the median tumor volume evaluation of MDA-MB-231 tumors in mice treated with placebo, compound **D** (reference) and **47-R.** These results are also showed in Figure 43.

Table 54

Days	Median Tumor Volume (mm^3)		
	Control	47-R	Compound D
0	149.4	150.5	149.6
2	240.0	225.3	217.2
5	325.1	323.2	284.5
7	407.8	405.0	310.0
9	514.8	495.9	325.5
12	648.1	594.1	268.4
14	799.0	769.5	237.7
16	1002.5	1009.5	261.2
19	1233.9	1298.0	251.3
21	1539.1	1580.7	219.9
23	2006.5	2006.5	221.8
26	2027.7	2032.1	245.5
28			320.3

Days	Median Tumor Volume (mm^3)		
	Control	47-R	Compound D
30			401.6
33			545.8
35			629.2
37			670.7
40			669.9
42			696.3
44			798.1
47			857.7
49			870.7
51			925.8
54			1005.4
56			1064.2
58			1235.6
61			1367.8
63			1553.7
65			2017.9

Example 35c. *In vivo* studies to determine the effect of 47-R in human lung cancer xenografts.

[0630] The aim of this study was to compare the antitumoral activity of **47-R** with the antitumoral activity of compound **D** (reference) by using three different xenograft models of human lung cancer. These models correspond to non-small cell lung cancer (H-460 cell line) and to small cell lung cancer (H526 and H82 cell lines).

[0631] Table 55 reports the median tumor volume evaluation of H460 tumors in mice treated with placebo, compound **D** (reference) and **47-R.** These results are also showed in **Figure 44.**

Table 55

Days	Median Tumor Volume (mm^3)		
	Control	47-R	Compound D
0	187.4	185.8	187.2
2	577.5	508.1	329.7
5	1352.0	979.3	559.4
7	1642.9	1280.0	756.5
9	2025.0	1543.1	971.9
12		1764.0	1370.9
14		1845.5	1626.8
16			2025.0

[0632] Table 56 reports the median tumor volume evaluation of H526 tumors in mice treated with placebo, compound **D** (reference) and **47-R.** These results are also showed in **Figure 45.**

Table 56

Days	Median Tumor Volume (mm^3)		
	Control	47-R	Compound D
0	217.2	211.5	216.1
2	410.7	367.9	240.9
4	778.5	583.7	99.3
7	1083.2	941.7	56.7
9	1371.0	1305.2	62.5
11	1782.0	1484.7	62.5
14	2025.0	2025.0	32.0
16			4.0
21			4.0
28			4.0
35			4.0
42			4.0
49			4.0
56			4.0
63			4.0
70			4.0
77			4.0
84			4.0
91			4.0
98			4.0
105			4.0
112			4.0
119			4.0
126			4.0
133			4.0
140			4.0
147			4.0
165			4.0
175			4.0
191			4.0
205			4.0

[0633] Table 57 reports the median tumor volume evaluation of H82 tumors in mice treated with placebo, compound D (reference) and 47-R. These results are also showed in Figure 46.

Table 57.

Days	Median Tumor Volume (mm^3)		
	Control	47-R	Compound D
0	171.6	169.0	169.4
2	439.4	371.6	340.6

Days	Median Tumor Volume (mm^3)		
	Control	47-R	Compound D
5	1024.7	888.8	443.3
7	1422.0	1314.2	496.2
9	1923.8	1811.0	614.1
12	2025.0	2055.4	665.5
14			1041.6
16			1151.2
19			1516.7
21			1748.0

Example 35d. *In vivo* studies to determine the effect of 47-R in human ovarian tumor xenografts.

[0634] The aim of this study was to compare the antitumoral activity of **47-R** with the antitumoral activity of compound **D** (reference) by using a xenograft model of human ovarian cancer.

[0635] The tumor model used in this study was A2780.

[0636] Table 58 reports the volume evaluation of A2780 tumors in mice treated with placebo, compound **D** (reference) and **47-R.** These results are also showed in **Figure 47.**

Table 58

Days	Median Tumor Volume (mm^3)		
	Control	47-R	Compound D
0	169.5	170.6	168.8
2	317.5	280.6	225.7
5	758.9	653.9	256.6
7	1351.9	848.7	473.8
9	1675.8	1569.1	633.6
12	2025.0	1764.0	822.8
14		1666.0	1129.3
16		2025.0	1198.6
19			1649.6
21			2025.0

Example 35e. *In vivo* studies to determine the effect of 47-R in human gastric tumor xenografts.

[0637] The aim of this study was to compare the antitumoral activity of **47-R** with the antitumoral activity of compound **D** (reference) by using a xenograft model of human gastric cancer.

[0638] The tumor model used in this study was HGC27.

[0639] Table 59 reports tumor volume growth of HGC27 tumors in mice treated with placebo, compound **D**

(reference), and **47-R.** These results are also showed in **Figure 48.**

Table 59

Days	Median Tumor Volume (mm^3)		
	Control	**47-R**	**Compound D**
0	200.7	194.0	194.0
2	429.0	359.4	324.2
5	835.5	774.8	561.6
7	1256.5	1155.4	504.2
9	1602.2	1474.7	584.2
12	2040.7	1870.2	767.7
14		2031.3	1056.8
16		2075.2	1440.2
19			1717.9
21			2043.4

Reference Example 36. *In vivo* studies to determine the effect of 32 (reference) in several xenograft models.

[0640] Compounds **32** (reference) and **ET-736** (reference) were provided in the form of freeze-dried vials of lyophilized product. Each vial was reconstituted with sterile water for injection to a concentration of 0.5 mg/mL. Further dilutions were made with 5% dextrose solution for injection to the dosing formulation concentration. The administered dose of **32** (reference) and **ET-736** (reference) was 0.5 mg/Kg.

[0641] Placebo was provided in the form of lyophilised product. Each vial (sucrose 200 mg + potassium dihydrogen phosphate 13.6 mg + phosphoric acid q.s. pH 3.8-4.5) was reconstituted with sterile water for injection (2 mL). Further dilutions were made with 5% dextrose solution for injection.

[0642] In these experiments, **32** (reference) and **ET-736** (reference), as well as placebo, were intravenously administered on a weekly schedule at a volume of 10 mL/Kg.

Reference Example 36a. *In vivo* studies to determine the effect of 32 (reference) in human fibrosarcoma xenografts.

[0643] The aim of this study was to evaluate the antitumoral activity of compound **32** (reference) by comparison with the antitumoral activity of **ET-736** (reference) by using a xenograft model of human sarcoma.

[0644] The tumor model used in this study was HT-1080 cell line.

[0645] Table 60 reports the total diameter (tumor + leg) evaluation of HT1080 tumors in mice treated with placebo, **ET-736** (reference) and **32** (reference). These results are also showed in **Figure 49.**

Table 60

Days	Total diameter (tumor + leg) (mm)		
	Control	**32**	**ET-736**
0	7.5	7.5	7.4
2	9.4	8.9	8.3

Days	Total diameter (tumor + leg) (mm)		
	Control	32	ET-736
5	11.4	8.2	7.1
7	12.1	8.8	7.6
9	13.2	10.0	7.4
12	14.5	8.8	7.0
14	15.2	10.8	7.1
16	15.9	11.8	7.4
19	18.0	12.0	8.4
21		14.0	8.6
23		13.8	10.0
27		13.6	10.9
30		15.5	13.2
33		18.0	14.3
35			15.2
37			15.8
40			16.6
42			18.0

Reference Example 36b. *In vivo* studies to determine the effect of 32 (reference) in human breast adenocarcinoma xenografts.

[0646] The aim of this study was to compare the antitumoral activities of **32** (reference) and **ET-736** (reference) by using a xenograft model of human breast cancer.

[0647] The tumor model used in this study was MDA-MB-231 cell line.

[0648] Table 61 reports the median tumor volume evaluation of MDA-MB-231 tumors in mice treated with placebo, **ET-736** (reference) and **32** (reference). These results are also showed in **Figure 50.**

Table 61

Days	Median Tumor Volume (mm^3)		
	Control	32	ET-736
0	149.4	150.2	150.0
2	240.0	233.6	237.7
5	325.1	310.6	302.1
7	407.8	386.1	364.9
9	514.8	437.5	404.6
12	648.1	493.4	395.4
14	799.0	560.3	398.3
16	1002.5	649.5	447.2
19	1233.9	853.0	485.0
21	1539.1	1017.5	536.3

Days	Median Tumor Volume (mm^3)		
	Control	32	ET-736
23	2006.5	1263.2	669.8
26	2027.7	1487.7	778.9
28		1726.6	1046.1
30		1892.6	1315.9
33		2082.8	1664.9
35			2007.7

Reference Example 36c. *In vivo* studies to determine the effect of 32 (reference) in human lung cancer xenografts.

[0649] The aim of this study was to compare the antitumoral activities of **32** (reference) and **ET-736** (reference) by using three different xenograft models of human lung cancer. These models correspond to non-small cell lung cancer (H-460 cell line) and to small cell lung cancer (H526 and H82 cell lines).

[0650] Table 62 reports the median tumor volume evaluation of H460 tumors in mice treated with placebo, **ET-736** (reference) and **32** (reference). These results are also showed in **Figure 51.**

Table 62

Days	Median Tumor Volume (mm^3)		
	Control	32	ET-736
0	187.4	183.9	185.8
2	577.5	455.2	457.8
5	1352.0	784.8	732.8
7	1642.9	837.4	930.1
9	2025.0	1044.3	1207.2
12	2025.0	1452.4	1568.0
14		1845.5	1845.5
16		2025.0	2025.0

[0651] Table 63 reports the median tumor volume evaluation of H526 tumors in mice treated with placebo, **ET-736** (reference) and **32** (reference). These results are also showed in **Figure 52.**

Table 63

Days	Median Tumor Volume (mm^3)		
	Control	32	ET-736
0	217.2	212.1	213.5
2	410.7	277.3	240.5
4	778.5	127.0	97.2
7	1083.2	95.0	48.8
9	1371.0	63.1	62.5
11	1782.0	62.5	62.5
14	2025.0	62.5	47.3

Days	Median Tumor Volume (mm^3)		
	Control	32	ET-736
16		62.5	32.0
21		4.0	4.0
28		4.0	4.0
35		55.3	4.0
42		85.3	4.0
49		185.6	4.0
56		169.1	4.0
63		62.5	4.0
70		88.9	4.0
77		280.6	4.0
84		694.2	199.8
91		1150.9	786.5

[0652] Table 64 reports the median tumor volume evaluation of H82 tumors in mice treated with placebo, **ET-736** (reference) and **32** (reference). These results are also showed in **Figure 53.**

Table 64

Days	Median Tumor Volume (mm^3)		
	Control	32	ET-736
0	171.6	171.6	170.0
2	439.4	309.4	334.4
5	1024.7	485.0	539.4
7	1422.0	708.4	836.4
9	1923.8	972.6	1013.1
12	2025.0	1101.6	1290.9
14		1339.6	1648.0
16		1430.3	
19		1885.7	

Reference Example 36d. *In vivo* studies to determine the effect of 32 (reference) in human ovarian tumor xenografts.

[0653] The aim of this study was to compare the antitumoral activities of **32** (reference) and **ET-736** (reference) by using a xenograft model of human ovarian cancer.

[0654] The tumor model used in this study was A2780.

[0655] Table 65 reports the volume evaluation of A2780 tumors in mice treated with placebo, **ET-736** (reference) and **32** (reference). These results are also showed in **Figure 54.**

Table 65

Days	Median Tumor Volume (mm^3)		
	Control	32	ET-736
0	169.5	168.6	168.8
2	317.5	262.9	251.2
5	758.9	572.7	382.6
7	1351.9	997.5	676.1
9	1675.8	1359.9	959.4
12	2025.0	1715.0	1241.5
14		2025.0	1582.7
16		2025.0	1646.4
19			1845.5
21			2025.0

Reference Example 36e. *In vivo* studies to determine the effect of 32 (reference) in human gastric tumor xenografts.

[0656] The aim of this study was to compare the antitumoral activities of **32** (reference) and **ET-736** (reference) by using a xenograft model of human gastric cancer.

[0657] The tumor model used in this study was HGC27.

[0658] **Table 66** reports tumor volume growth of HGC27 tumors in mice treated with placebo, **ET-736** (reference) and **32** (reference). These results are also showed in **Figure 55.**

Table 66

Days	Median Tumor Volume (mm^3)		
	Control	32	ET-736
0	200.7	194.8	195.9
2	429.0	386.3	359.2
5	835.5	551.3	537.6
7	1256.5	579.2	553.5
9	1602.2	665.8	604.7
12	2040.7	701.1	627.4
14		814.5	648.0
16		959.9	687.6
19		1312.4	760.0
21		1626.8	792.4
23		1737.3	818.9
26			1026.1
28			1354.9

Reference Example 37. *In vivo* studies to determine the effect of 35 (reference) in several xenograft models.

[0659] Compound 35 (reference) was provided in the form of freeze-dried vials of lyophilized product. Each vial was reconstituted with sterile water for injection to a concentration of 0.5 mg/mL. Further dilutions were made with 5% dextrose solution for injection to the dosing formulation concentration. The administered dose **of 35** (reference) was 0.25 mg/Kg.

[0660] PM01183 (reference) was provided in the form of vials of lyophilized product. Each vial was reconstituted with sterile water for injection to a concentration of 0.5 mg/mL. Further dilutions were made with 5% glucose or 0.9% sodium chloride solution for injection to the dosing formulation concentration. The administered dose of **PM01183** (reference) was 0.18 mg/Kg.

[0661] Placebo was provided in the form of vials of lyophilised product each vial (sucrose 200 mg + potassium dihydrogen phosphate 13.6 mg + phosphoric acid q.s. pH 3.8-4.5) was reconstituted with sterile water for injection (2 mL). Further dilutions were made with 5% dextrose solution for injection.

[0662] In this experiment, compound **35** (reference) and **PM01183** (reference), as well as placebo were intravenously administered on a weekly schedule at a volume of 10 mL/Kg.

Reference Example 37a. *In vivo* studies to determine the effect of 35 (reference) in human fibrosarcoma xenografts.

[0663] The aim of this study was to evaluate the antitumoral activities of compound **35** (reference) and **PM01183** (reference) by using a xenograft model of human sarcoma.

[0664] The tumor model used in this study was HT-1080 cell line.

[0665] Table 67 reports the total diameter (tumor + leg) evaluation of HT1080 tumors in mice treated with placebo, **PM01183** (reference) and **35** (reference). These results are also showed in **Figure 56.**

Table 67

Days	Total diameter (tumor + leg) (mm)		
	Control	PM01183	35
0	8.4	8.4	8.3
2	10.9	9.8	9.4
5	14.8	9.7	8.7
7	15.9	11.4	8.0
9	18.0	12.7	9.9
12		13.7	11.4
14		14.6	12.5
16		15.5	13.2
19		15.6	14.6
21		18.0	15.7
23			18.0

Reference Example 37b. *In vivo* studies to determine the effect of 35 (reference) in human breast adenocarcinoma xenografts.

[0666] The aim of this study was to compare the antitumoral activities of **35** (reference) and **PM01183** (reference) by using a xenograft model of human breast cancer.

[0667] The tumor model used in this study was MDA-MB-231 cell line.

[0668] Table 68 reports the median tumor volume evaluation of MDA-MB-231 tumors in mice treated with placebo, **PM01183** (reference) and **35** (reference). These results are also showed in **Figure 57.**

Table 68

Days	Median Tumor Volume (mm^3)		
	Control	35	PM01183
0	132.6	132.7	134.3
4	194.1	193.6	177.2
7	248.2	179.1	186.3
11	377.6	276.7	250.7
14	461.3	286.0	266.1
18	679.2	384.5	327.7
21	753.2	436.8	391.0
25	909.2	554.3	493.1
28	1090.7	647.0	627.3
32	1433.4	817.5	789.0
36	1887.5	1156.7	1022.0
39	1785.2	1387.6	1294.2
42	2081.5	1595.3	1643.3
46	2137.5	1689.9	1658.9
49		2044.2	1938.0

Reference Example 37c. *In vivo* studies to determine the effect of 35 (reference) in human lung cancer xenografts.

[0669] The aim of this study was to compare the antitumoral activities of **35** (reference) and **PM01183** (reference) by using a xenograft model of human lung cancer.

[0670] The tumor model used in this study was H460 cell line.

[0671] Table 69 reports the median tumor volume evaluation of H460 tumors in mice treated with placebo, **PM01183** (reference) and **35** (reference). These results are also showed in **Figure 58.**

Table 69

Days	Median Tumor Volume (mm^3)		
	Control	PM01183	35
0	197.0	196.3	197.2
2	529.5	457.0	415.3
4	1057.4	861.5	750.8

Days	Median Tumor Volume (mm^3)		
	Control	PM01183	35
7	1582.5	1280.2	1242.3
9	2094.8	1424.9	1536.3
11		1969.9	1728.7
14			2080.9

Reference Example 37d. *In vivo* studies to determine the effect of 35 (reference) in human ovarian tumor xenografts.

[0672] The aim of this study was to compare the antitumoral activities of **35** (reference) and **PM01183** (reference) by using a xenograft model of human ovarian cancer.

[0673] The tumor model used in this study was A2780.

[0674] Table 70 reports the volume evaluation of A2780 tumors in mice treated with placebo, **PM01183** (reference) and **35** (reference). These results are also showed in **Figure 59.**

Table 70

Days	Median Tumor Volume (mm^3)		
	Control	PM01183	35
0	163.4	163.6	163.6
2	287.1	236.5	189.9
4	568.7	463.2	284.3
7	1211.3	986.3	606.4
9	1633.7	1451.4	946.9
11	2047.8	2062.0	1394.2
14			2067.7

Reference Example 37e. *In vivo* studies to determine the effect of 35 (reference) in human gastric tumor xenografts.

[0675] The aim of this study was to compare the antitumoral activities of **35** (reference) and **PM01183** (reference) by using a xenograft model of human gastric cancer.

[0676] The tumor model used in this study was HGC27.

[0677] Table 71 reports volume growth of HGC27 tumors in mice treated with placebo, **PM01183** (reference) and **35** (reference). These results are also showed in **Figure 60.**

Table 71

Days	Median Tumor Volume (mm^3)		
	Control	35	PM01183
0	178.3	182.3	177.6

Days	Median Tumor Volume (mm^3)		
	Control	35	PM01183
2	409.0	382.2	395.6
5	907.4	610.8	572.4
7	1283.6	775.5	766.6
9	1664.0	988.0	950.7
12	1692.4	1005.6	972.0
14	2102.8	1531.7	1199.4
16		1866.3	1353.1

Example 38. ***In vivo*** **studies to determine the effect of 12-S and 12-R in human prostate xenografts.**

[0678] **12-S** and **12-R** were provided in the form of freeze-dried vials of lyophilized product. Each vial was reconstituted with water for infusion to a concentration of 0.5 mg/mL. Further dilutions were made with 5% dextrose solution for injection to the dosing formulation concentration. The administered doses **of 12-S** and **12-R** were 0.25 mg/kg and 0.05 mg/kg respectively.

[0679] Placebo was provided in the form of lyophilised cake containing 100 mg Sucrose + Potassium dihydrogen phosphate 6.8 mg + Phosphoric acid q.s. pH 3.8-4.5 which was reconstituted with water for infusion.

[0680] In these experiments, **12-S** and **12-R,** as well as placebo, were intravenously administered once per week for 3 consecutive weeks, on Days 0, 7 and 14, whenever it was possible.

[0681] The aim of this study was to compare the antitumoral activity of **12-S** and **12-R** by using a xenograft model of human prostate cancer.

[0682] The tumor model used in this study was PC-3 cell line.

[0683] Table 72 reports the median tumor volume evaluation of PC-3 tumors in mice treated with placebo, **12-S** and **12-R.** These results are also showed in Figure 61.

Table 72

Median Tumor Volume (mm^3)			
Days	**Control**	**12-R**	**12-S**
0	128.0	129.0	128.0
2	149.6	136.2	141.5
4	197.0	144.2	143.7
7	250.9	172.2	183.9
11	291.6	183.6	208.1
14	326.5	205.2	270.7
16	361.9	256.0	286.3
18	397.0	325.7	336.1
21	476.9	322.2	357.1
23	506.1	407.8	400.8
25	526.7	419.9	443.6

Median Tumor Volume (mm^3)			
Days	**Control**	**12-R**	**12-S**
29	593.6	459.1	523.4
32	769.5	512.1	652.6
35	875.3	579.2	689.7
37	900.0	613.8	692.2
39	977.8	764.1	726.9
42	1061.5	785.0	823.7
44	1463.4	845.5	864.2
46	1612.8	748.0	1182.8
49	1809.2	808.7	1219.2
51	2030.9	855.8	1331.9
56		1125.2	1335.2

Reference Example 39. *In vivo* studies to determine the effect of 4-S (reference) in human prostate xenografts.

[0684] The aim of this study was to compare the antitumoral activity of **4-S** (reference) by using three different xenograft models of human prostate cancer. These models correspond to PC-3, DU-145 and 22Rv1 cell lines.

[0685] Compound 4-S (reference) was provided in the form of freeze-dried vials of lyophilized product. Each vial was reconstituted with sterile water for injection to a concentration of 0.5 mg/mL. Further dilutions were made with 5% dextrose solution for injection to the dosing formulation concentration. The administered dose of 4-S (reference) varied depending on the study, being 1.25 mg/Kg when the tumor model was PC-3, 1.00 mg/Kg when the tumor model was DU-145 and 0.75 mg/Kg when the tumor model was 22Rv1, respectively.

[0686] Placebo was provided in the form of lyophilised cake containing 100 mg Sucrose + Potassium dihydrogen phosphate 6.8 mg + Phosphoric acid q.s. pH 3.8-4.5 which was reconstituted with water for infusion.

[0687] In these experiments, **4-S** (reference), as well as placebo were intravenously administered once per week for 3 consecutive weeks, on Days 0, 7 and 14, whenever it was possible.

[0688] Table 73 reports the median tumor volume evaluation of PC-3 tumors in mice treated with placebo and **4-S** (reference). These results are also showed in Figure 62.

Table 73

Median Tumor Volume (mm^3)		
Days	**Control**	**4-S**
0	140.5	141.3
2	178.6	130.7
4	233.1	147.6
7	284.6	157.7
9	331.7	200.9
11	433.7	192.8
14	500.4	210.8

Median Tumor Volume (mm^3)		
Days	**Control**	**4-S**
16	570.8	255.5
18	680.3	261.1
21	850.1	282.4
23	928.5	382.2
25	915.7	451.6
28	1187.5	611.1
30	1270.1	762.3
32	1327.1	821.6
35	1373.6	1045.6

[0689] Table 74 reports the median tumor volume evaluation of DU-145 tumors in mice treated with placebo and **4-S** (reference). These results are also showed in Figure 63.

Table 74

Median Tumor Volume (mm^3)		
Days	**Control**	**4-S**
0	127.4	126.2
3	180.9	102.4
5	248.8	119.5
7	320.4	149.5
10	384.6	216.8
12	441.0	181.4
14	519.6	237.7
17	601.0	204.4
19	660.8	210.9
24	740.7	300.0
26	798.6	378.4
28		587.0
31		650.3

[0690] Table 75 reports the median tumor volume evaluation of 22Rv1 tumors in mice treated with placebo and **4-S** (reference). These results are also showed in Figure 64.

Table 75

Median Tumor Volume (mm^3)		
Days	**Control**	**4-S**
0	174.6	173.6
3	307.2	70.3
5	511.5	63.1
7	739.1	76.7
10	955.2	49.1
12	1286.1	59.8

Median Tumor Volume (mm^3)		
Days	**Control**	**4-S**
14	1385.8	74.9
17	1791.1	55.1
19	2025.0	64.9
24		138.4
26		186.9
28		242.0
31		392.5
33		561.8
35		799.3
38		1107.0
40		1426.4
42		1685.5
45		2025.0

[0691] Reference Example 40. *In vivo* studies to determine the effect of **39-S** (reference) in human prostate xenografts.

[0692] The aim of this study was to compare the antitumoral activity **of 39-S** (reference) by using three different xenograft models of human prostate cancer. These models correspond to PC-3, DU-145 and 22Rv1 cell lines.

[0693] Compound 39-S (reference) was provided in the form of freeze-dried vials of lyophilized product. Each vial was reconstituted with sterile water for injection to a concentration of 0.5 mg/mL. Further dilutions were made with 5% dextrose solution for injection to the dosing formulation concentration. The administered dose of **39-S** (reference) varied depending on the study, being 1.25 mg/Kg when the tumor model was PC-3, 1.00 mg/Kg when the tumor model was DU-145 and 0.75 mg/Kg when the tumor model was 22Rv1, respectively.

[0694] Placebo was provided in the form of lyophilised cake containing 100 mg Sucrose + Potassium dihydrogen phosphate 6.8 mg + Phosphoric acid q.s. pH 3.8-4.5 which was reconstituted with water for infusion.

[0695] In these experiments, **39-S** (reference), as well as placebo, were intravenously administered once per week for 3 consecutive weeks, on Days 0, 7 and 14, whenever it was possible.

[0696] Table 76 reports the median tumor volume evaluation of PC-3 tumors in mice treated with placebo and **39-S** (reference). These results are also showed in Figure 65.

Table 76

Median Tumor Volume (mm^3)		
Days	**Control**	**39-S**
0	181.9	182.3
2	254.8	222.6
4	308.7	244.0
7	344.5	269.3
9	396.8	295.8
11	439.2	315.0

Median Tumor Volume (mm^3)		
Days	**Control**	**39-S**
14	542.7	356.9
16	619.0	388.0
18	721.3	400.1
21	908.1	503.3
23	1039.1	556.0
25	1117.0	579.6
28	1232.3	694.9
30	1778.6	811.1
32	2018.1	1027.1
35		1194.3
37		1495.0
39		1710.7
42		2066.2

[0697] Table 77 reports the median tumor volume evaluation of DU-145 tumors in mice treated with placebo and **39-S** (reference). These results are also showed in Figure 66.

Table 77

Median Tumor Volume (mm^3)		
Days	**Control**	**39-S**
0	156.8	179.9
2	198.3	199.9
4	253.9	222.2
7	325.8	340.5
9	385.1	354.1
11	462.2	349.7
14	483.8	429.1
16	599.0	454.8
18	664.0	449.7
21	816.9	517.5
23	861.3	568.5
25	977.9	629.4
28	973.6	775.7

[0698] Table 78 reports the median tumor volume evaluation of 22Rv1 tumors in mice treated with placebo and **39-S** (reference). These results are also showed in Figure 67.

Table 78

Median Tumor Volume (mm^3)		
Days	**Control**	**39-S**
0	174.6	173.5
3	307.2	93.0

Median Tumor Volume (mm^3)		
Days	**Control**	**39-S**
5	511.5	96.8
7	739.1	115.2
10	955.2	108.2
12	1286.1	128.4
14	1385.8	155.6
17	1791.1	173.4
19	2025.0	210.2
24		358.8
26		456.5
28		645.2
31		1049.5
33		1439.4
35		2025.0

REFERENCES CITED IN THE DESCRIPTION

Cited references

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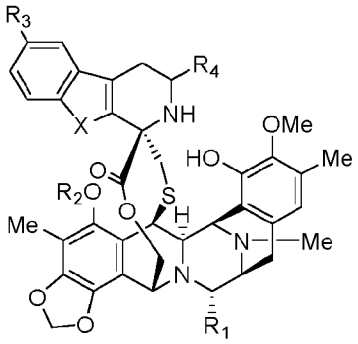
ANTITUMORFORBINDELSER

PATENTKRAV

1. Forbindelse med formlen IE, eller et farmaceutisk acceptabelt salt eller en ester deraf:



IE

hvor:

X er -NH- eller -O-;

R_1 er -OH eller -CN;

R_2 er en -C(=O)R^a-gruppe;

R_3 er hydrogen eller en -OR^b-gruppe;

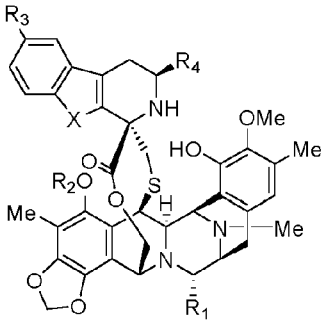
R_4 vælges blandt -CH_2NH_2 og -$CH_2NHProt^{NH}$;

R^a vælges blandt hydrogen, substitueret eller usubstitueret C_1-C_{12}-alkyl, substitueret eller usubstitueret C_2-C_{12}-alkenyl, og substitueret eller usubstitueret C_2-C_{12}-alkynyl;

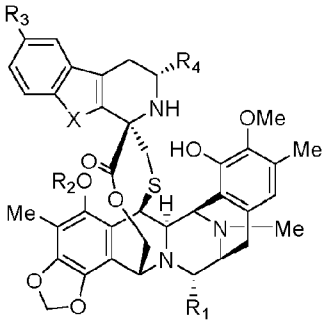
R^b vælges blandt substitueret eller usubstitueret C_1-C_{12}-alkyl, substitueret eller usubstitueret C_2-C_{12}-alkenyl og substitueret eller usubstitueret C_2-C_{12}-alkynyl; og

$Prot^{NH}$ er en beskyttelsesgruppe for amino.

2. Forbindelse ifølge krav 1 valgt blandt formula IEa eller IEb, eller et farmaceutisk acceptabelt salt eller en ester deraf:



IEa



IEb

hvor:

X er -NH- eller -O-;

R_1 er -OH eller -CN;

R_2 er en -C(=O)R^a-gruppe;

R_3 er hydrogen eller en -OR^b-gruppe;

R_4 vælges blandt $-CH_2NH_2$ og $-CH_2NHProt^{NH}$;

R^a vælges blandt hydrogen, substitueret eller usubstitueret C_1-C_{12}-alkyl, substitueret eller usubstitueret C_2-C_{12}-alkenyl, og substitueret eller usubstitueret C_2-C_{12}-alkynyl;

R^b vælges blandt substitueret eller usubstitueret C_1-C_{12}-alkyl, substitueret eller usubstitueret C_2-C_{12}-alkenyl, og substitueret eller usubstitueret C_2-C_{12}-alkynyl; og

$Prot^{NH}$ er en beskyttelsesgruppe for amino.

3. Forbindelse ifølge et hvilket som helst som af kravene 1 eller 2, hvor X er -NH-.

4. Forbindelse ifølge et hvilket som helst som af kravene 1 eller 2, hvor X er -O-.

5. Forbindelse ifølge et hvilket som helst som af kravene 1 til 4, hvor R_4 er $-CH_2NH_2$.

6. Forbindelse ifølge et hvilket som helst som af kravene 1 til 5,hvor R_1 er -OH.

7. Forbindelse ifølge et hvilket som helst som af kravene 1 til 6, hvor R_2 er en $-C(=O)R^a$ gruppe, hvor R^a er substitueret eller usubstitueret C_1-C_6-alkyl; fortrinsvis hvor R^a vælges blandt substitueret eller usubstitueret methyl, substitueret eller usubstitueret ethyl, substitueret eller usubstitueret *n*-propyl, substitueret eller usubstitueret isopropyl, substitueret eller usubstitueret n-butyl, substitueret eller usubstitueret isobutyl, substitueret eller usubstitueret sec-butyl og substitueret eller usubstitueret tert-butyl.

8. Forbindelse ifølge krav 7, hvor R_2 er acetyl.

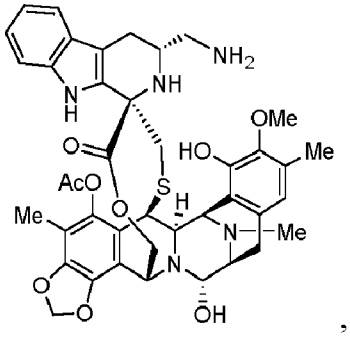
9. Forbindelse ifølge et hvilket som helst som af kravene 1 til 8, hvor R_3 er hydrogen eller $-OR^b$, hvor R^b er substitueret eller usubstitueret C_1-C_6-alkyl; fortrinsvis hvor R^b vælges blandt substitueret eller usubstitueret methyl, substitueret eller usubstitueret ethyl, substitueret eller usubstitueret n-propyl, substitueret eller usubstitueret isopropyl, substitueret eller usubstitueret n-butyl, substitueret eller usubstitueret isobutyl, substitueret eller usubstitueret sec-butyl og substitueret eller usubstitueret tert-butyl.

10. Forbindelse ifølge krav 9, hvor R_3 er hydrogen.

11. Forbindelse ifølge krav 9, hvor R_3 er $-OR^b$, hvor R^b er substitueret eller usubstitueret C_1-C_6-alkyl; fortrinsvis hvor R^b vælges blandt substitueret eller usubstitueret methyl, substitueret eller usubstitueret ethyl, substitueret eller usubstitueret n-propyl, substitueret eller usubstitueret isopropyl, substitueret eller usubstitueret n-butyl, substitueret eller usubstitueret isobutyl, substitueret eller usubstitueret sec-butyl og substitueret eller usubstitueret tert-butyl.

12. Forbindelse ifølge krav 11, hvor R_3 er methoxy.

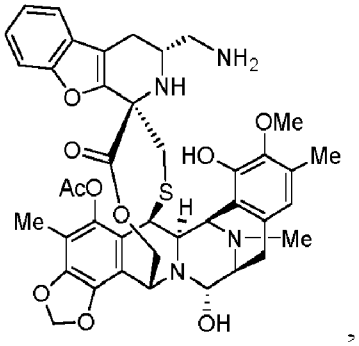
13. Forbindelse ifølge krav 1 med formlen:



,

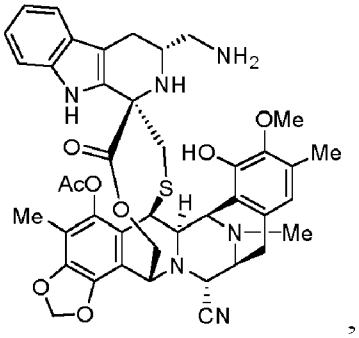
eller et farmaceutisk acceptabelt salt eller en ester deraf.

14. Forbindelse ifølge krav 1 med formlen:



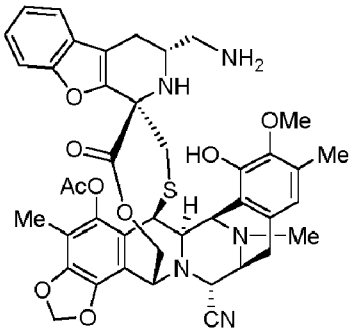
eller et farmaceutisk acceptabelt salt eller en ester deraf.

15. Forbindelse ifølge krav 1 med formlen:



eller et farmaceutisk acceptabelt salt eller en ester deraf.

16. Forbindelse ifølge krav 1 med formlen:



eller et farmaceutisk acceptabelt salt eller en ester deraf.

17. Forbindelse ifølge et hvilket som helst som af kravene 1 til 16, hvor saltet vælges blandt hydrochlorid, hydrobromid, hydroiodid, sulfat, nitrat, phosphat, acetat, trifluoracetat, maleat, fumarat, citrat, oxalat, succinat, tartrat, malat, mandelat, methansulfonat, *p*-toluensulfonat, natrium, kalium, calcium, ammonium, ethylendiamin, ethanolamin, N,N-dialkylenethanolamin, triethanolamin og basiske aminosyrer.

18. Farmaceutisk sammensætning, der omfatter en forbindelse ifølge et hvilket som helst som af kravene 1 til 17 eller et farmaceutisk acceptabelt salt eller en ester deraf og en farmaceutisk acceptabel bærer.

19. Doseringsform, der omfatter en farmaceutisk sammensætning ifølge krav 18.

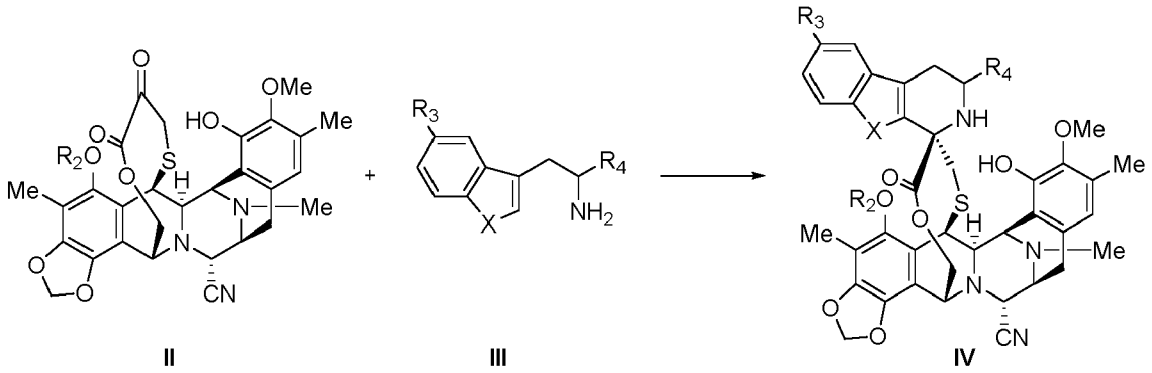
20. Forbindelse ifølge et hvilket som helst som af kravene 1 til 17, eller et farmaceutisk acceptabelt salt eller en ester deraf, eller en sammensætning ifølge krav 18, eller en doseringsform ifølge krav 19, til anvendelse som et lægemiddel.

21. Forbindelse ifølge et hvilket som helst som af kravene 1 til 17, eller et farmaceutisk acceptabelt salt eller en ester deraf, eller en sammensætning ifølge krav 18, eller en doseringsform ifølge krav 19, til anvendelse i cancerbehandlingen.

22. Forbindelse, sammensætning eller doseringsform til anvendelse ifølge krav 21, hvor canceren vælges blandt lungecancer indbefattende ikke-småcellet lungecancer og småcellet lungecancer, coloncancer, colorektal cancer, brystcancer, pancreascancer, sarkom, ovariecancer, prostatacancer og gastisk cancer; fortrinsvis hvor canceren vælges blandt lungecancer indbefattende ikke-småcellet lungecancer og småcellet lungecancer, brystcancer, pancreascancer og colorektal cancer.

23. Fremgangsmåde til opnåelse af en forbindelse som defineret i et hvilket som helst af kravene 1 til 17 eller et farmaceutisk acceptabelt salt eller en ester deraf:

omfattende trinnet med omsætning af en forbindelse med formlen II med en forbindelse med formlen III for at give forbindelse med formlen IV:



II III IV

hvor (når det er tilladt i henhold til mulige substituentgrupper):

X er -NH- eller -O-;

R_2 er en -C(=O)R^a-gruppe;

R_3 er hydrogen eller en -OR^b-gruppe;

R_4 er -CH_2NHProt^{NH};

R^a vælges blandt hydrogen, substitueret eller usubstitueret C_1-C_{12}-alkyl, substitueret eller usubstitueret C_2-C_{12}-alkenyl, substitueret eller usubstitueret C_2-C_{12}-alkynyl;

R^b vælges blandt substitueret eller usubstitueret C_1-C_{12}-alkyl, substitueret eller usubstitueret C_2-C_{12}-alkenyl, og substitueret eller usubstitueret C_2-C_{12}-alkynyl; og

Prot^{NH} er en beskyttelsesgruppe for amino;

og som eventuelt omfatter det yderligere trin med erstatning af cyangruppen i forbindelsen med formlen IV med en hydroxygruppe for at give en forbindelse med formlen IE, hvor R_1 er OH.

24. Kit, der omfatter en terapeutisk virksom mængde af en forbindelse ifølge et hvilket som helst som af kravene 1 til 17 eller et farmaceutisk acceptabelt salt eller en ester deraf og en farmaceutisk acceptabel bærer; hvilket kit eventuelt endvidere omfatter instruktioner om anvendelse af forbindelsen i cancerbehandlingen, og mere fortrinsvis en cancer valgt blandt lungecancer, indbefattende ikke-småcellet lungecancer og småcellet lungecancer, coloncancer, brystcancer, pancreascancer, sarkom, ovariecancer, prostatacancer, colorektal cancer og gastrisk cancer.

DRAWINGS

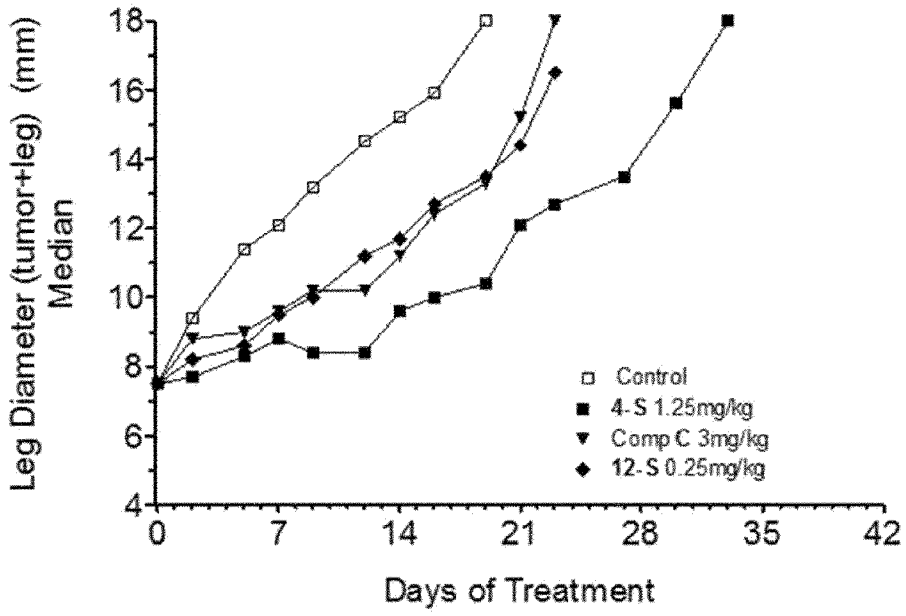
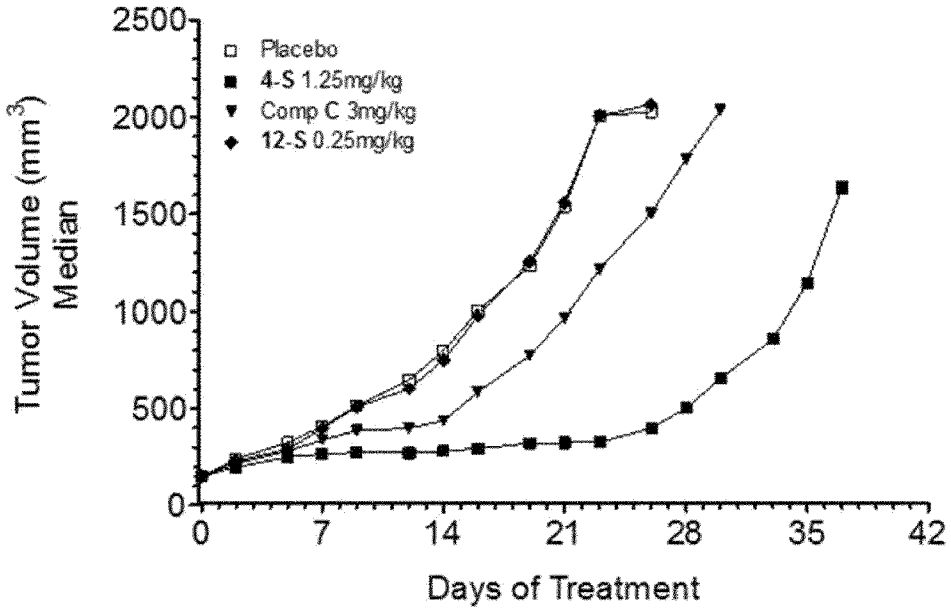


Figure 1



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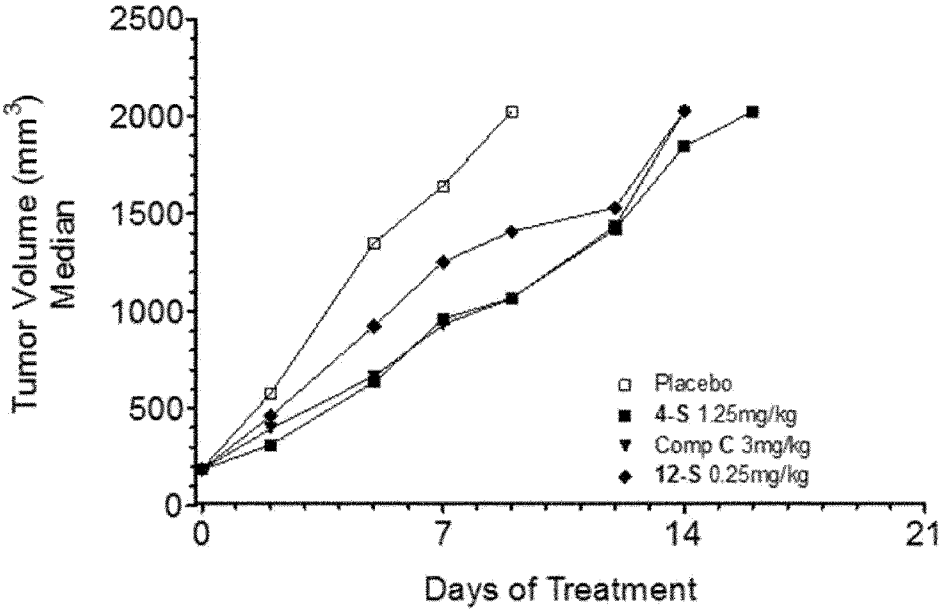
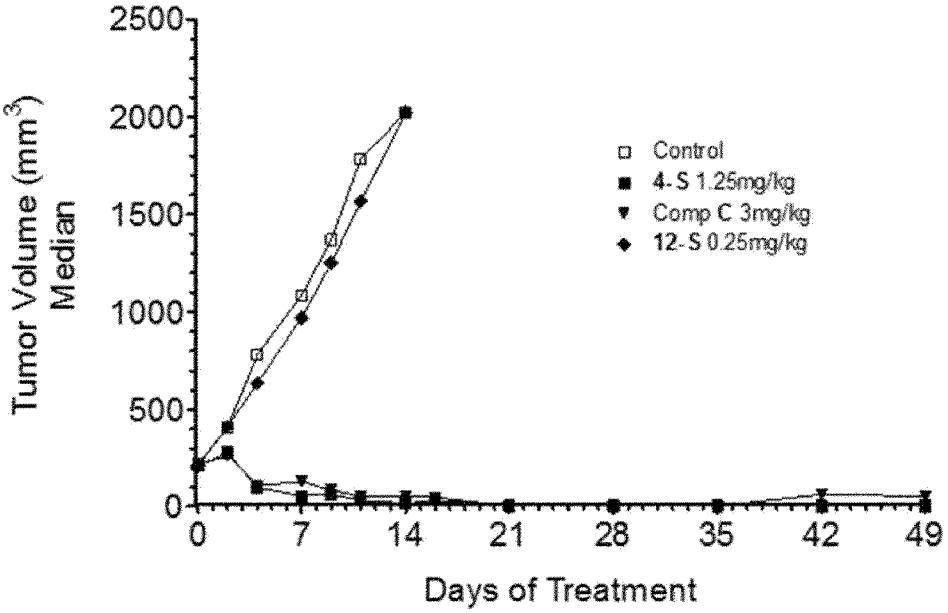


Figure 3



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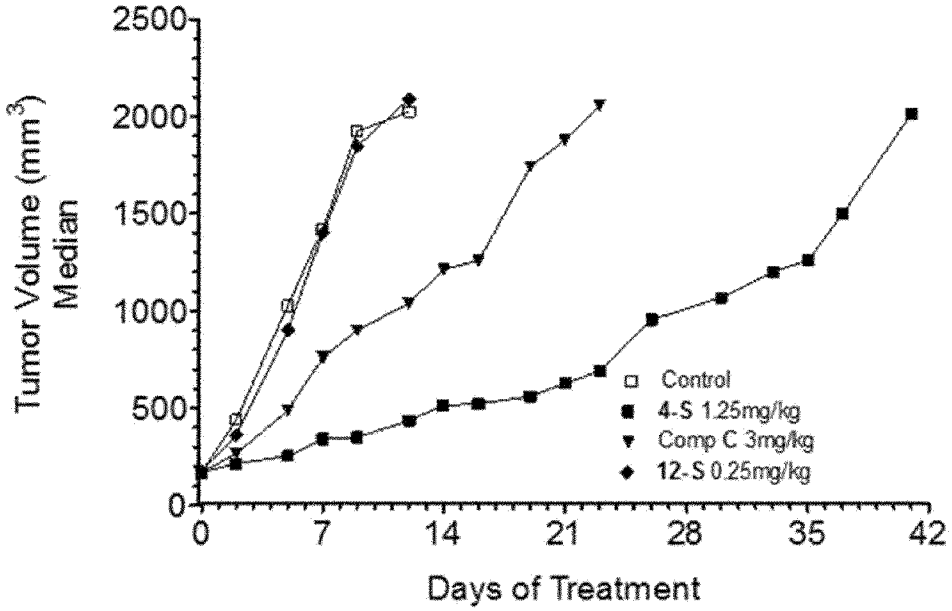

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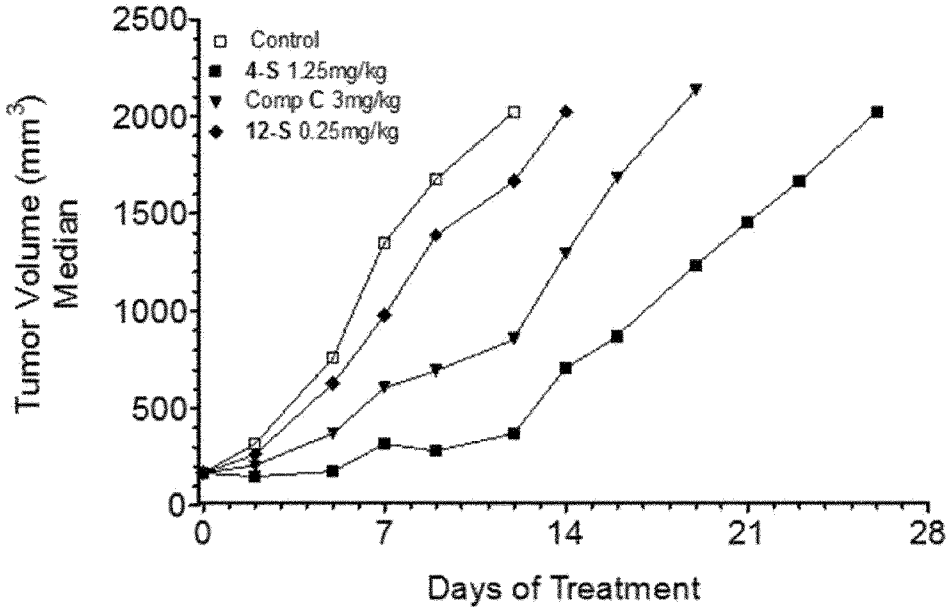

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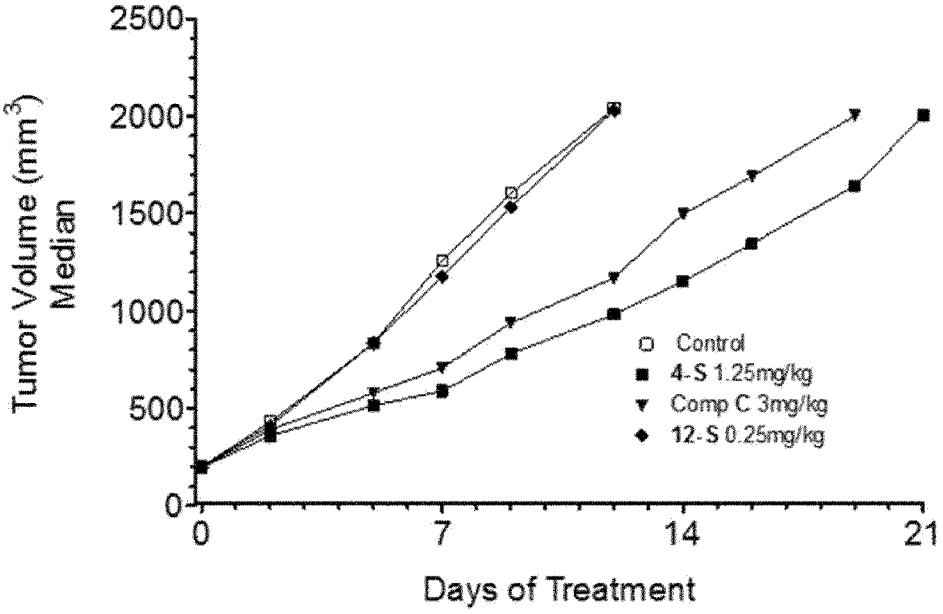

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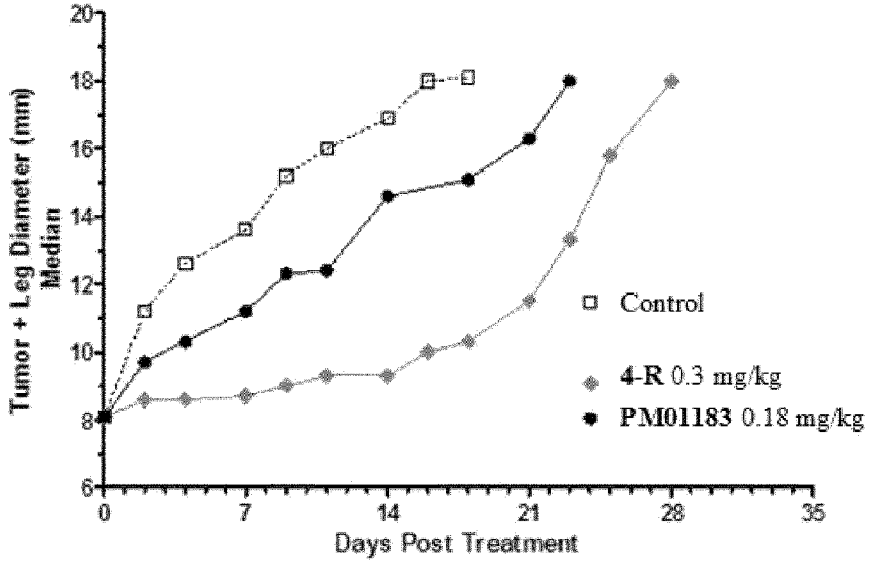

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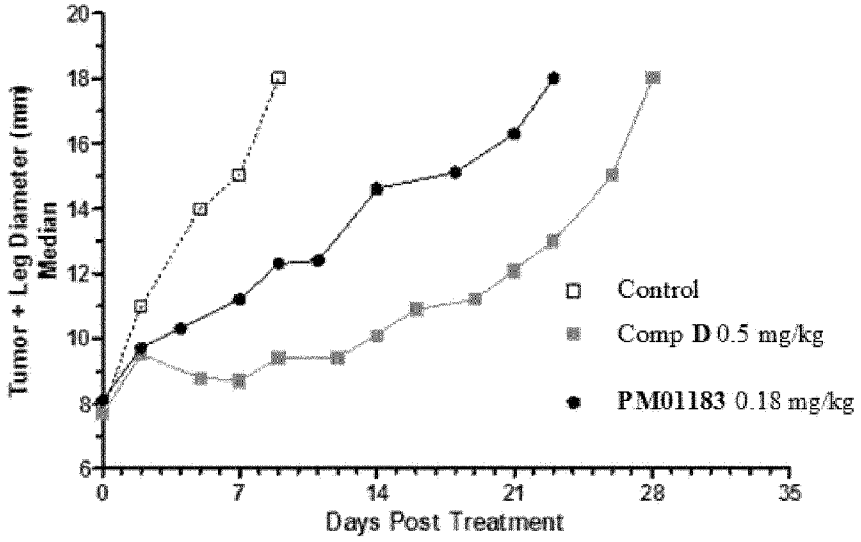

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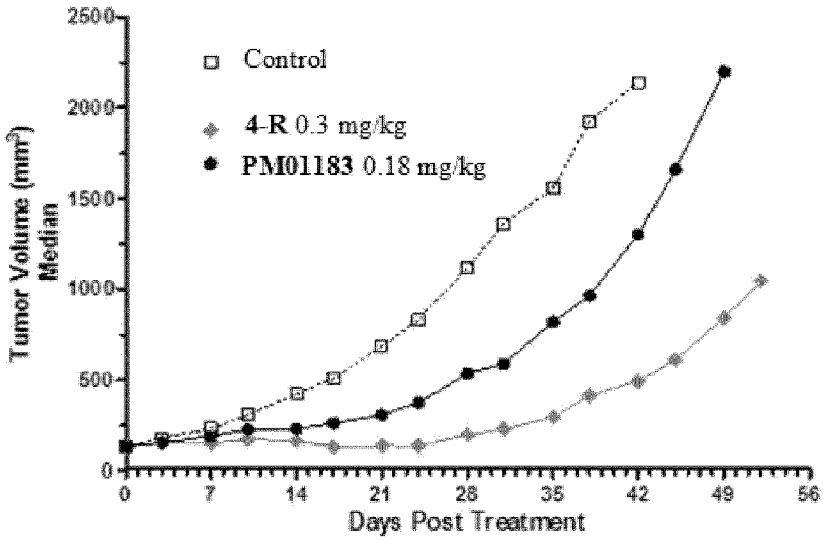

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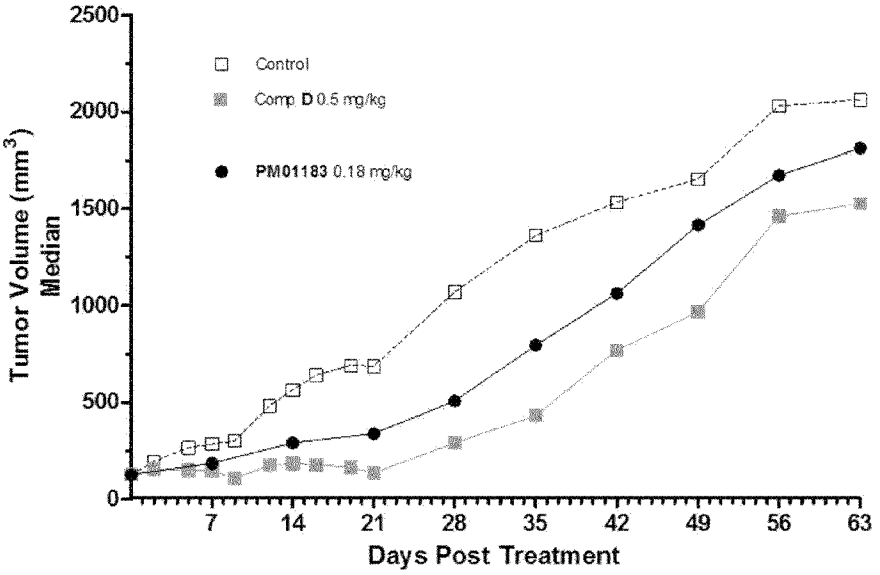

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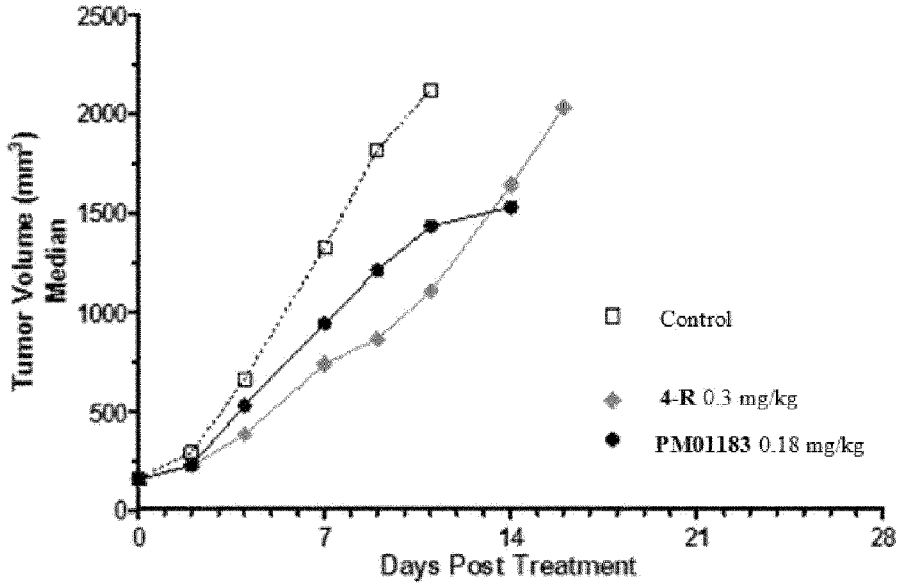

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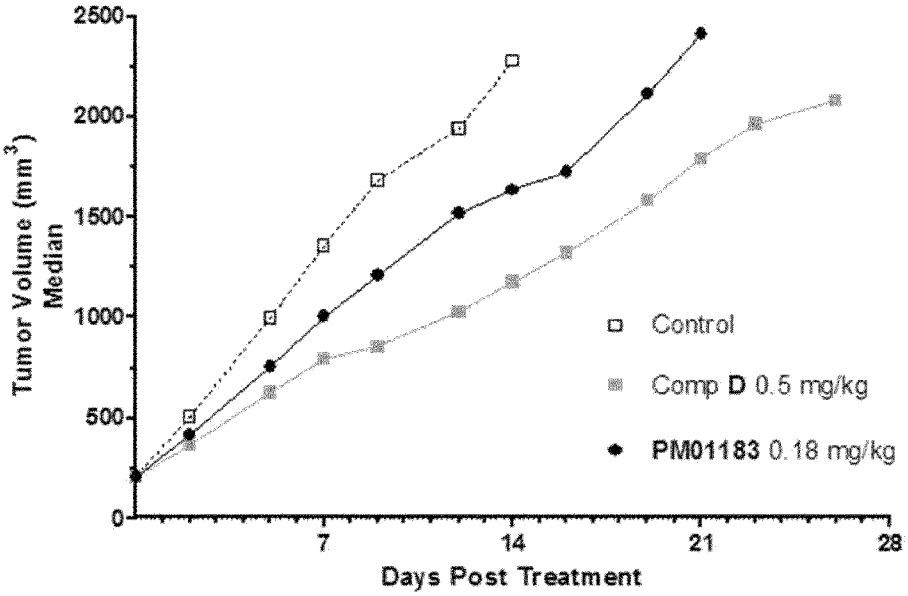

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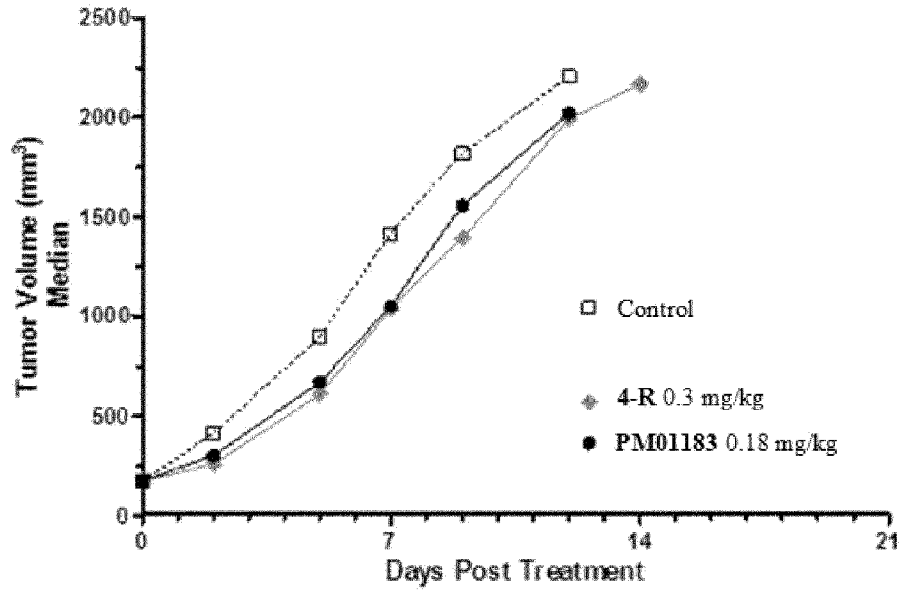

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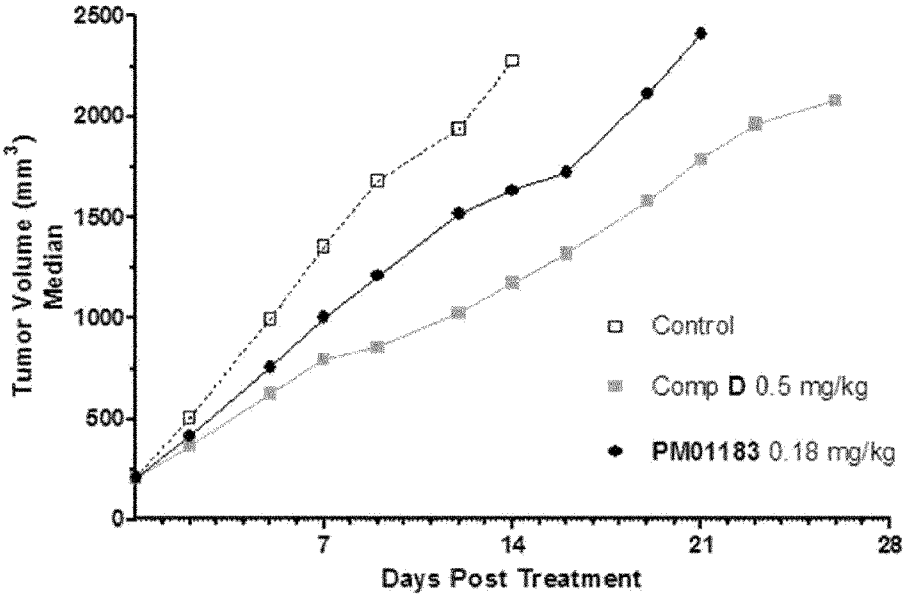

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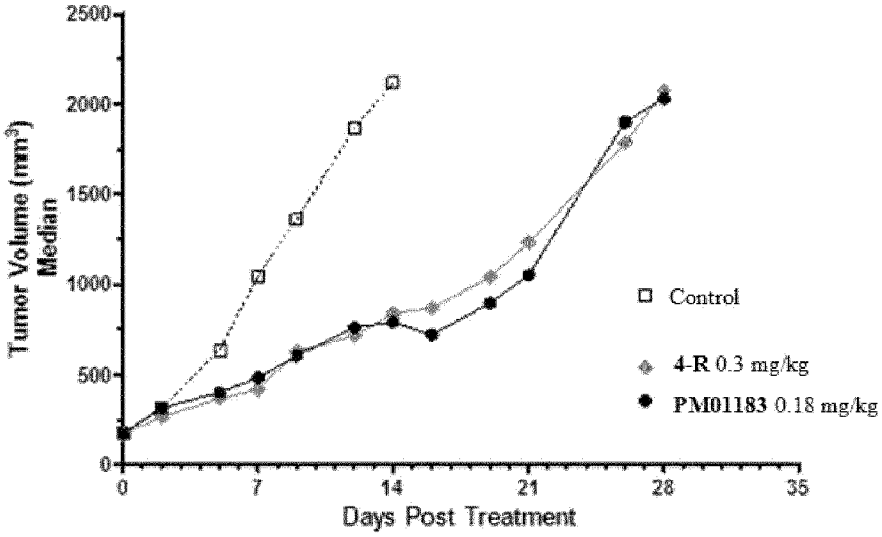

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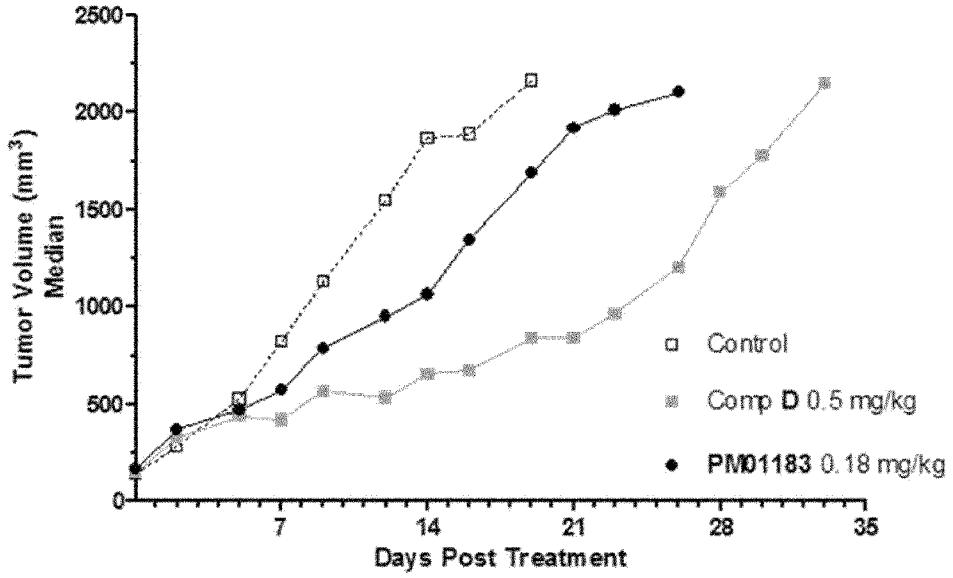

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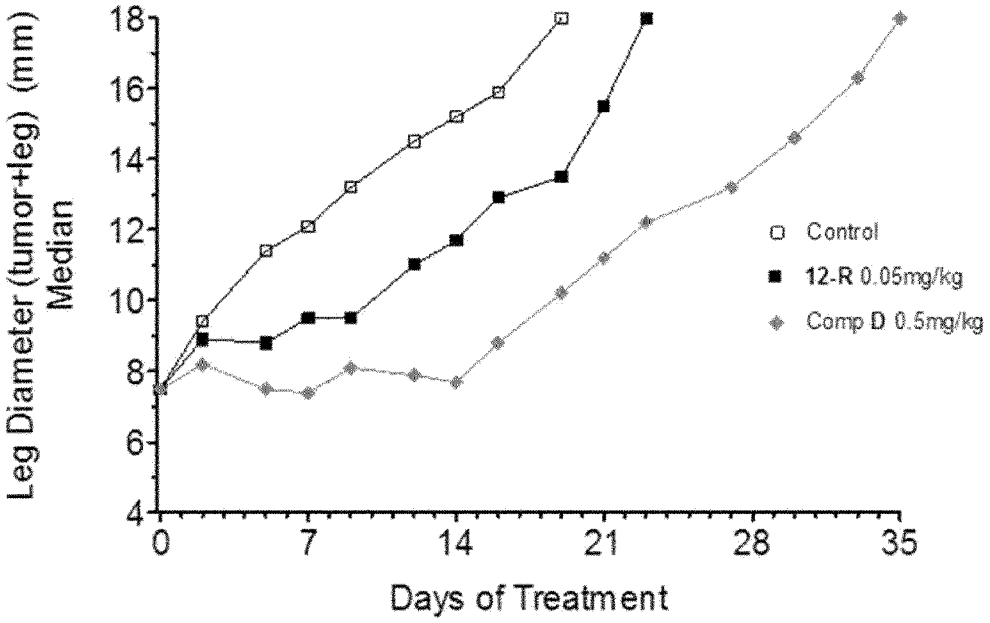

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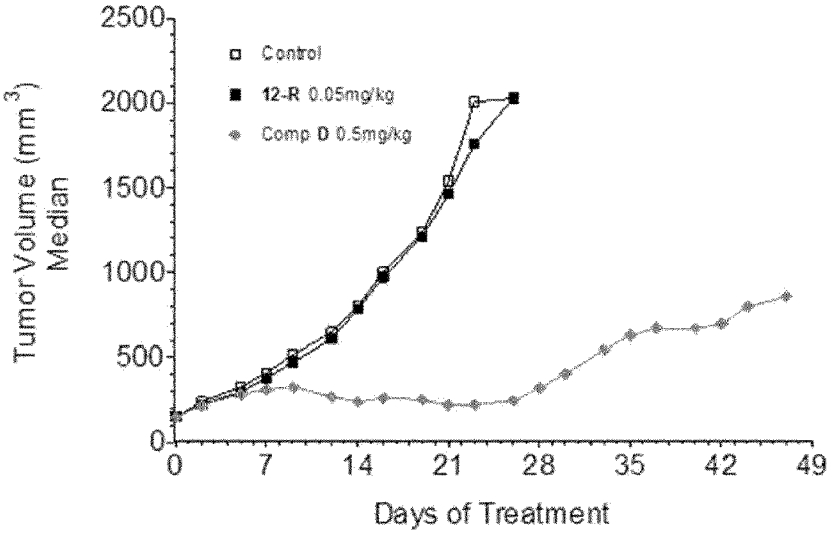

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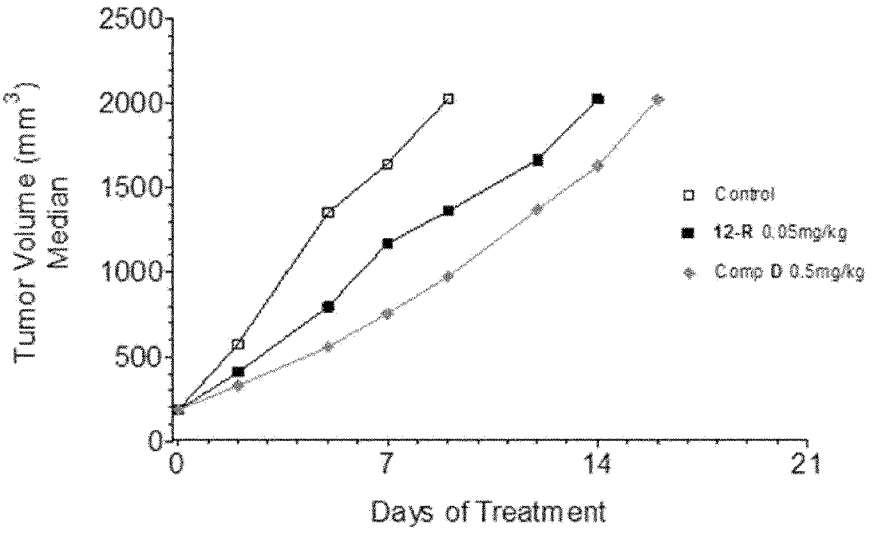

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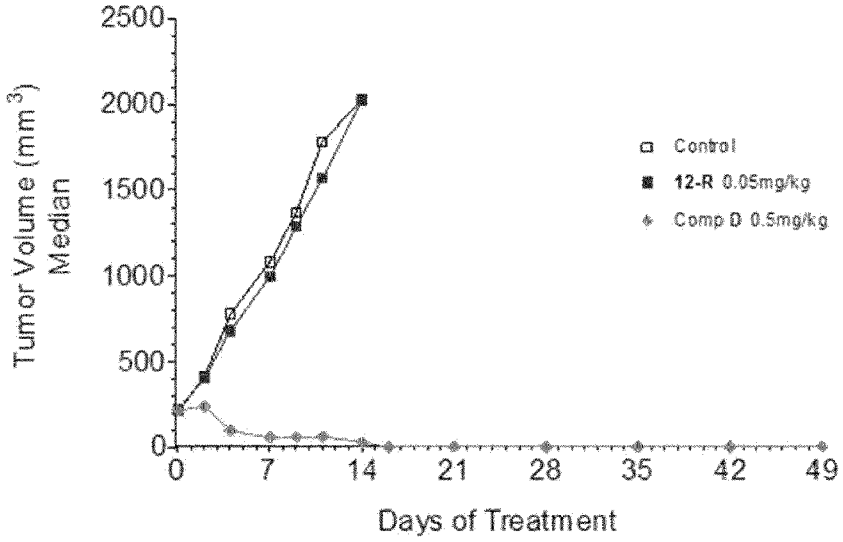

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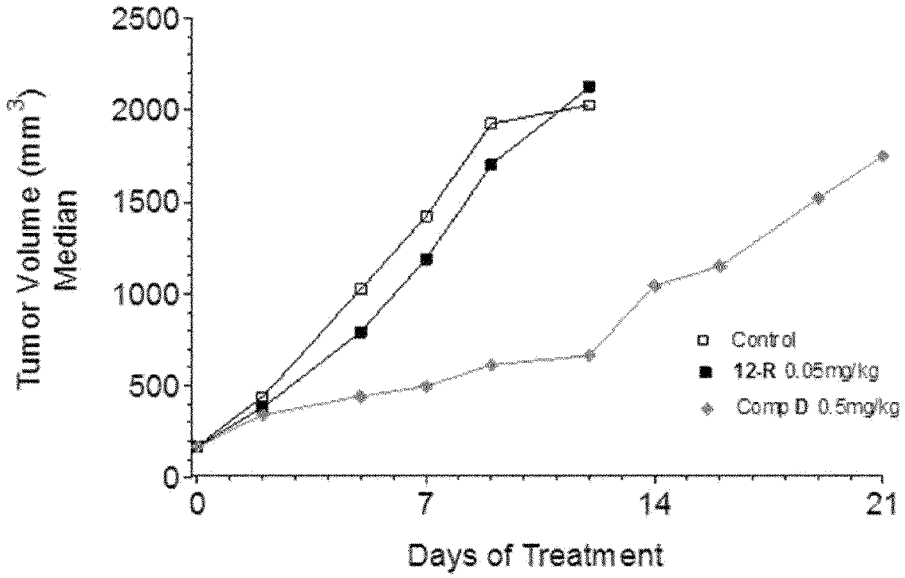

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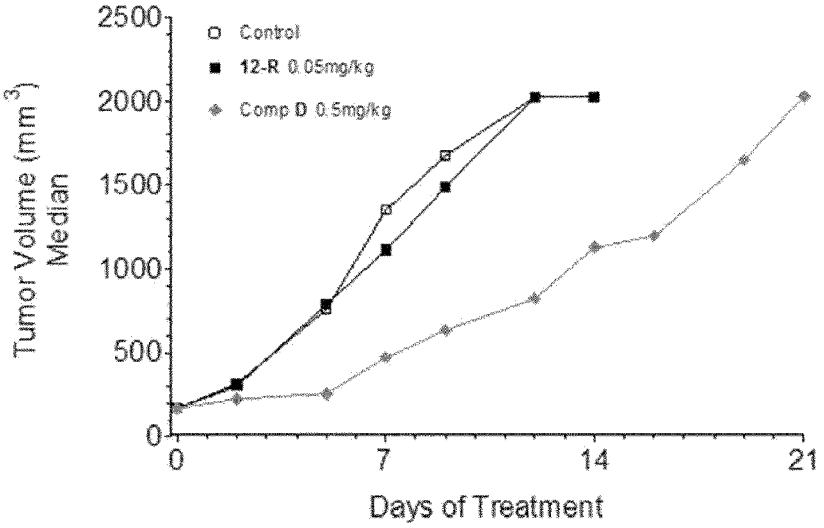

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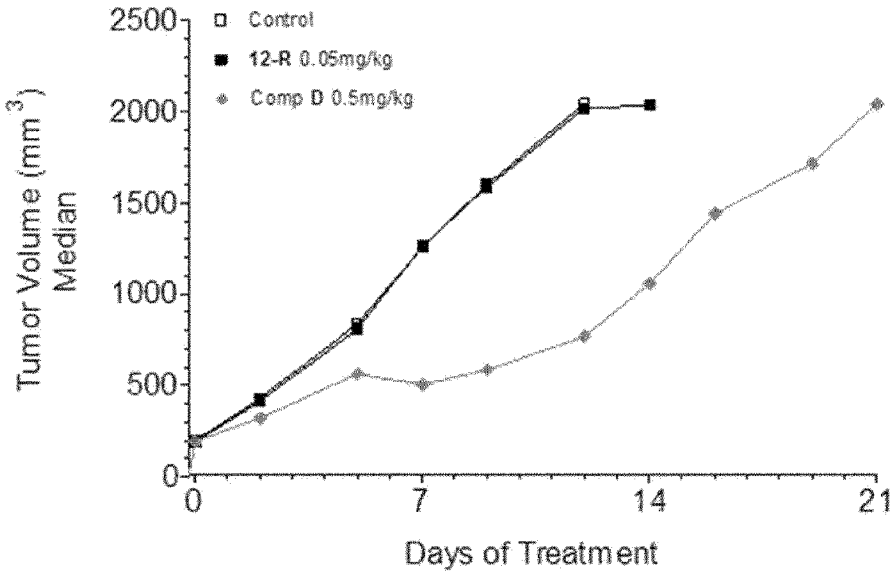

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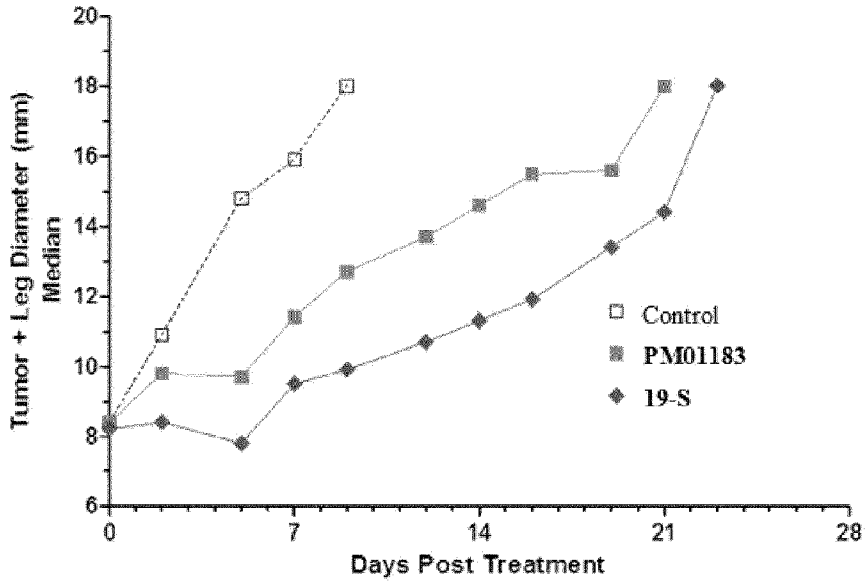

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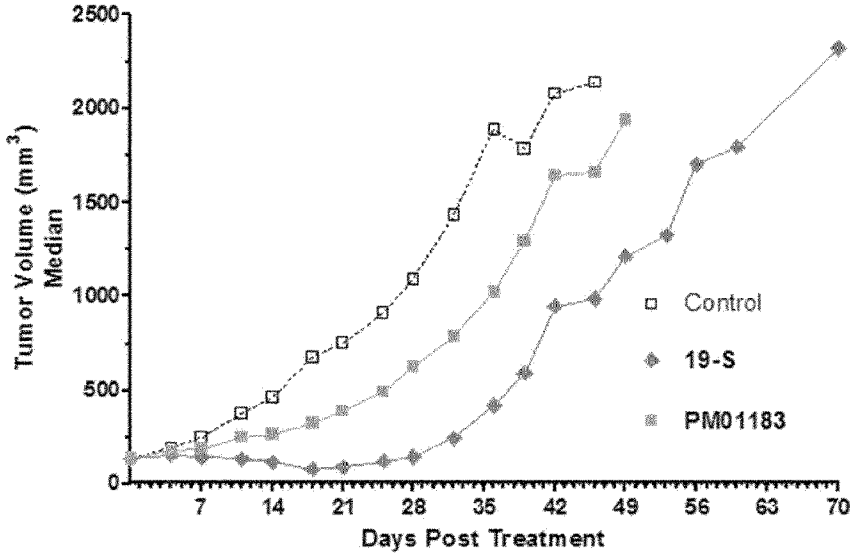

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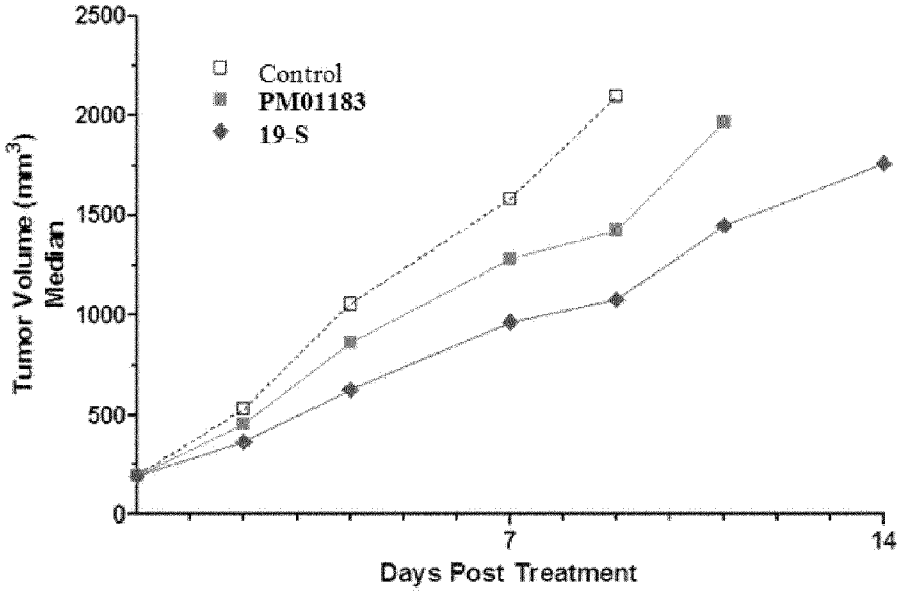

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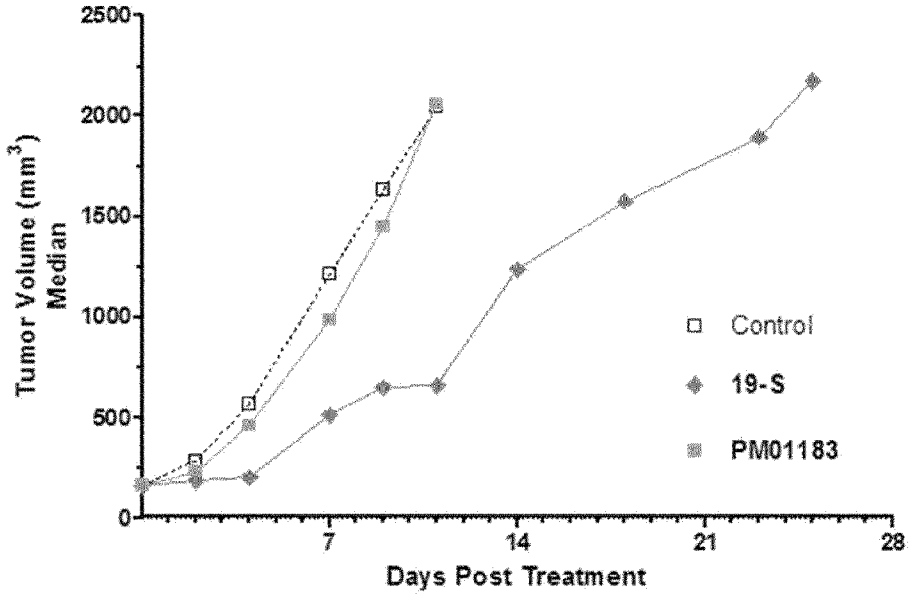

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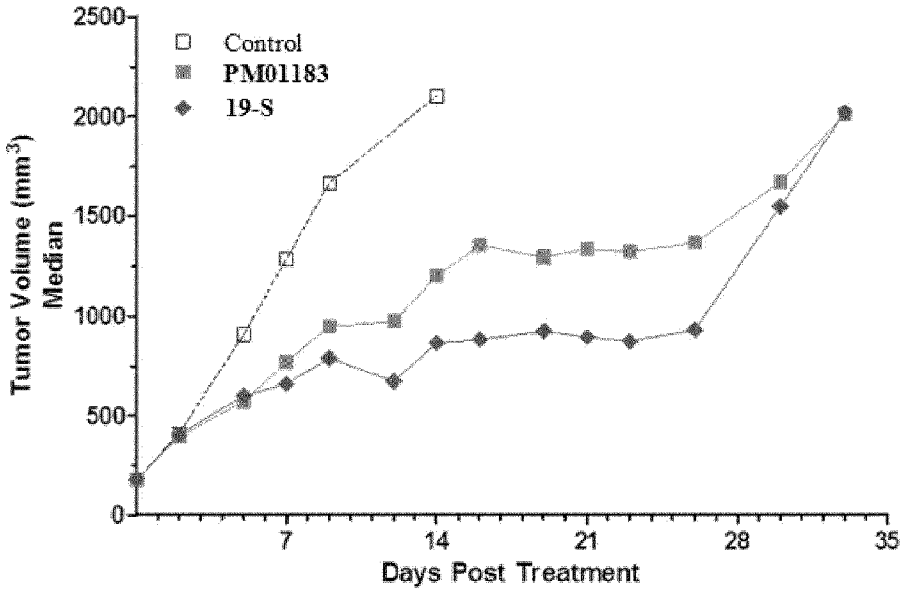

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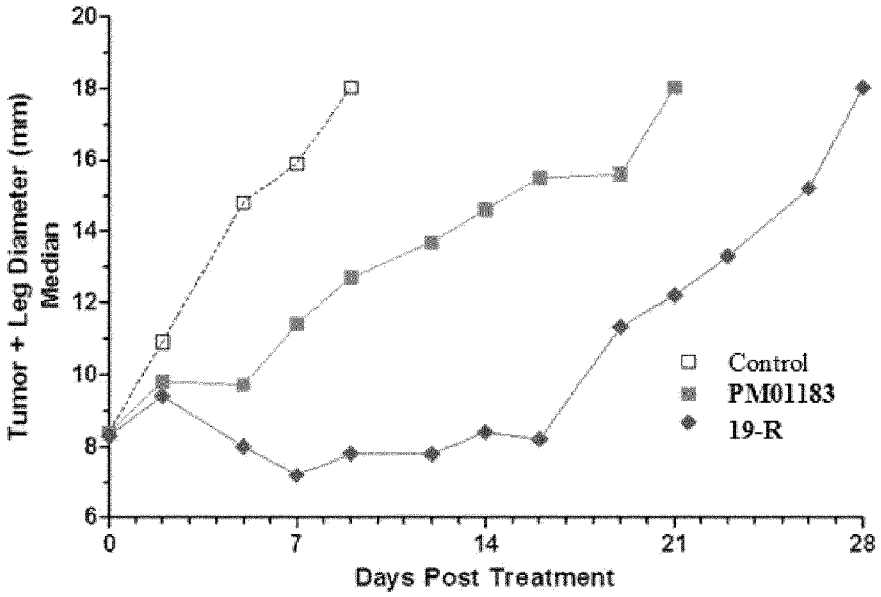

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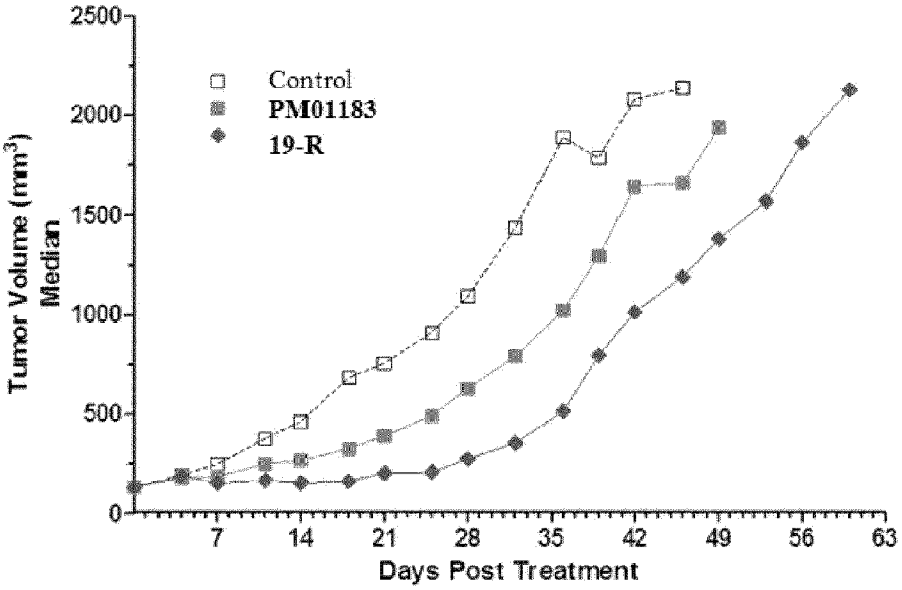

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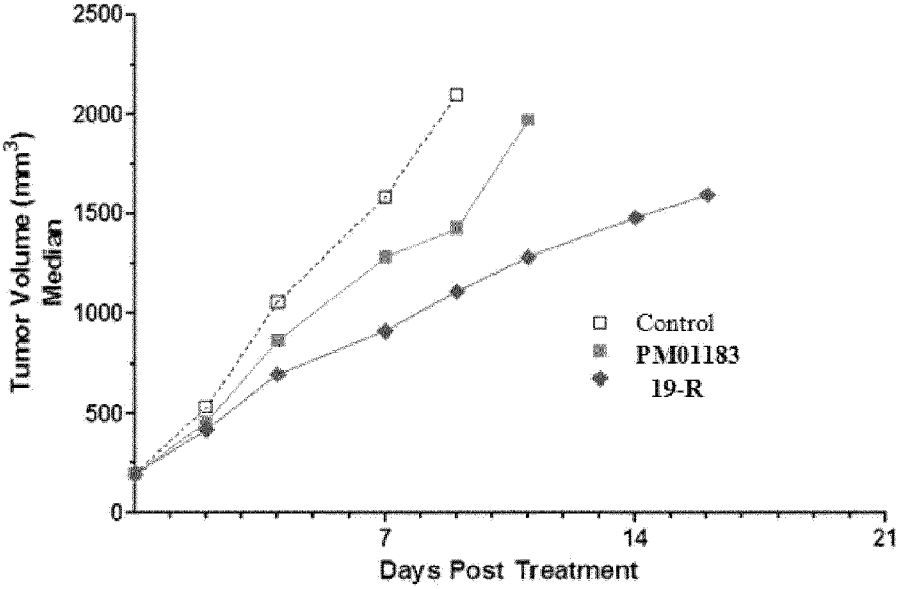

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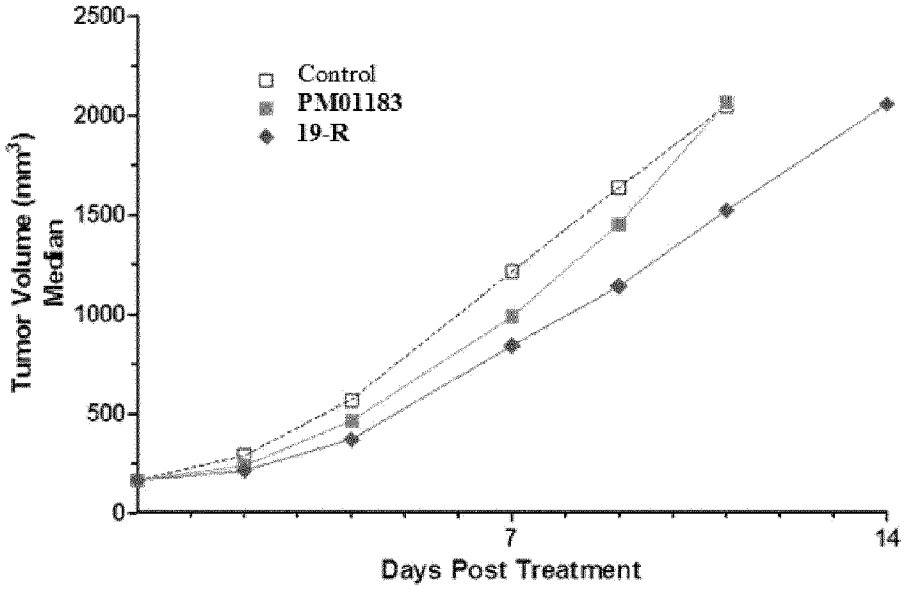

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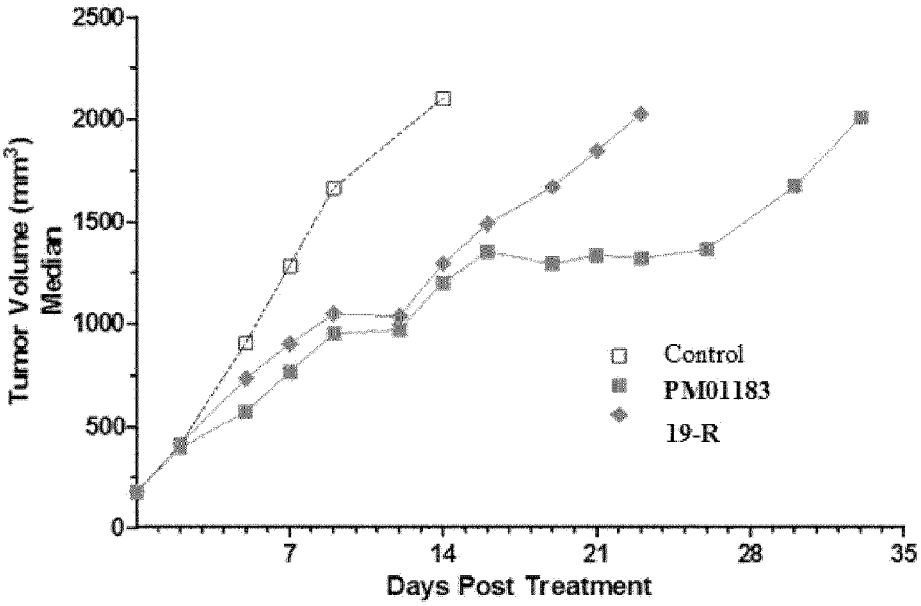

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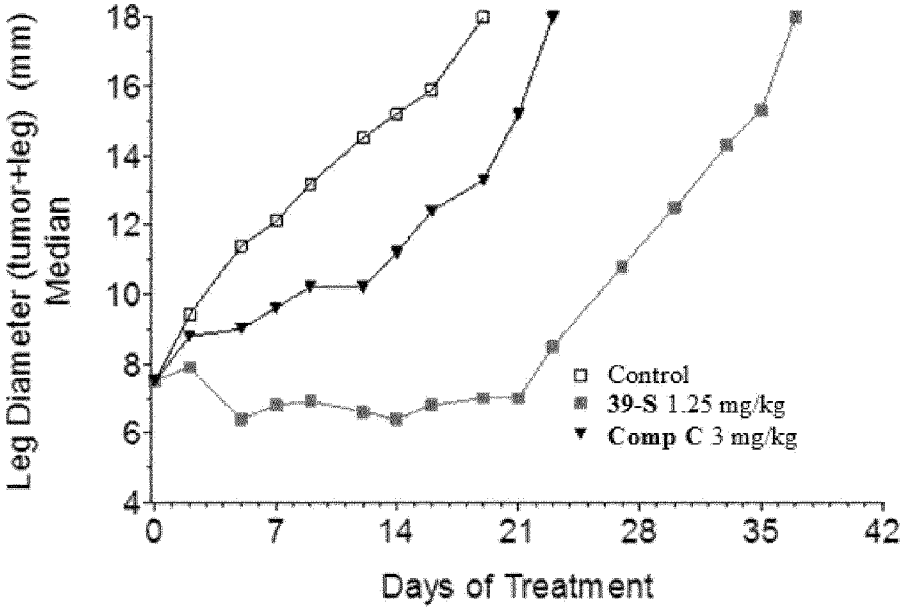

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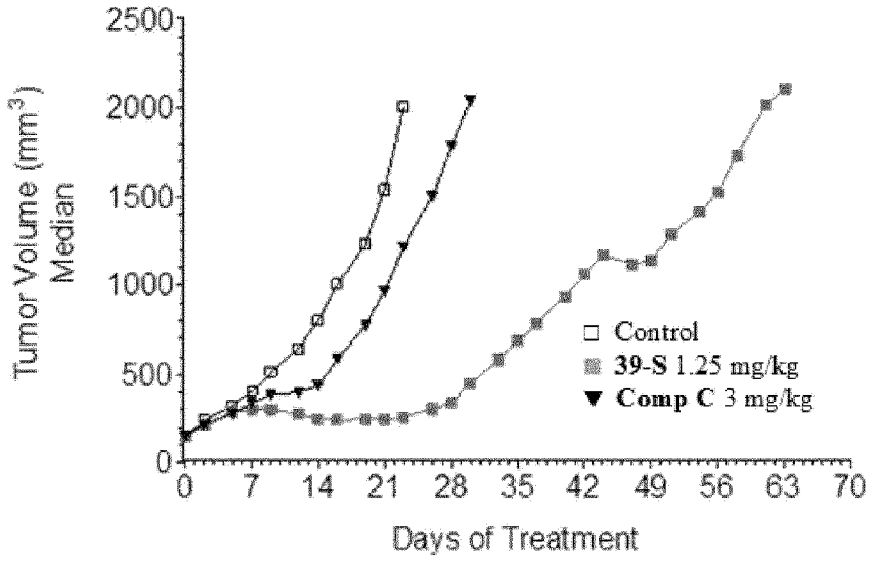

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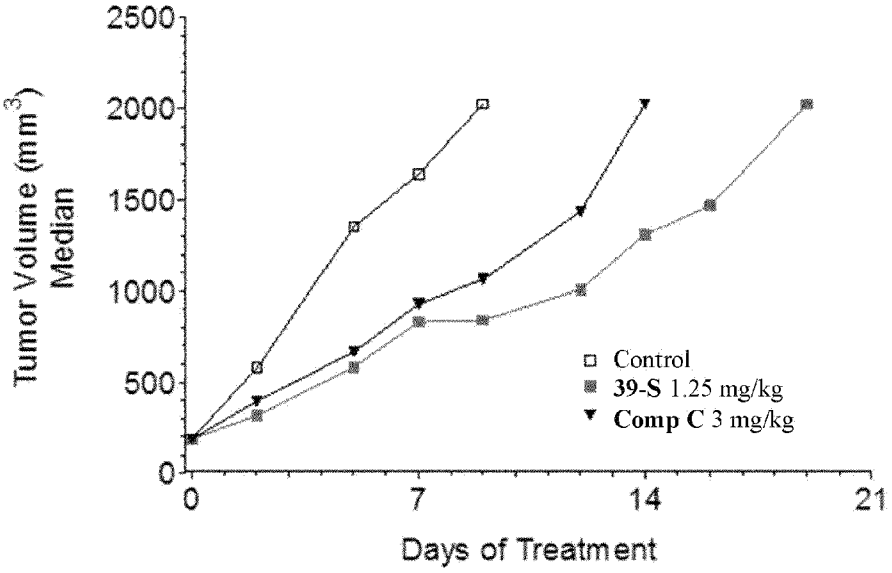

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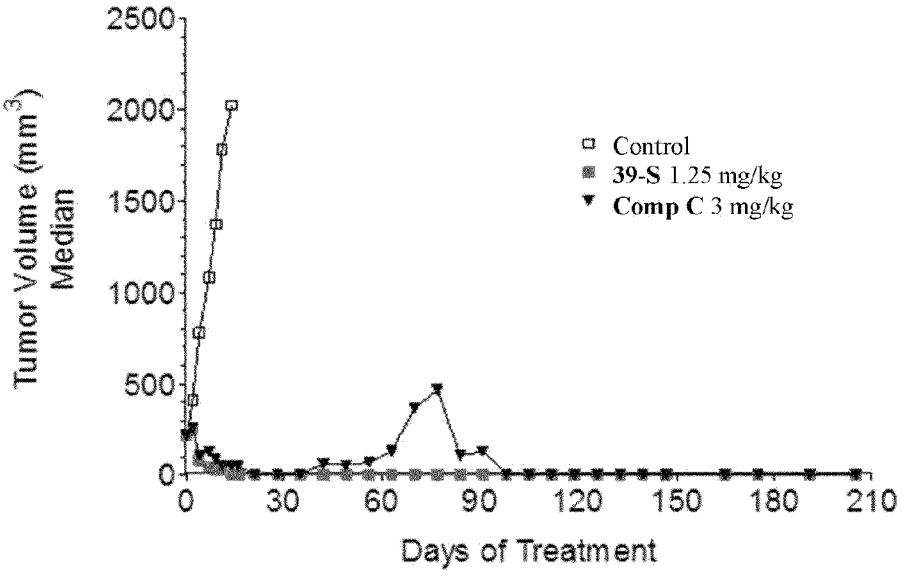

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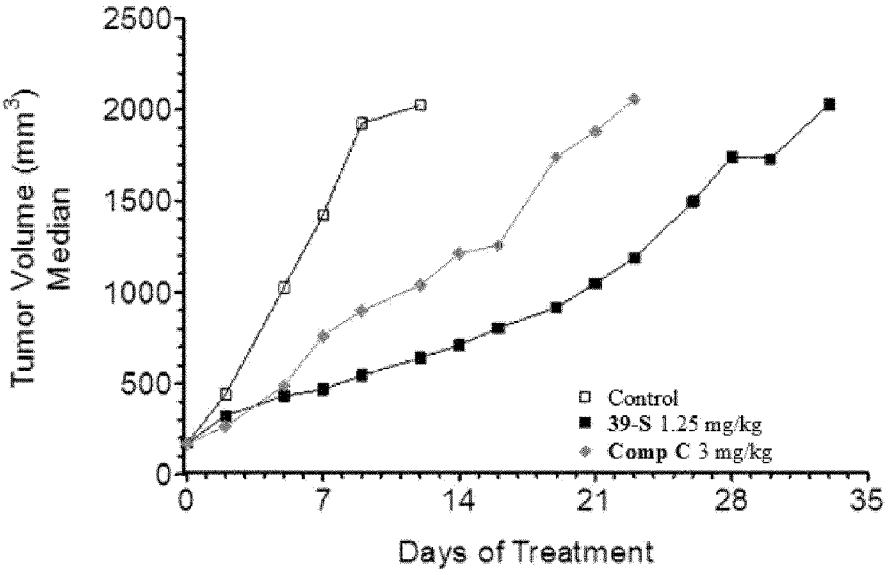

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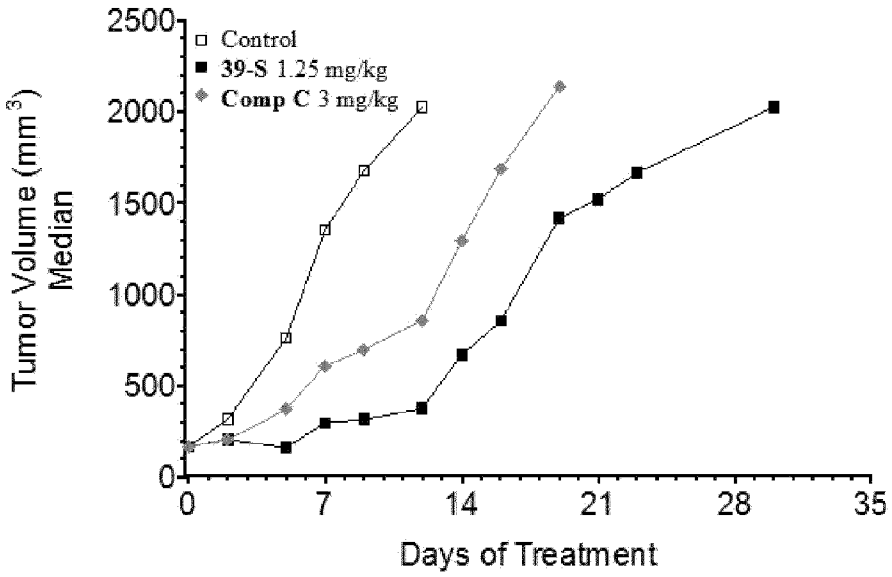

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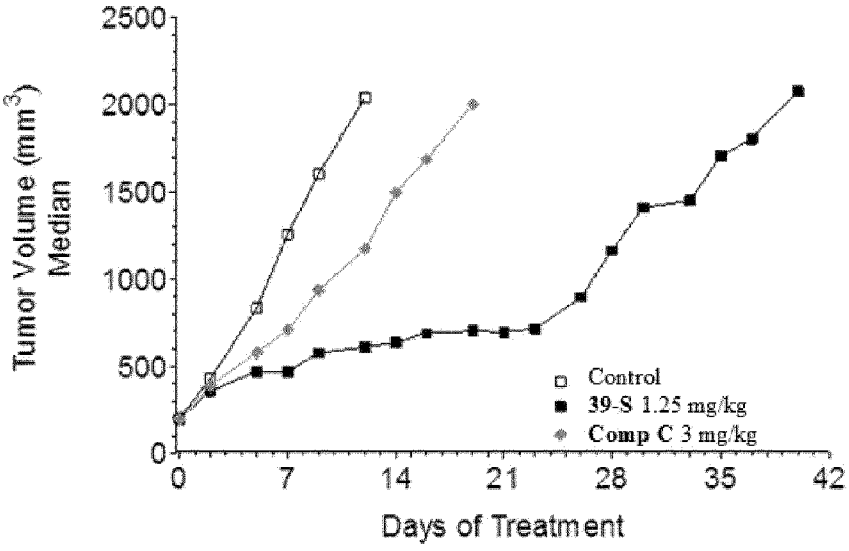

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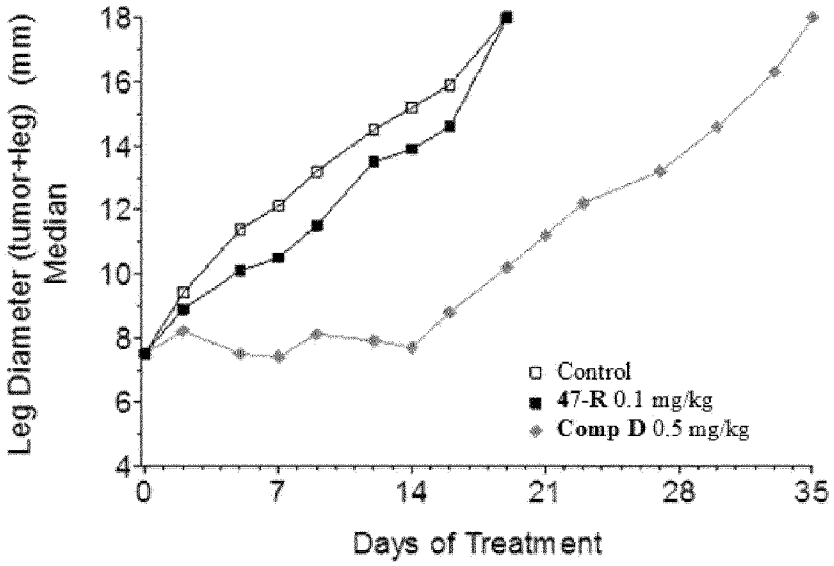

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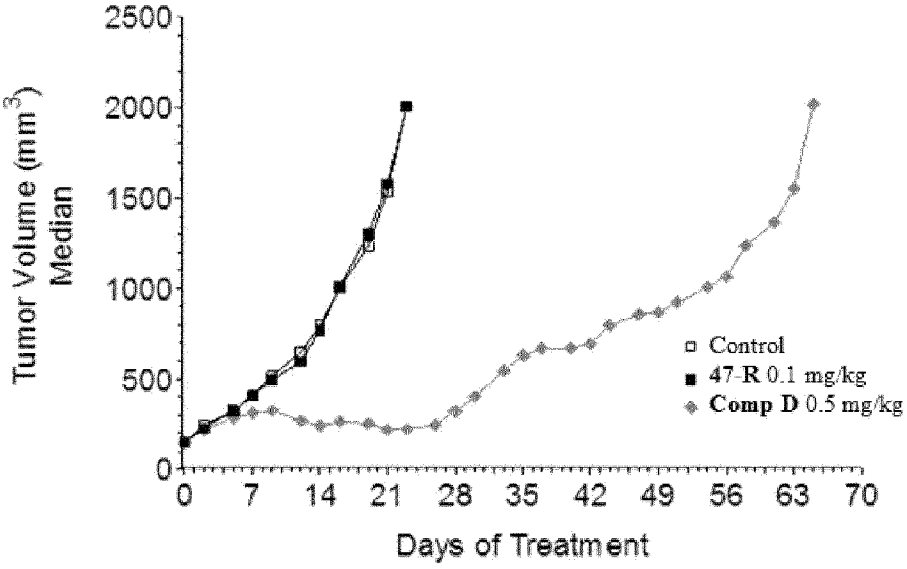

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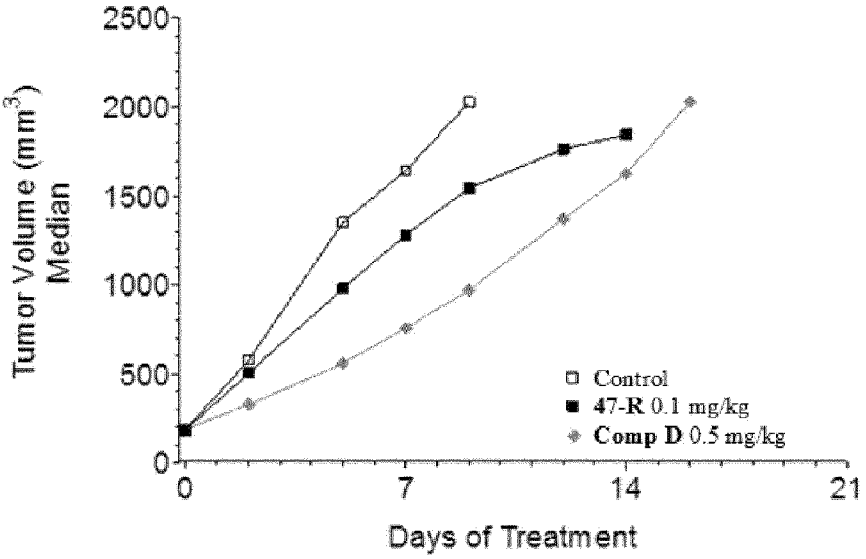

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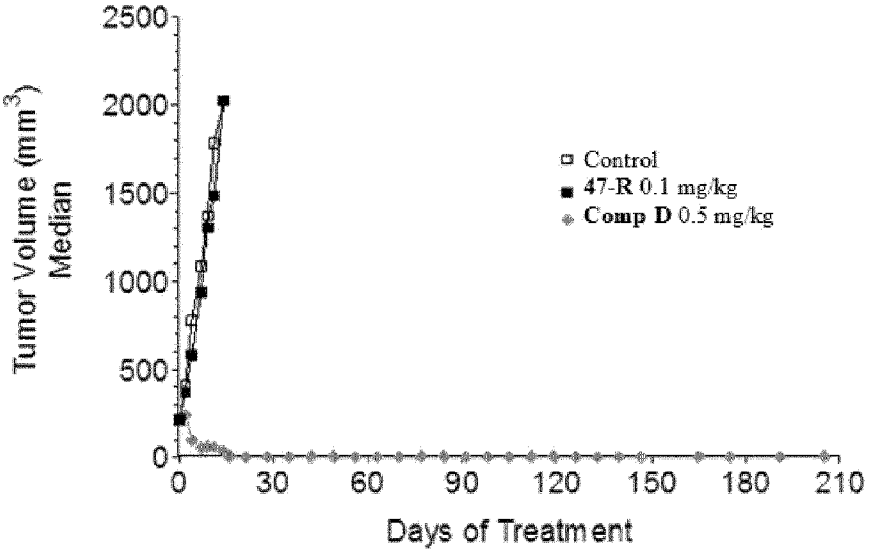

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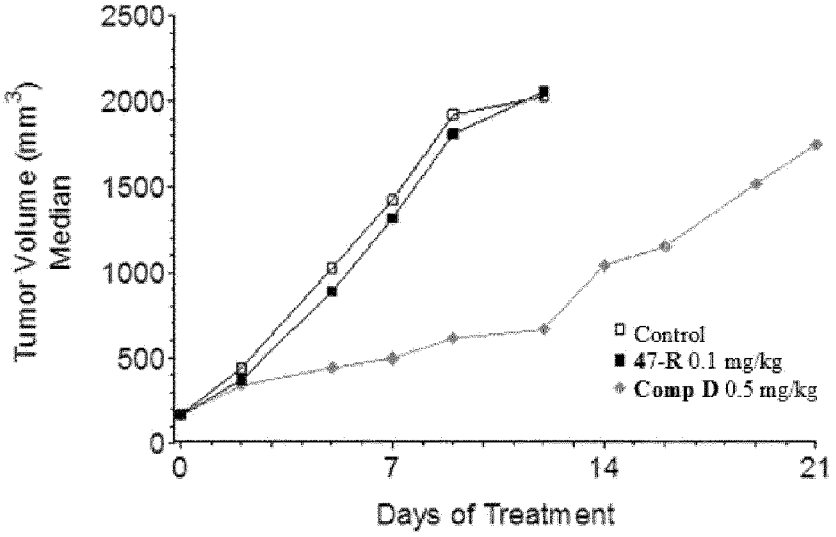

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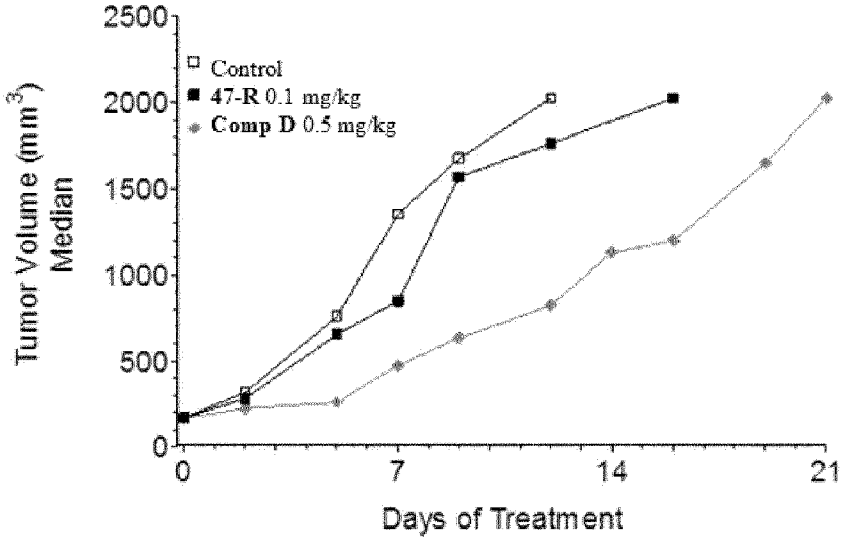


Figure 47

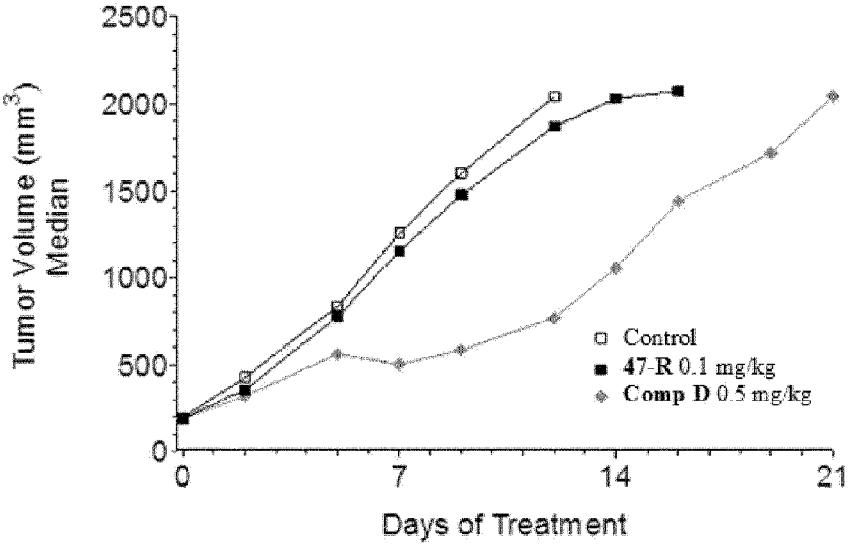


Figure 48

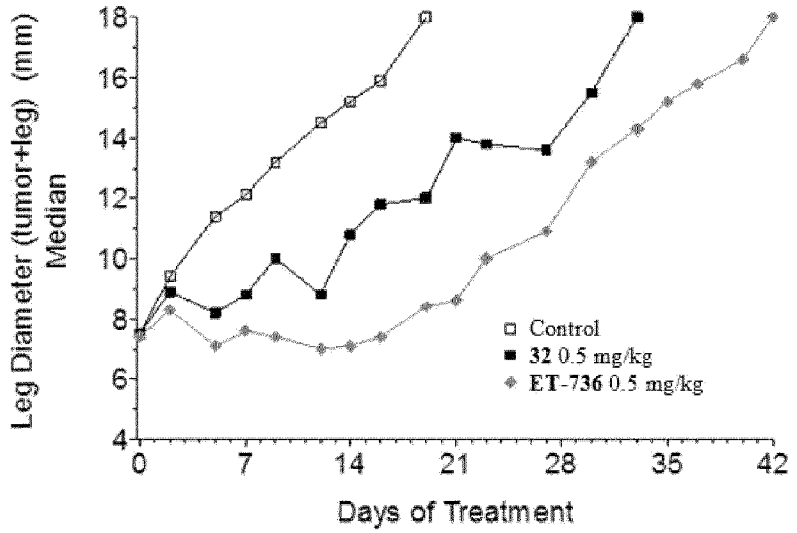


Figure 49

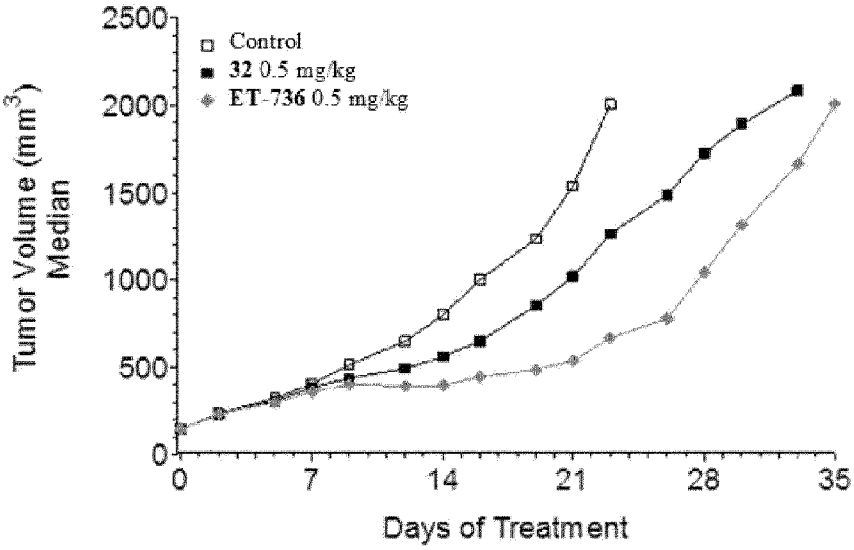
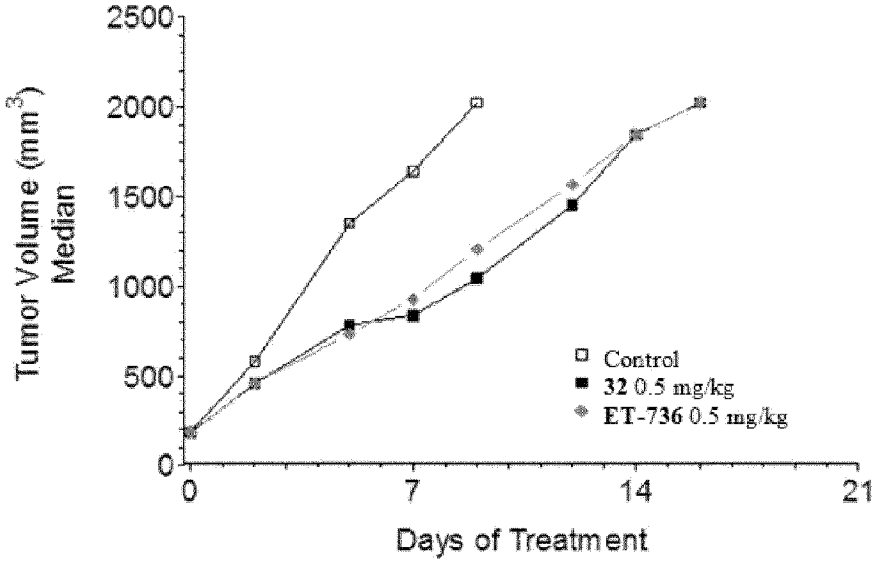


Figure 50



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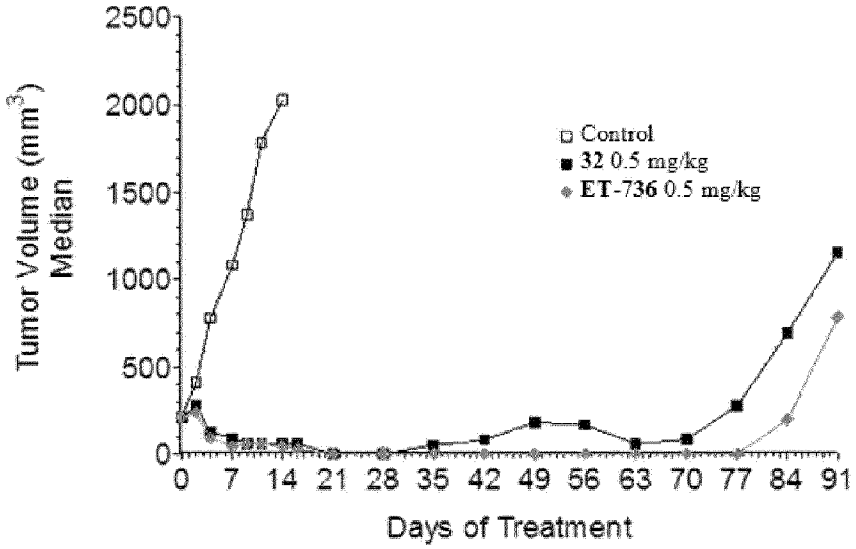

Figure 52

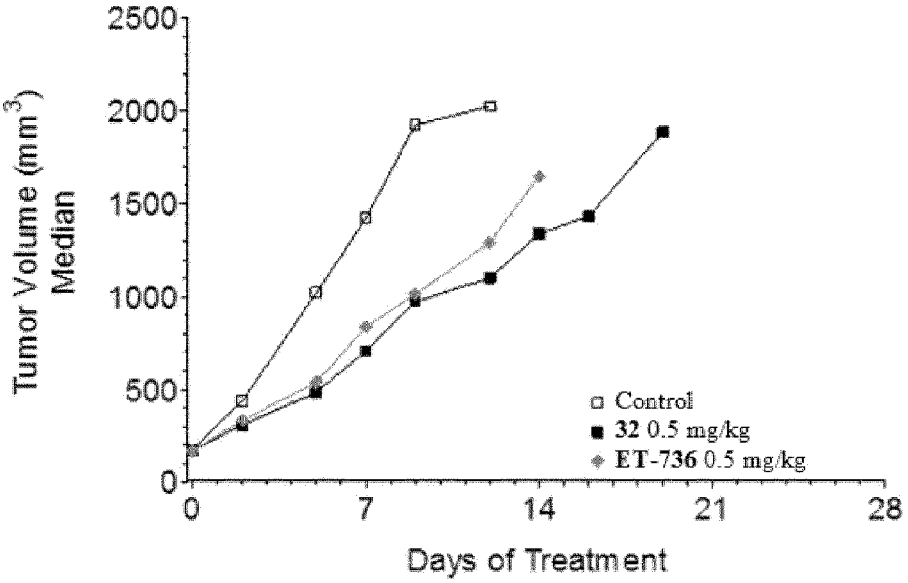

Figure 53

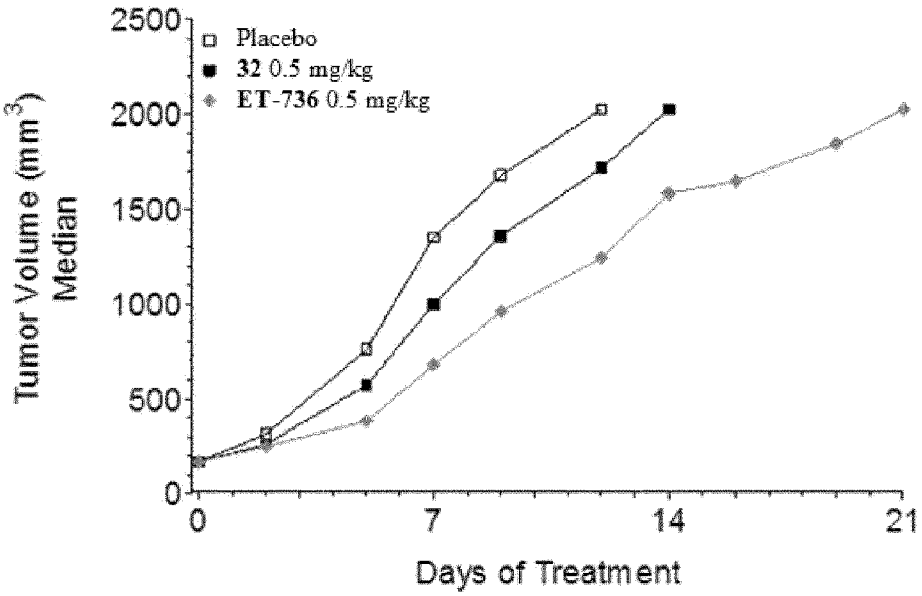

Figure 54

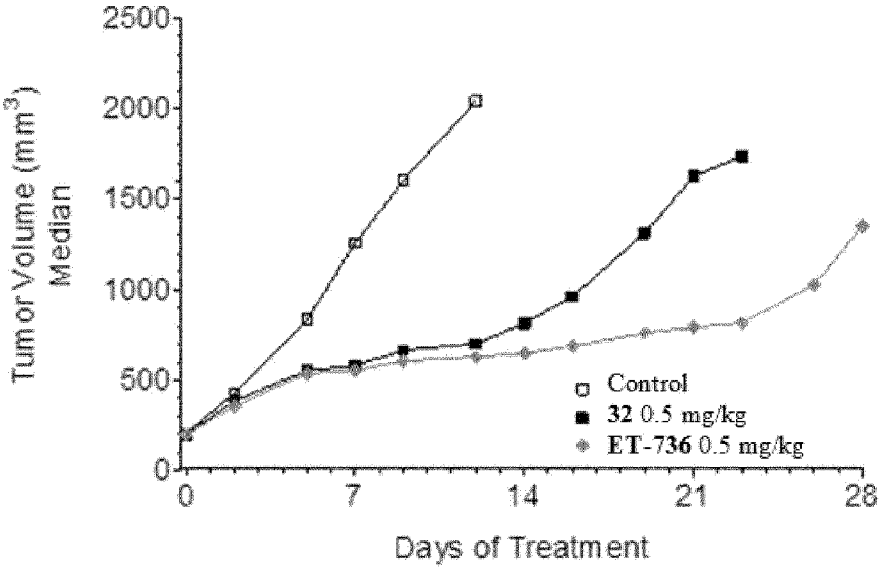

Figure 55

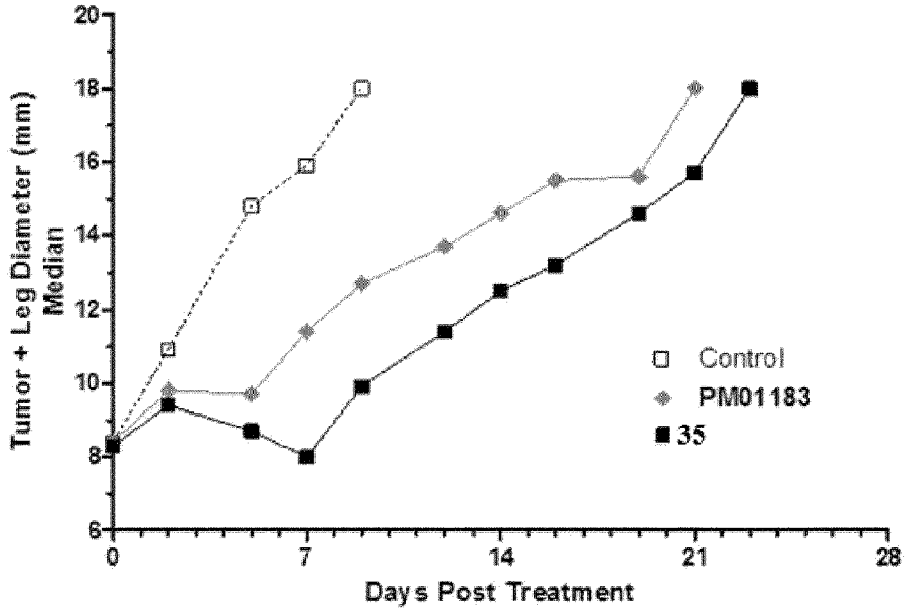

Figure 56

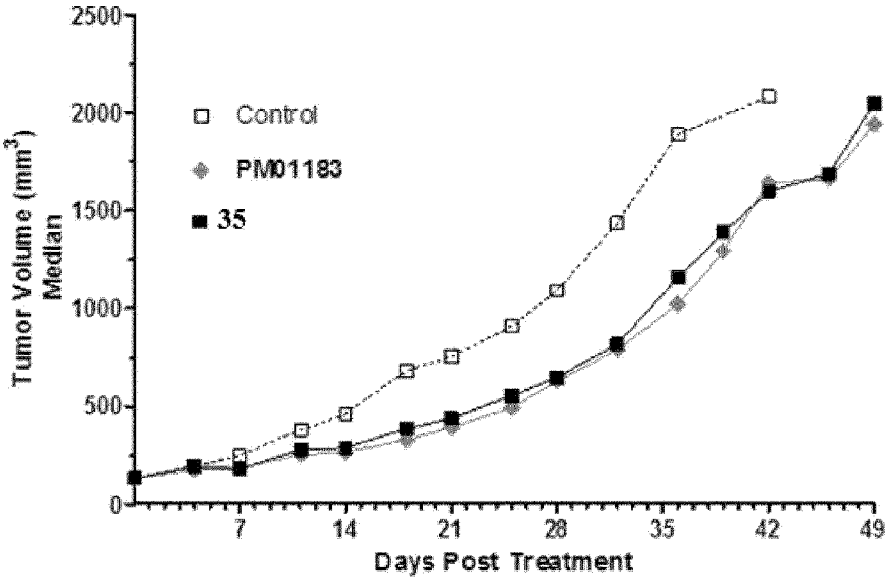

Figure 57

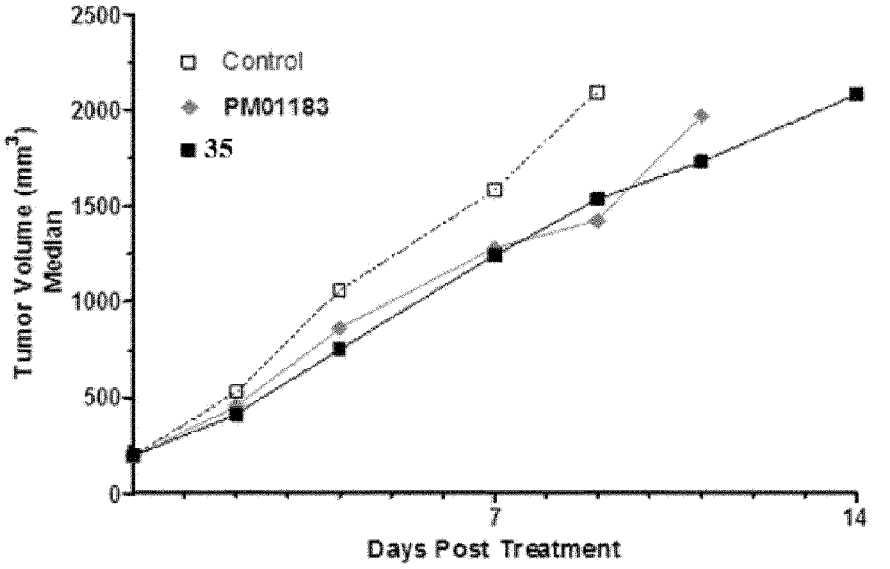

Figure 58

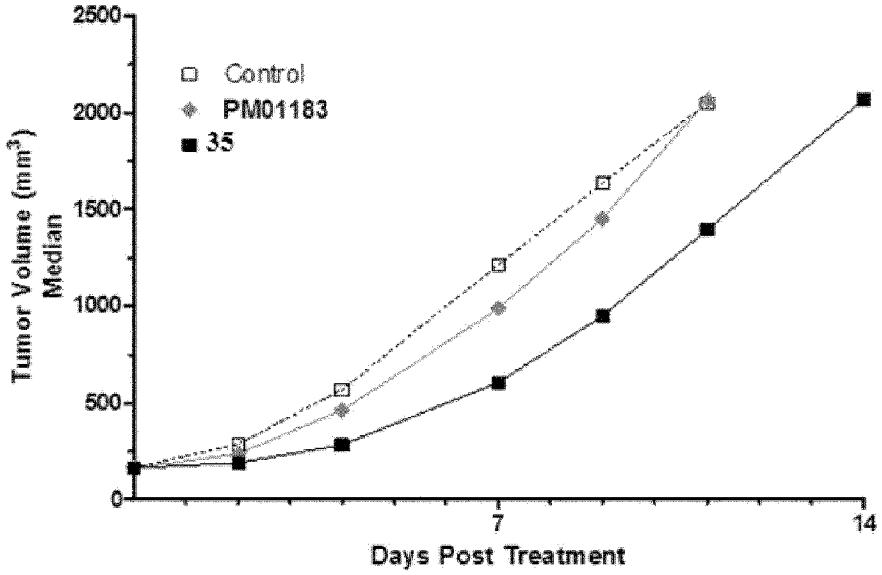

Figure 59

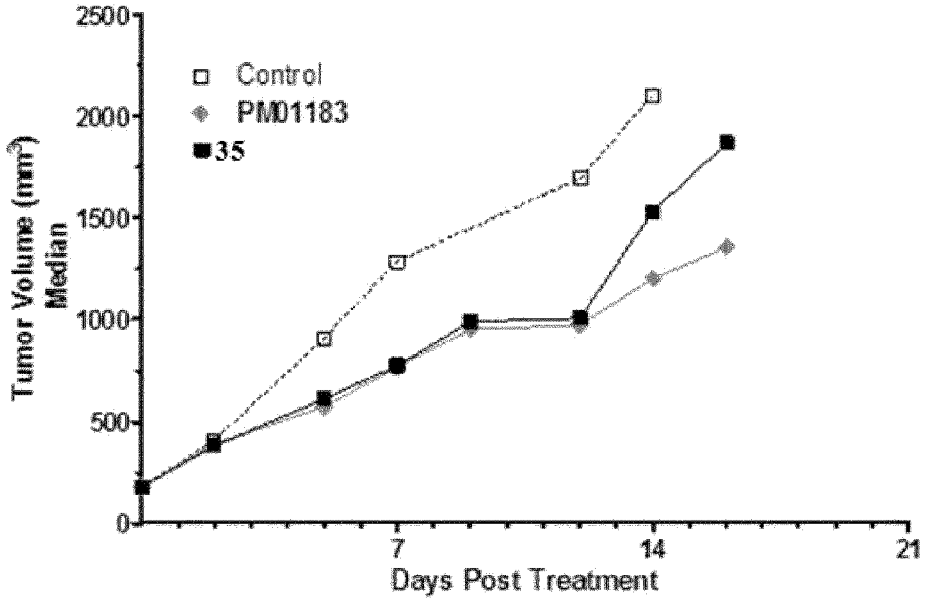

Figure 60

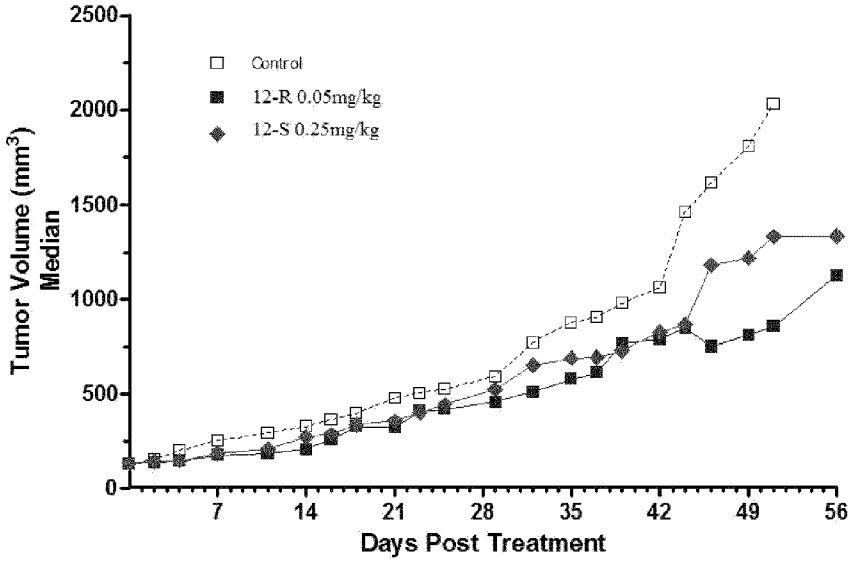

Figure 61

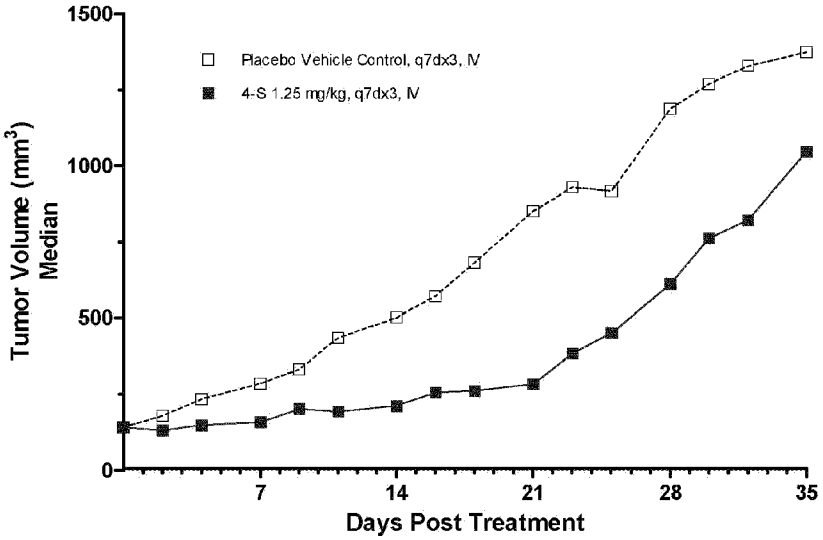

Figure 62

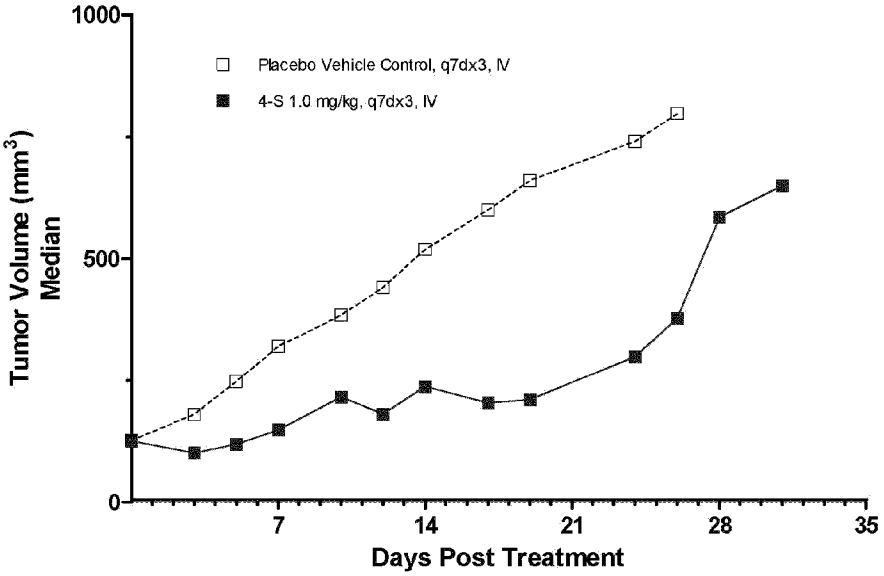

Figure 63

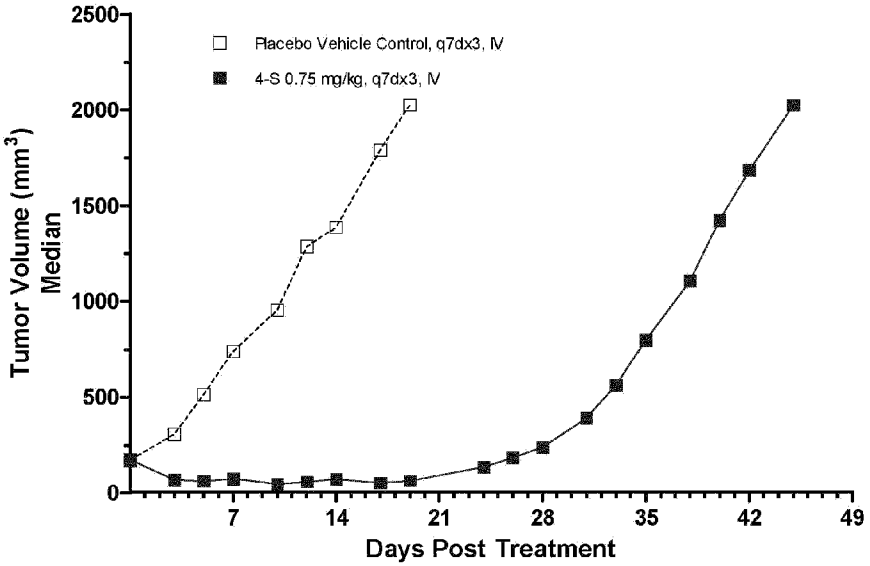

Figure 64

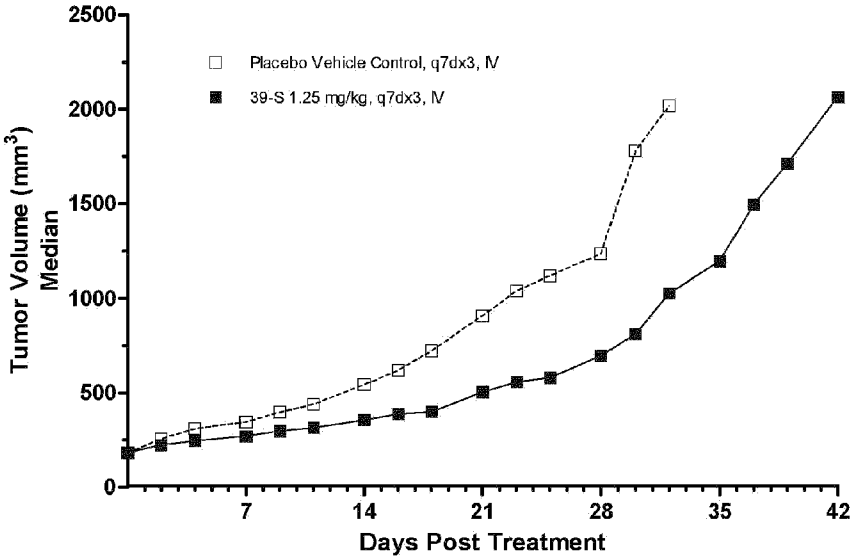

Figure 65

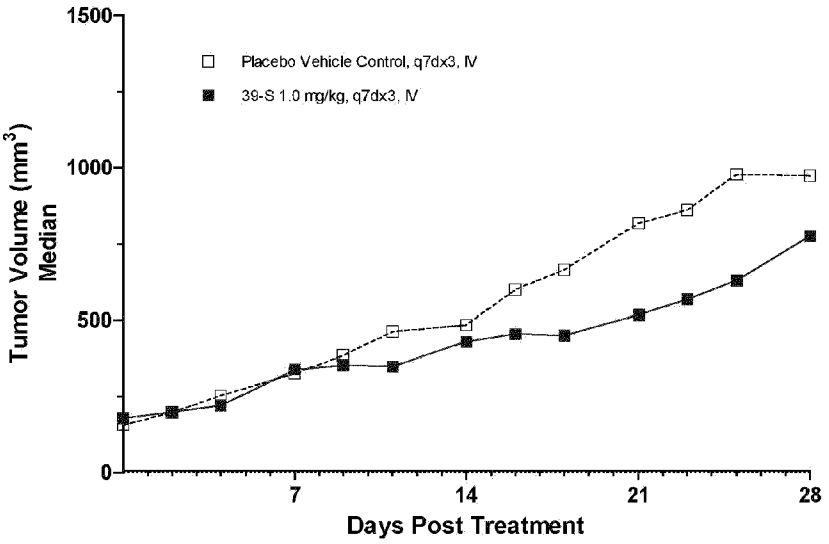

Figure 66

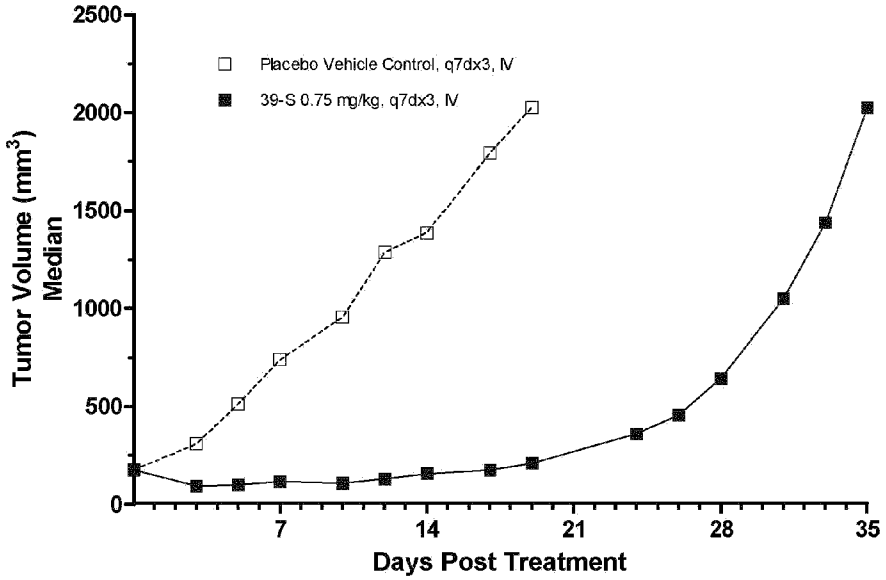
Placebo Vehicle Control, q7dx3, IV
39-S 0.75 mg/kg, q7dx3, IV
Tumor Volume (mm3)
Median
2500
2000
1500
1000
500
0
7
14
21
28
35
Days Post Treatment

Figure 67