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(57) Abstract

The invention features derivatives of the tetrapeptide Ser-Asp-Lys-Pro and the use thereof to inhibit the proliferation of hematopoietic cells.

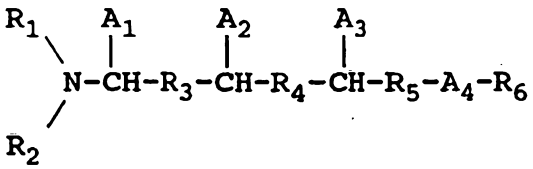
PEPTIDE INHIBITORS OF HEMATOPOIETIC CELL PROLIFERATION

Background of the Invention

The tetrapeptide N-Acetyl-Ser-Asp-Lys-Pro (AcSDKP) was originally isolated from fetal calf bone marrow. Lenfant, et al., Proc. Natl. Acad. Sci. USA 86:779-782 (1989). AcSDKP is a negative regulator of hematopoietic stem cell proliferation, preventing stem cell recruitment into the S-phase. Frindel, et al., Exp. Hematol. 5:74-76 (1977). AcSDKP appears to exert this function by blocking the action of stem cell-specific proliferation stimulators. Robinson, et al., Cell Proliferation 25:623-32 (1992). Phase-specific anticancer drugs (e.g., Ara-C or cisplatin) or radiation act on cells committed to proliferation, irrespective of whether the cell is malignant. Thus, administration of AcSDKP in conjunction with cytotoxic therapy, protect normal hematopoietic progenitor cells in the quiescent state.

Summary of the Invention

In one aspect, the invention features compounds of the formula:



wherein

A_1 is the identifying group of the D- or L- isomer of Ser;

A_2 is the identifying group of the D- or L- isomer of Asp or Glu;

A_3 is the identifying group of the D- or L- isomer of Lys, Arg, or Orn;

A_4 is the D- or L- isomer of Pro;

R_1 is H, C_{1-12} alkyl, C_{7-20} arylalkyl, R_7CO, or $R_7OC(O)$, where R_7 is C_{1-12} alkyl, C_{7-20} arylalkyl, or C_{1-12} alkyl or C_{7-20} arylalkyl substituted, e.g., one to three times, with OH, CO_2H, or NH_2;

R_2 is H, C_{1-12} alkyl, or C_{7-20} arylalkyl;

each of R_3 and R_4, independently, is CO-NH, CH_2-NH, CH_2-S, CH_2-O, CO-CH_2, CH_2-CO, O-CH_2-CH_2;

R_5 is CO or CH_2; and

R_6 is OH, NH_2, C_{1-12} alkoxy, or NH-Y-CH_2-Z, where Y is a bond or C_{1-12} hydrocarbon, e.g., branched or straight chain, moiety and Z is H, OH, CO_2H, or $CONH_2$; provided that if R_6 is OH, R_3 is CO-NH, and R_4 is CO-NH, then R_5 is CH_2; or a pharmaceutically acceptable salt thereof.

Examples of compounds of the invention are the following:

CH_3CO-Ser-Ψ(CH_2NH)-Asp-Lys-Pro-OH (Analog 1);

CH_3CO-Ser-Asp-Ψ(CH_2NH)-Lys-Pro-OH (Analog 2);

CH_3CO-Ser-Asp-Lys-Ψ(CH_2N)Pro-OH (Analog 3);

CH_3CO-Ser-Ψ(CH_2NH)-Asp-Lys-Pro-NH_2;

CH_3CO-Ser-Asp-Ψ(CH_2NH)-Lys-Pro-NH_2;

CH_3CO-Ser-Asp-Lys-Ψ(CH_2N)Pro-NH_2;

H-Ser-Ψ(CH_2NH)-Asp-Lys-Pro-OH;

H-Ser-Asp-Ψ(CH_2NH)-Lys-Pro-OH;

H-Ser-Asp-Lys-Ψ(CH_2N)-Pro-OH;

$HOOCCH_2CH_2CO$-Ser-Ψ(CH_2NH)-Asp-Lys-Pro-OH;

$HOOCCH_2CH_2CO$-Ser-Asp-Ψ(CH_2NH)-Lys-Pro-OH;

$HOOCCH_2CH_2CO$-Ser-Asp-Lys-Ψ(CH_2N)Pro-OH;

H-Ser-Ψ(CH_2NH)-Asp-Lys-Pro-NH_2;

H-Ser-Asp-Ψ(CH_2NH)-Lys-Pro-NH_2;

H-Ser-Asp-Lys-Ψ(CH_2N)-Pro-NH_2;

$HOOCCH_2CH_2CO$-Ser-Ψ(CH_2NH)-Asp-Lys-Pro-NH_2;

$HOOCCH_2CH_2CO$-Ser-Asp-Ψ(CH_2NH)-Lys-Pro-NH_2;

$HOOCCH_2CH_2CO$-Ser-Asp-Lys-Ψ(CH_2N)Pro-NH_2;

CH_3CO-Ser-Asp-Lys-Pro-NH_2;

H-Ser-Asp-Lys-Pro-NH_2;

CH_3CO-Ser-Asp-Lys-Pro-$NHCH_3$;

H-Ser-Asp-Lys-Pro-$NHCH_3$;

$HOOCCH_2CH_2CO$-Ser-Asp-Lys-Pro-$NHCH_3$; and

$HOOCCH_2CH_2CO$-Ser-Asp-Lys-Pro-NH_2.

With the exception of the N-terminal amino acid and Pro, all abbreviations (e.g., Asp) of amino acids in this disclosure stand for the structure of -NH-CH(R)-CO-, wherein R is a side chain "identifying group" of an amino acid (e.g., CH_2OH for Ser, CH_2COOH for Asp, CH_2CH_2COOH for Glu, $CH_2CH_2CH_2NHC(NH_2)NH_2$ for Arg, $(CH_2)_3NH_2$ for Orn, and $(CH_2)_4NH_2$ for Lys). For the N-terminal amino acid, the abbreviation stands for the structure of =N-CH(R)-CO- or -NH-CH(R)-CO-,
wherein R is the identifying group of the amino acid. Pro is the abbreviation of prolyl. By non-peptide bond or pseudopeptide bond is meant that, where the α-amino group of proline is not involved, the peptide CO-NH bond between two amino acid residues is replaced with a non-peptide bond, e.g., CH_2-NH, CH_2-S, CH_2-O, CO-CH_2, CH_2-CO, or CH_2-CH_2 (symbolized by Ψ(CH_2-NH) or the like); or that, where the α-amino group of proline is involved, the carbonyl group of the peptide bond is replaced with CH_2 (symbolized by Ψ(CH_2-N)). C_{1-12} alkyl and C_{1-12} alkoxy may be straight chained or branched, e.g., methyl, ethyl, propyl, isopropyl, methoxy, ethoxy, propoxy, or isopropoxy. C_{7-20} arylalkyl may be straight chained or branched, e.g., benzyl, napthyl, or phenylethyl.

The compounds of the present invention can be used to inhibit the proliferation of hematopoietic cells. The compounds of the invention can be used to protect hematopoietic cells (e.g., stem cells) during treatment with cytotoxic agents (e.g., chemotherapy) or radiation (e.g., radiotherapy). The compounds of the invention may

be administered prior to the administration of the cytotoxic agent or radiation and continued through the duration of the cytotoxic treatment or radiation.

The compounds of this invention can be provided in the form of pharmaceutically acceptable salts. Acceptable salts include, but are not limited to, acid addition salts of inorganic acids such as hydrochloride, sulfate, phosphate, diphosphate, hydrobromide, and nitrate; or salts of organic acids such as acetate, maleate, fumarate, tartrate, succinate, citrate, lactate, methanesulfonate, p-toluenesulfonate, palmoate, salicylate, oxalate, and stearate. Also within the scope of the present invention, where applicable, are salts formed from bases such as sodium or potassium hydroxide. For further examples of pharmaceutically acceptable salts see, "Berge et al.", J. Pharm. Sci. 66:1 (1977).

A therapeutically effective amount (e.g., an amount effective to reduce the proliferation of hematopoietic cells) of a compound of this invention and a pharmaceutically acceptable carrier substance (e.g., magnesium carbonate, lactose, or a phospholipid with which the therapeutic compound can form a micelle) together form a therapeutic composition (e.g., a pill, tablet, capsule, or liquid) for administration (e.g., orally, intravenously, transdermally, pulmonarily, vaginally, subcutaneously, nasally, ionphoretically, or intratracheally) to a subject in need of the compound. The pill, tablet, or capsule can be coated with a substance capable of protecting the composition from the gastric acid or intestinal enzymes in the subject's stomach for a period of time sufficient to allow the composition to pass undigested into the subject's small intestine. The therapeutic composition can also be in the form of a biodegradable or sustained release formulation for subcutaneous or intramuscular

administration. See, e.g., U.S. Patents 3,773,919 and 4,767,628 and PCT Application No. WO 94/00148. Continuous administration can also be obtained using an implantable or external pump (e.g., INFUSAID™ pump) to administer the therapeutic composition.

The dose of a compound of the present invention for protecting hematopoietic cells varies depending upon the manner of administration, the age and the body weight of the subject, and the condition of the subject to be treated, and ultimately will be decided by the attending physician or veterinarian. Such an amount of the compound as determined by the attending physician or veterinarian is referred to herein as a "therapeutically effective amount." The compound of the present invention may also be administered with a cytotoxic agent or radiation. Examples of cytotoxic agents include, but are not limited to, daunorubicine, cyclophosphamide, taxol, 5-fluorouracil, dioxorubicine, cisplatin, methotrexate, cytosine, arabinoside, mitomycin C, prednisone, vindesine, carboplatinum, vincristine, or 3'-azido-3'deoxythymidine (AZT). The compound of the present invention may also be administered with an angiotensin converting enzyme (ACE) inhibitor. Examples of ACE inhibitors are listed in Jackson, et al., Renin and Angiotensin, in Goodman & Gillman's, The Pharmacological Basis of Therapeutics, 9th ed., eds. Hardiman, et al. (McGraw Hill, 1996).

Also contemplated within the scope of this invention is a compound covered by the above generic formula for use in protection of hematopoietic cells during cytotoxic treatment, e.g., chemotherapy, viral treatment, or radiation treatment.

Other features and advantages of the present invention will be apparent from the detailed description and from the claims.

Detailed Description of the Invention

It is believed that one skilled in the art can, based on the description herein, utilize the present invention to its fullest extent. The following specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Also, all publications, patent applications, patents, and other references mentioned herein are incorporated by reference.

Synthesis

The synthesis of short peptides are well examined in the art. The peptides of the invention were synthesized using the following general synthesis procedure.

All protected amino acids were purchased from Bachem (Bobendorf, Switzerland), Calbiochem (San Diego, CA), or Nova Biochem (La Jolla, CA). Mass spectra were obtained using a mass spectrometer (MS50) using a xenon fast atom bombardment (FAB) gun using glycerol, thioglycerol, or nitrobenzyl alcohol as a matrix. Thin-layer chromatography (TLC) was performed on silica gel precoated plates (60F 254, Merck, Darmstadt, Germany). The following solvent systems were used: A) dichloromethane/methanol, 95/5; B) dichloromethane/methanol 9/1; C) ethyl acetate/heptane, 1/1; D) n-butanol/acetic acid/water, 4/1/1; and E) n-butanol/acetic acid/water/pyridine, 1/1/1/1. UV light, ninhydrin, and/or Pataki reagent were used for detection. Protected peptides were purified by chromatography on Merck silica gel 60 (40-60μm) columns. All reagents and

solvents were of analytical grade and used as supplied except for tetrahydrofuran (THF) which was either distilled from sodium/benzophenone or filtered through a column of basic alumina immediately prior to use and dimethylformamide (DMF) which was distilled from ninhydrin under reduced pressure and stored over 4 angstrom molecular sieves. Protected peptides were characterized by their fast atom bombardment (FAB) or secondary ion mass spectra (SIMS). High pressure liquid chromatography (HPLC) purifications were performed on a reverse phase Beckmann Ultrasphere C-18 column (5μ particle size, 10 x 250 mm; Beckman, Fullerton, CA) using either a gradient or an isocratic elution with a mixture of acetonitrile and water containing 0.1% trifluoroacetic acid (TFA) at 3 ml/min flow rate. Elution was monitored by recording absorbance at 210 nm. Pure peptides were characterized by their FAB or SI mass spectrum. HPLC analysis for purity control was performed on a Novapak column C-18, 5μm (3.9 x 150 mm; Waters, Milford, MA) with a solvent system consisting of a binary system of water and acetonitrile containing 0.1% TFA at 1 ml/min flow rate with monitoring at 210 nm. The solvent program involved the following linear gradients: 1) 0% to 50% acetonitrile over 50 min, 2) 0% to 80% acetonitrile over 40 min. k values are reported in the two solvent systems.

The following is the description of the synthesis of N-Ac-Ser-Asp-$\psi(CH_2NH)$-Lys-Pro-OH (Analog 2). The abbreviations Ac, Z, Boc, t-But, and Bzl mean, respectively, acetyl, benzyloxycarbonyl, *tert*-butoxycarbonyl, tert-butyl, and benzyl.

(1) N-α-(Z)-N-ε-(Boc)-L-lysyl-L-proline-tert-butylester

To a stirred solution of Z-Lys(Boc)-OH (2.66 g,

7 mmol) in THF (35 ml), cooled to -15°C, was added N-methylmorpholine (0.77 ml, 7 mmol) followed by isobutylchloroformate (0.98 ml, 7 mmol). The solution was stirred at -15°C for 5 min and then cooled to -20°C. Proline tert-butyl ester (1.32 g, 7.7 mmol), dissolved in DMF, was added. The temperature was maintained at -10°C for 1 h, and the solution was then allowed to warm up to room temperature. After stirring for 5 h, the reaction mixture was concentrated under reduced pressure. The residue was then dissolved in ethyl acetate (200 ml) and 5% citric acid (50 ml). The aqueous layer was extracted with ethyl acetate (50 ml). The pooled organic layers were washed with water, 5% sodium bicarbonate, and brine, dried over Na_2SO_4, and concentrated under reduced pressure to afford the protected dipeptide as an oil (3.6 g; yield: 96%).

(2) N-ε-(Boc)-L-lysyl-L-proline-tert-butylester

The oil from step (1) (1.08 g; 2 mmol) was dissolved in ethanol (40 ml). 10% palladium on carbon catalyst (0.120 g) was added, and the suspension was stirred for 4 hours 30 min under an atmosphere of hydrogen. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure (0.672 g; yield: 84%).

(3) N-α-(Z)-β-(t-But)-L-aspartyl N,O-dimethyl hydroxamate

This compound was prepared and converted to the corresponding aldehyde as previously described by Martinez, et al., J. Med. Chem., 28:1878 (1985).

(4) N-α-(Z)-β-(O-t-But)-L-asparty-Ψ(CH_2NH)-N-ε-(Boc)-L-lysyl-L-proline-tert-butylester

The aldehyde obtained in step (3) (2 mmol) was added to a solution of the dipeptide from step (2) (1 mmol) in methanol (MeOH) containing 1 percent of acetic acid (7 ml). Sodium cyanoborohydride (0.094 g) was added in portions over 30 min. After 2 hours 30 min, the reaction mixture was cooled on a ice-water bath and under stirring, and a cool saturated sodium bicarbonate solution was added at 0°C followed by ethyl acetate. The aqueous phase was extracted with ethyl acetate. The pooled organic layers were washed with water, dried over Na_2SO_4, and then concentrated under reduced pressure. The crude product was chromatographed on silica gel using CH_2Cl_2/MeOH (99/1) and CH_2Cl_2/MeOH (98/2) as eluents to give the desired product (yield: 56%).

(5) β-(O-t-But)-L-aspartyl-Ψ(CH_2NH)-N-ε-(t-Boc)-L-lysyl-L-proline-tert-butylester

The compound obtained in step (4) (0.5 mmol) was dissolved in ethanol (13 mL). 10% Palladium on carbon catalyst (0.040 g) was added, and the suspension was stirred for 24 hours under an atmosphere of hydrogen. Additional catalyst in water (1 ml) was added. After 24 hours, the catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure (0.277 g; yield: 100%).

(6) N-α-(Z)-β-(O-t-But)-L-seryl-β-(O-t-But)-L-aspartyl-Ψ(CH_2NH)-N-ε-(Boc)-L-lysyl-L-proline-tert-butylester

To a stirred solution of Z-(O-t-But)-Ser-OH (0.132 g, 0.45 mmol) in THF (2.5 ml) cooled to -15°C, was added N-methylmorpholine (0.050 ml, 0.45 mmol) followed by isobutylchloroformate (0.063 ml, 0.45 mmol). The solution was stirred at -15°C for 5 min then cooled to -20°C. The tripeptide of step (5) dissolved in the minimum amount of dichloromethane was added. The

temperature was maintained at -10°C for 1 hour, then allowed to warm up to room temperature. After stirring for 5 hours, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate and 5% citric acid. The aqueous layer was extracted with ethyl acetate (AcOEt) (50 ml). The pooled organic layers were washed with water, 5% sodium bicarbonate, and brine, and then dried over Na_2SO_4 and concentrated under reduced pressure to afford a white foam (0.351 g). The crude product was chromatographed on silica gel using AcOEt/Hexane (1/1) as an eluant (0.233 g; yield: 70%).

(7) β-(O-t-But)-L-seryl-β-(O-t-But)-L-aspartyl-Ψ(CH_2NH)-N-ε-(Boc)-L-lysyl-L-proline-tert-butylester

The compound obtained in step (6) (0.2 mmol) was dissolved in 10% ethanol (4.4 ml). 10% Palladium on carbon catalyst (0.035 g) was added, and the suspension was stirred under an atmosphere of hydrogen overnight. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure (0.110 g).

(8) N-α-(acetyl)-β-(O-t-But)-L-seryl-β-(O-t-But)-L-aspartyl-Ψ(CH_2NH)-N-ε-(Boc)-L-lysyl-L-proline-tert-butyl ester

The amine of step (7) (0.110 g, 0.16 mmol) was dissolved in DMF (0.4 ml) and reacted with acetylimidazole (0.026 g, 0.24 mmol). After stirring for 3 hours, the reaction mixture was diluted with ethyl acetate. The organic phase was then washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product (0.120 g) was chromatographed on silica gel using AcOEt/MeOH(99/1) as an eluent (0.090 g; yield: 76%).

(9) N-α-(acetyl)-L-seryl-L-aspartyl-Ψ(CH_2NH)-L-lysyl-L-proline-OH

The compound obtained in step (8) (0.086 g, 0.12 mmol) was dissolved in TFA/CH_2Cl_2 (0.4 ml). The solution was stirred for 2 hours 30 min at room temperature. The reaction mixture was concentrated under reduced pressure. The residue was triturated with dry ether and dried under vacuum after removal of ether. Purification by HPLC on a C18 column using the following gradient solvent system: 0% at 3% acetonitrile over 10 min, 3% acetonitrile over 15 min with a flow rate of 3 ml/min (k'(1)= 8.17; k'(2)= 8) yielded the desired N-acetylated reduced tetrapeptide.

The following is the synthesis of CH_3CO-Ser-Asp-Lys-Pro-NH_2. (Analog 9)

(1) N-α-Benzyloxycarbonyl-N-ε-tert-Butoxycarbonyl-L-lysyl-L-proline-benzyl-ester

To a stirred solution of Z-Lys(Boc)-OH (1.54 g, 4 mmol) in THF (20 mL), cooled to -15ºC, was added 0.5 mL (4 mmol) N-Methylmorpholine followed by 0.44 mL (4 mmol) isobutylchloroformate. The solution was stirred at -15ºC for 5 min and then cooled to -20ºC. Benzylester proline hydrochloride (1.06 g; 4.4 mmol), in suspension in DMF (6 mL), was added followed by N-Methylmorpholine (0.48 mL, 4.4 mmol). The temperature was maintained below -10ºC for one hour and then allowed to warm up to room temperature. After 5 hours of stirring, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate (120 mL) and 5% citric acid (60 mL). The aqueous phase was extracted with ethyl acetate (60 mL). The combined organic layers were washed with water, 5% sodium bicarbonate, and brine, then dried over Na_2SO_4, and concentrated under reduced pressure to afford the product as a syrup. The crude

product was chromatographed on silica gel using CH_2Cl_2/MeOH (99/1) as an eluant to give 1. Yield: 1.76 g (77%), R_f(CH_2Cl_2/MeOH, 98/2) - 0.22; R_f (AcOEt/ Heptane, 1/1) = 0.24, MS (FAB)m/z = 590 (MNa+), 568 (MH+), 512 (MH+-But), 468 (MH+-Boc), 434 MH+-Z), 378 (MH+-Boc-Bzl), 334 (MH+-Boc-Z).

(2) N-ε-tert-Butoxycarbonyl-L-lysyl-L-proline

Product from step (1) (1 g; 1.75 mmol) was dissolved in 10% aqueous methanol (33 mL). 10% palladium on carbon catalyst (0.200 g) was added, and the suspension was stirred under an atmosphere of hydrogen overnight. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure. Yield: 0.565 g (94%); R_f (n-Butanol/Acetic acid/Water, 4/1/1) = 0.55. MS (FAB) m/z = 366 (MNa+), 344 (MH+), 288 (MH+-BUt), 244 (MH+-Boc).

(3) N-α-Benzyloxycarbonyl-β-O-tert-butyl-L-aspartyl-N-ε-tert-Butoxycarbonyl-L-lysyl-L-proline

To a stirred solution of Z-L-Asp(O-t-But)-OH (0.323 g, 1 mmol) in THF (5 mL), cooled to -15ºC, was added 0.11 mL (1 mmol) of N-Methylmorpholine, followed by 0.14 ml (1 mmol) isobutylchloroformate. The solution was stirred at -15ºC for 5 minutes and then cooled to -20ºC. The product of step (2) was added in solution in DMF (2.5 mL). After 5 hours stirring, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate (50 mL) and 5% citric acid (25 mL). The aqueous phase was extracted with ethyl acetate (25 mL). The combined organic layers were washed with water and brine, then dried over Na_2SO_4, and concentrated, under reduced pressure, to afford a white foam. The crude product was chromatographed on silica gel using CH_2Cl_2/MeOH/AcOH (97/3/0.5) as an eluant. Yield: 0.450 g (60%), R_f (CH_2Cl_2/MeOH/AcOH, 97/3/0.5) = 0.11 MS (FAB) m/z

= 693 (M2Na+-H), 671 (MNa+), 615 (MNa+-But), 549 (MH+-Boc), 537 (MNa+-Z), 515 (MNa+-But-Boc), 493 (MH+-But-Boc), 437 (MNa+-Boc-Z).

(4) N-α-Benzyloxycarbonyl-β-O-tert-butyl-L-aspartyl-N-ε-tert-Butoxycarbonyl-L-lysyl-L-proline amide

To a stirred solution of the product of step (3) (0.129 g, 0.2 mmol) in THF (5 mL), cooled to -15ºC, was added 0.022 mL (0.2 mmol) of N-Methylmorpholine followed by 0.028 mL (0.2 mmol) isobutylchloroformate. The solution was stirred at -15ºC for 5 minutes, and then cooled to -20ºC. 0.2 mL of a cold 34% ammonia solution was added.

After one hour stirring, at a temperature below -10ºC, a further hour at a temperature below 0ºC, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate and 5% citric acid. The aqueous phase was extracted with ethyl acetate. The combined organic layers were washed with water and brine, then dried over Na_2SO_4, and concentrated under reduced pressure to afford a white foam. The crude product was chromatographed on silica gel using CH_2Cl_2/MeOH (95/5) as an eluant. Yield: 0.105 g (81%). R_f (CH_2Cl_2/MeOH, 95/5) = 0.24; R_f (AcOEt/MeOH, 99/1) = 0.45. MS (FAB) m/z = 670 (MNa+), 648 (MH+), 614 (MNa+-But), 548 (MH+-Boc), 534 (MH+-ProNH_2), 514 (MH+-Z), 492 (MH+-But-Boc).

(5) β-O-tert-butyl-L-aspartyl-N-ε-tert-Butoxycarbonyl-L-lysyl-L-proline amide

The product of step (4) (0.152 g; 0.23 mmol) was dissolved in methanol (6 mL), 10% palladium in carbon catalyst (0.030 g) was added, and the suspension was stirred under an atmosphere of hydrogen for 2 hours. The catalyst was removed by filtration on a Celite pad, and

the filtrate was concentrated under reduced pressure. Yield: 0.111 g (94%); R_f (CH_2Cl_2/MeOH, 95/5) = 0.08; R_f (CH_2Cl_2/MeOH, 9/1) = 0.35. MS (FAB) m/z = 536 (MNa+), 514 (MH+), 480 (MNa+-But), 458 (MH+-But), 414 (MH+-Boc), 400(MH+-ProNH$_2$).

(6) N-α-Benzyloxycarbonyl-L-seryl-β-O-tert-butyl-L-aspartyl-N-ε-tert-Butoxycarbonyl-L-lysyl-L-proline amide

To a stirred solution of Z-L-Ser-OH (0.045 g, 0.19 mmol) in THF (1 mL), cooled to -15ºC, was added 0.021 mL (0.19 mmol) N-Methylmorpholine followed by 0.026 mL (0.19 mmol) isobutylchloroformate. The solution was stirred at -15ºC for 5 minutes and then cooled to -20ºC. The product of step (5) (0.105 g, 0.2 mmol) was added in solution in DMF (1 mL).

After 5 hours stirring, the reaction mixture was concentrated under reduced pressure. The reaction mixture was dissolved in ethyl acetate (50 mL) and 5% citric acid (25 mL). The aqueous phase was extracted with ethyl acetate (25 mL). The combined organic layers were washed with water and brine, then dried over Na_2SO_4, and concentrated, under reduced pressure. The crude product was purified on a silica gel column using CH_2Cl_2/MeOH (94/6) as an eluant. Yield: 0.140 g (80%). R_f (CH_2Cl_2/MeOH, 95/5) = 0.18; R_f (CH_2Cl_2/MeOH, 9/1) = 0.43. MS (FAB) m/z = 757 (MNa+), 735 (MH+), 701 (MNa+-But), 635 (MH+-Boc), 601 (MH+-Z), 579 (MH+-But-Boc), 501 (MH+-Boc-Z), 465 (MH+-Boc-But-ProNH$_2$).

(7) N-α-acetyl-L-seryl-β-O-tert-butyl-L-aspartyl-N-ε-tert-Butoxycarbonyl-L-lysyl-L-proline amide

The product of step (6) (0.080 g, 0.011 mmol) was dissolved in AcOEt (2 mL). 10% palladium carbon catalyst (0.016 g) and acetylimidazole (0.014 g, 0.013 mmol) was added, and the suspension was stirred under an atmosphere

of hydrogen overnight. The catalyst was removed by filtration on a Celite pad, and the filtrate was concentrated under reduced pressure. The crude product was purified on a gel column using CH_2Cl_2/MeOH (9/1) as an eluant. Yield: 0.050 g (71%); R_f (CH_2Cl_2/MeOH, 9/1) = 0.29. MS (FAB) m/z = 665 (MNa+), 643 (MH+), 609 (MNa+-But), 543 (MH+-Boc), 487 (MH+-But-Boc), 373 (MH+-Boc-But-ProNH_2).

(8) N-α-acetyl-L-seryl-L-aspartyl-L-lysyl-L-proline amide

The product of step (7) (0.039 g, 0.06 mmol) in solution in 200 µl trifluoroacetic acid, containing 20 µl of water, was stirred at room temperature for 95 minutes. The reaction mixture was concentrated under reduced pressure, and the residue was triturated twice with dry ether. After removal of ether, the solid white residue was taken up in 1.5 ml water and lyophilized. The crude peptide was purified by HPLC on C-18 column (Beckman Ultrasphere ODS (10 x 250 mm)) using an elution consisting of two solvents (A: H_2O/0.1% TFA, B: acetonitrile 0.1% TFA; 100% to over 20 minutes; tR = 13 minutes) with a flow rate of 3 ml.min-1. The collected fraction was lyophilized and analyzed by HPLC on a Waters Nova-Pak C-18 column (Waters, Milford, MA), 4 µ, 80 λ (3.9 x 150 mm) with two different elution programs using the same solvent system as above and a 1 ml.min-1 flow rate. k = 8.4, 100 to 50% A over 50 min.; k = 7.1, 100 to 20% A over 40 min.; MS (FAB)m/z = 509 (MNa+), 487 (MH+).

Other substitutions may similarly be added to the N-terminus of the peptide by similar methods known in the art. For example, N-α-(HOOCCH_2CH_2CO)-β-(O-t-But)-L-Ser-β-(O-t-But)-L-aspartyl-Ψ(CH_2NH)-N-ε-(Boc)-L-lysyl-L-proline-OH may be synthesized by mixing the amine of step

(7) above dissolved in the minimum amount of CH_2Cl_2 with a solution of succinic anhydride dissolved in THF. The reaction is stirred at room temperature and then the mixture is evaporated under reduced pressure. The residue is dissolved in AcOEt and washed with 5% citric acid, water, brine, and then dried over Na_2SO_4. The resulting compound may then be deprotected to yield the desired product.

Other peptides of the invention can be prepared in an analogous manner by a person of ordinary skill in the art.

Biological activity of AcSDKP analogues

The activity of the compounds of the invention was evaluated by their ability to inhibit the *in vitro* entry into S-phase of murine primitive hematopoietic cells: "HPP-CFC". In order to trigger the quiescent stem cells into cycle, normal murine bone marrow cells (5×10^6 cells/ml in Dulbecco's medium) were incubated with the same volume of either stimulatory medium (conditioned medium of bone marrow cells obtained from sublethally irradiated mice, 4.5 GY whole body X-1 irradiated upon dose), or with Dulbecco's medium as control. Test compounds were added at the beginning of the incubation at a final concentration of 2×10^{-9} M. Incubations were performed in pair tubes at 37°C for 3 h. One hour before the end of the incubation, cells in S-phase were killed by adding cytosine arabinoside (Ara-C) at a final concentration of 25 µg/ml in the first set of tubes. Dulbecco's medium is added in the other tubes as control. Incubation with Ara-C leads to the death of cells which have been triggered into S-phase. Therefore, cells which have been prevented to cycle by the action of analogues will be insensitive to the phase-specific toxicity of

Ara-C. Cells were washed twice prior to subsequent HPP-CFC assay.

HPP-CFC were studied using a bilayer semi-solid agar assay as described by Robinson, et al., Cell Prolif. 25:623-632, 1992. Two milliliters of Dulbecco's medium containing 20% horse serum, 10% conditioned medium from the WEHI 3B myelomonocytic leukaemic cell line (as a source of IL-3/multi-CSF), 10% conditioned medium from L929 fibroblast cell line (as a source of M-CSF/CSF1), 0.5% melted agar, 2 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin were aliquoted into 55 mm diameter non-tissue culture grade plastic petri-dishes as the underlayer. Two milliliters of Dulbeccos's medium supplemented with 20% horse serum, 0.3% melted agar, 2 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin containing 4 x 10^4 bone marrow cells were then aliquoted over prepared underlayers. Quadruplicate cultures were incubated for 14 days at 37°C in a fully humidified atmosphere with 5% CO_2. Twelve hours before the end of the culture, 1 ml of a colorless 1 mg/ml 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) solution in saline was added, allowing the staining of viable cells by INT processing into a red derivative which precipitates inside cells. HPP-CFC derived macroscopic colonies were defined as those above 2 mm in diameter and scored. Table I lists the percent decrease of HPP-CFC derived macroscopic colonies entering the S-phase induced by the test compounds.

TABLE I

TEST COMPOUND	PERCENT DECREASE OF HPP-CFC IN S-PHASE
Analog 1	61.2
Analog 2	67.6

Analog 3	70.8

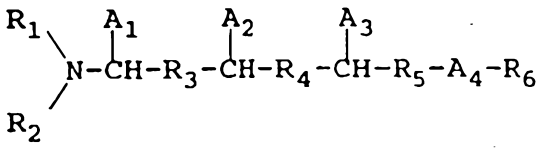
Other Embodiments

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, that the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the claims.

What is claimed is:

Claims

1. A compound of the formula:



wherein

A_1 is the identifying group of the D- or L-isomer of Ser;

A_2 is the identifying group of the D- or L-isomer of Asp or Glu;

A_3 is the identifying group of the D- or L-isomer of Lys, Arg, or Orn;

A_4 is the D- or L- isomer of Pro;

R_1 is H, C_{1-12} alkyl, C_{7-20} arylalkyl, R_7CO, or $R_7OC(O)$, where R_7 is C_{1-12} alkyl, C_{7-20} arylalkyl, or C_{1-12} alkyl or C_{7-20} arylalkyl substituted with OH, CO_2H, or NH_2;

R_2 is H, C_{1-12} alkyl, or C_{7-20} arylalkyl;

each of R_3 and R_4, independently, is CO-NH, CH_2-NH, CH_2-S, CH_2-O, CO-CH_2, CH_2-CO, or CH_2-CH_2;

R_5 is CO or CH_2; and

R_6 is OH, NH_2, C_{1-12} alkoxy, or NH-Y-CH_2-Z, where Y is a bond or C_{1-12} hydrocarbon moiety and Z is H, OH, CO_2H, or $CONH_2$; provided that if R_6 is OH, R_3 is CO-NH, and R_4 is CO-NH, then R_5 is CH_2; or a pharmaceutically acceptable salt thereof.

2. A compound of claim 1, wherein A_2 is the identifying group of the D- or L-isomer of Asp and A_3 is the identifying group of the D- or L-isomer of Lys.

3. A compound of claim 2, wherein
A_1 is the identifying group of L-Ser;
A_2 is the identifying group of L-Asp;
A_3 is the identifying group of L-Lys; and
A_4 is the identifying group of L-Pro.

4. A compound of claim 3, wherein each of R_3 and R_4, independently, is CO-NH or CH_2-NH.

5. A compound of claim 4, wherein R_1 is H or R_7CO (where R_7 is C_{1-12} alkyl or C_{1-12} alkyl substituted with OH) and R_2 is H.

6. A compound of claim 5, further provided that if R_3 is CO-NH and R_4 is CO-NH, then R_5 is CH_2.

7. A compound of claim 6, wherein R_1 is H, CH_3CO, or $HOOCCH_2CH_2CO$ and R_6 is OH or NH_2.

8. A compound of claim 1 of the formula:
CH_3CO-Ser-$\Psi(CH_2NH)$-Asp-Lys-Pro-OH; or
a pharmaceutically acceptable salt thereof.

9. A compound of claim 1 of the formula:
CH_3CO-Ser-Asp-$\Psi(CH_2NH)$-Lys-Pro-OH; or
a pharmaceutically acceptable salt thereof.

10. A compound of claim 1 of the formula:
CH_3CO-Ser-Asp-Lys-$\Psi(CH_2N)$Pro-OH;or
a pharmaceutically acceptable salt thereof.

11. A compound of claim 1 of the formula:

CH_3CO-Ser-Ψ(CH_2NH)-Asp-Lys-Pro-NH_2;

CH_3CO-Ser-Asp-Ψ(CH_2NH)-Lys-Pro-NH_2;

CH_3CO-Ser-Asp-Lys-Ψ(CH_2N)Pro-NH_2;

H-Ser-Ψ(CH_2NH)-Asp-Lys-Pro-OH;

H-Ser-Asp-Ψ(CH_2NH)-Lys-Pro-OH;

H-Ser-Asp-Lys-Ψ(CH_2N)-Pro-OH;

$HOOCCH_2CH_2CO$-Ser-Ψ(CH_2NH)-Asp-Lys-Pro-OH;

$HOOCCH_2CH_2CO$-Ser-Asp-Ψ(CH_2NH)-Lys-Pro-OH;

$HOOCCH_2CH_2CO$-Ser-Asp-Lys-Ψ(CH_2N)Pro-OH;

H-Ser-Ψ(CH_2NH)-Asp-Lys-Pro-NH_2;

H-Ser-Asp-Ψ(CH_2NH)-Lys-Pro-NH_2;

H-Ser-Asp-Lys-Ψ(CH_2N)-Pro-NH_2;

$HOOCCH_2CH_2CO$-Ser-Ψ(CH_2NH)-Asp-Lys-Pro-NH_2;

$HOOCCH_2CH_2CO$-Ser-Asp-Ψ(CH_2NH)-Lys-Pro-NH_2; and

$HOOCCH_2CH_2CO$-Ser-Asp-Lys-Ψ(CH_2N)Pro-NH_2; or

a pharmaceutically acceptable salt thereof.

12. A compound of claim 5, wherein R_6 is NH_2 or NH-Y-CH_2-Z (where Y is a C_{1-12} hydrocarbon moiety and Z is H).

13. A comppound of claim 12, wherein R_3 and R_4 are CO-NH and R_5 is CO.

14. A compound of caim 13, wherein R_1 is H, CH_3CO, or $HOOCCH_2CH_2CO$.

15. A compound of claim 14 of the formula:

CH_3CO-Ser-Asp-Lys-Pro-NH_2; or

a pharmaceutically acceptable salt thereof.

16. A compound of claim 14, of the formula:

H-Ser-Asp-Lys-Pro-NH_2;

CH_3CO-Ser-Asp-Lys-Pro-$NHCH_3$;

H-Ser-Asp-Lys-Pro-$NHCH_3$;

$HOOCCH_2CH_2CO$-Ser-Asp-Lys-Pro-$NHCH_3$; and

$HOOCCH_2CH_2CO$-Ser-Asp-Lys-Pro-NH_2; or

a pharmaceutically acceptable salt thereof.

17. A method of protecting hematopoietic cells in a subject undergoing chemotherapy or radiotherapy, said method comprising administering to said subject a therapeutically effective amount of a compound of claim 1, said amount being effective to reduce the proliferation of hematopoietic cells during said chemotherapy or radiotherapy.

18. A method of claim 17, wherein said compound is of the formula:

CH_3CO-Ser-Ψ(CH_2NH)-Asp-Lys-Pro-OH;

CH_3CO-Ser-Asp-Ψ(CH_2NH)-Lys-Pro-OH;

CH_3CO-Ser-Asp-Lys-Ψ(CH_2NH)-Pro-OH;

CH_3CO-Ser-Asp-Lys-Pro-NH_2; or

a pharmaceutically acceptable salt thereof.

19. A method of inhibiting the proliferation of hematopoietic cells in a patient, said method comprising administering to said patient a compound of claim 1.

20. A method of claim 19, wherein said compound is of the formula:

CH_3CO-Ser-Ψ(CH_2NH)-Asp-Lys-Pro-OH; or

a pharmaceutically acceptable salt thereof.