



US 20160243062A1

(19) **United States**

(12) **Patent Application Publication**  
**Fahl**

(10) **Pub. No.: US 2016/0243062 A1**

(43) **Pub. Date: Aug. 25, 2016**

(54) **SYNTHESIS AND GROWTH REGULATORY  
ACTIVITY OF A PROTOTYPE MEMBER OF A  
NEW FAMILY OF AMINOTHIOLE  
RADIOPROTECTORS**

(60) Provisional application No. 61/713,050, filed on Oct.  
12, 2012.

**Publication Classification**

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(51) **Int. Cl.**  
**A61K 31/145** (2006.01)

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(52) **U.S. Cl.**  
CPC ..... **A61K 31/145** (2013.01)

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(57) **ABSTRACT**

(21) Appl. No.: **15/146,020**

(22) Filed: **May 4, 2016**

**Related U.S. Application Data**

(62) Division of application No. 13/957,176, filed on Aug.  
1, 2013.

The synthesis, growth inhibition and radioprotective activity of the PrC-210 aminothiol, 3-(methyl-amino)-2-((methylamino)methyl)propane-1-thiol, and its polyamine and thiolated polyamine progenitors are reported. All of the molecules significantly inhibited growth of cultured normal human fibroblasts. The combination of an ROS-scavenging thiol group and a positively charged alkyl-amine backbone provided the most radioprotective aminothiol molecule.

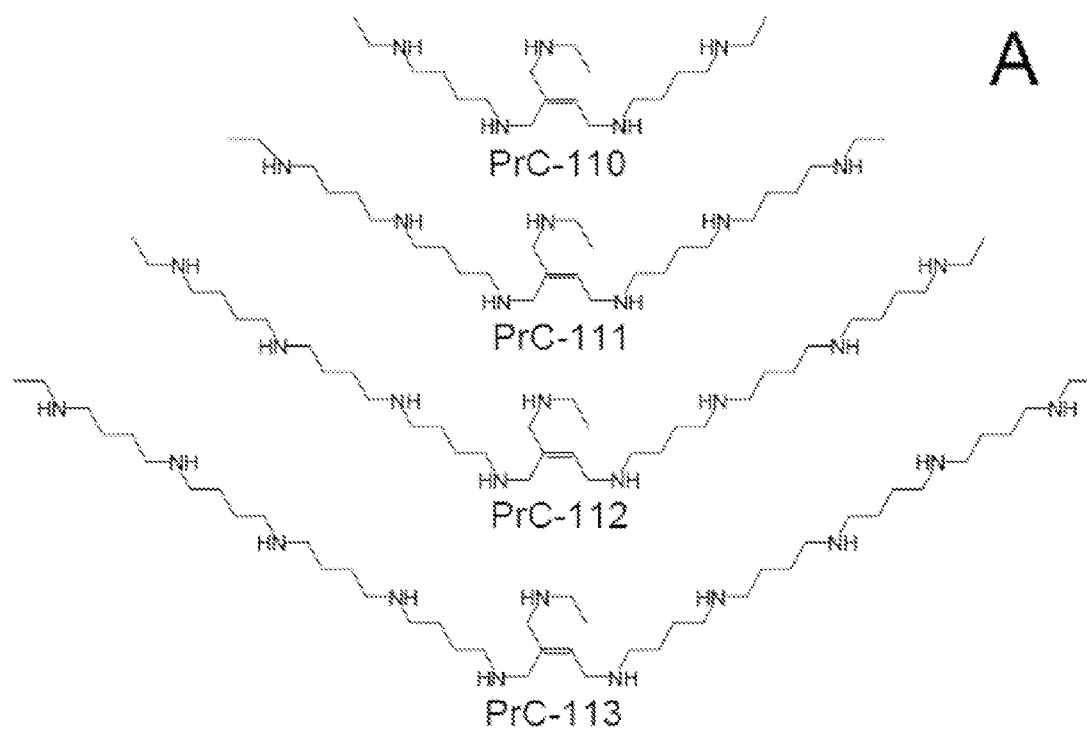


Figure 1

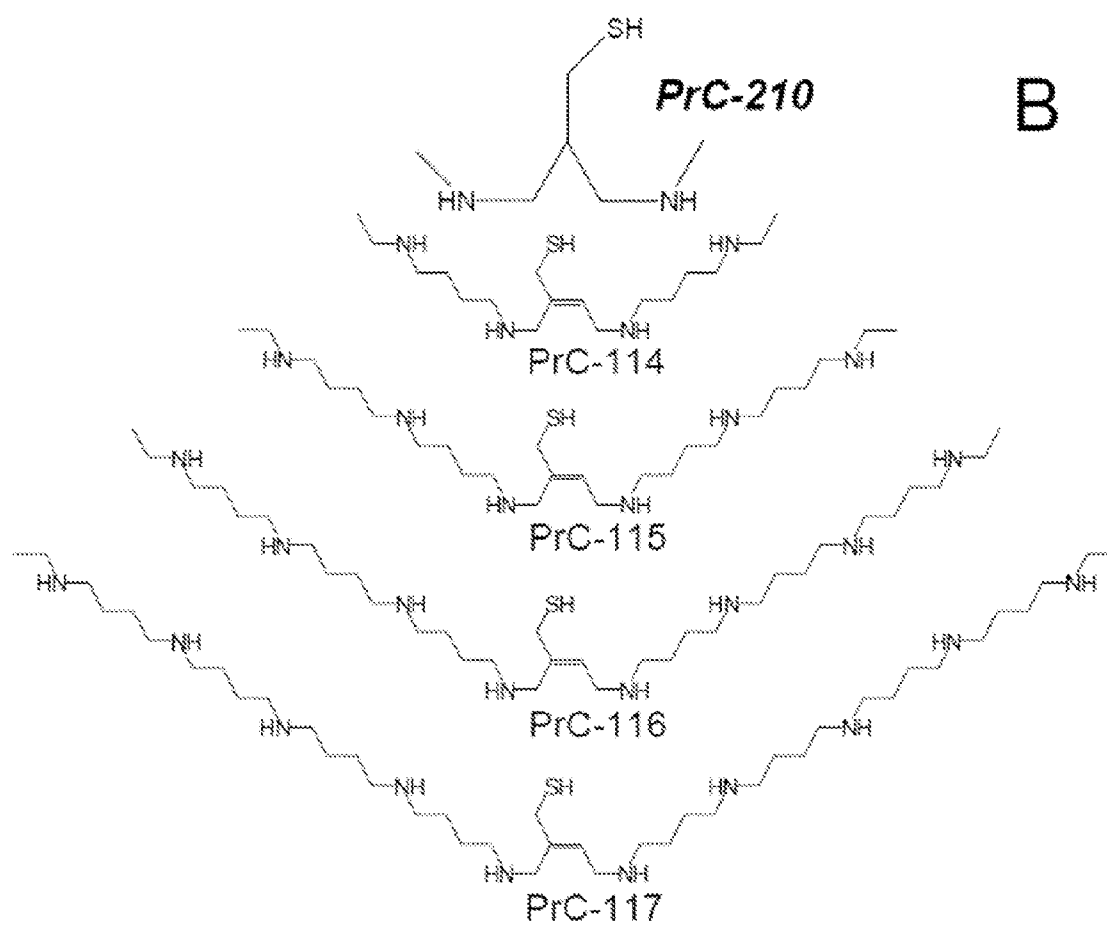


Figure 1 Continued

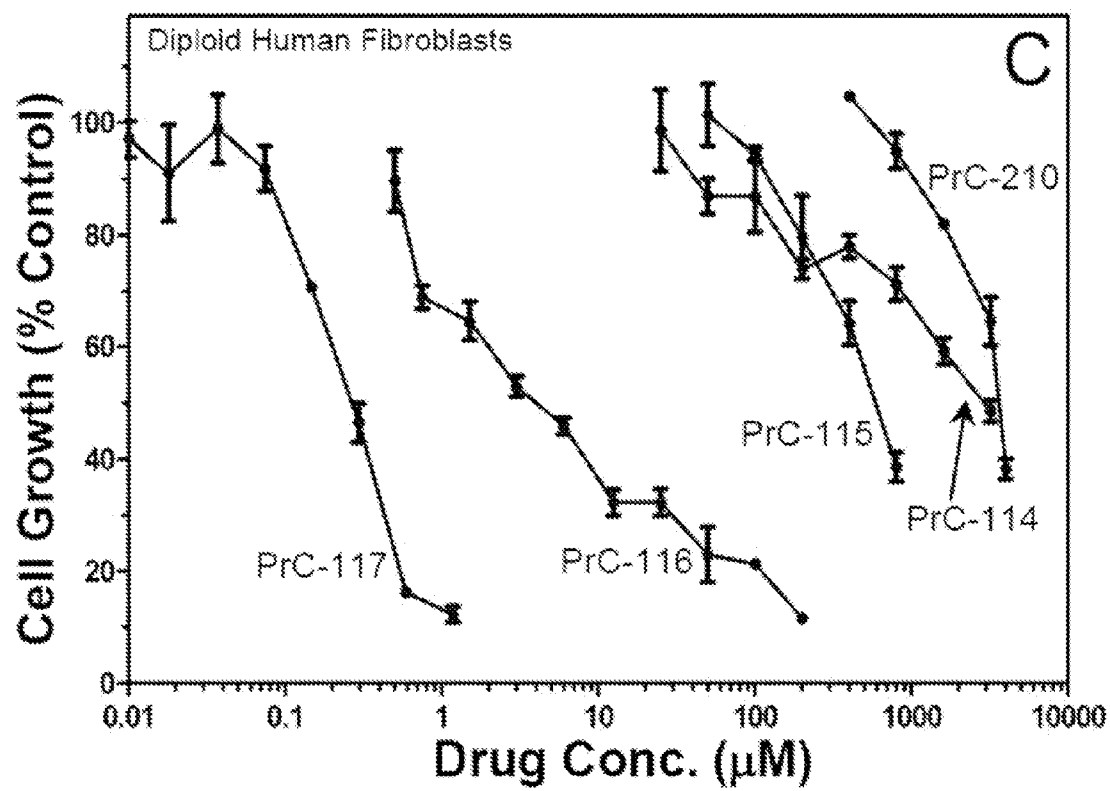


Figure 1 Continued

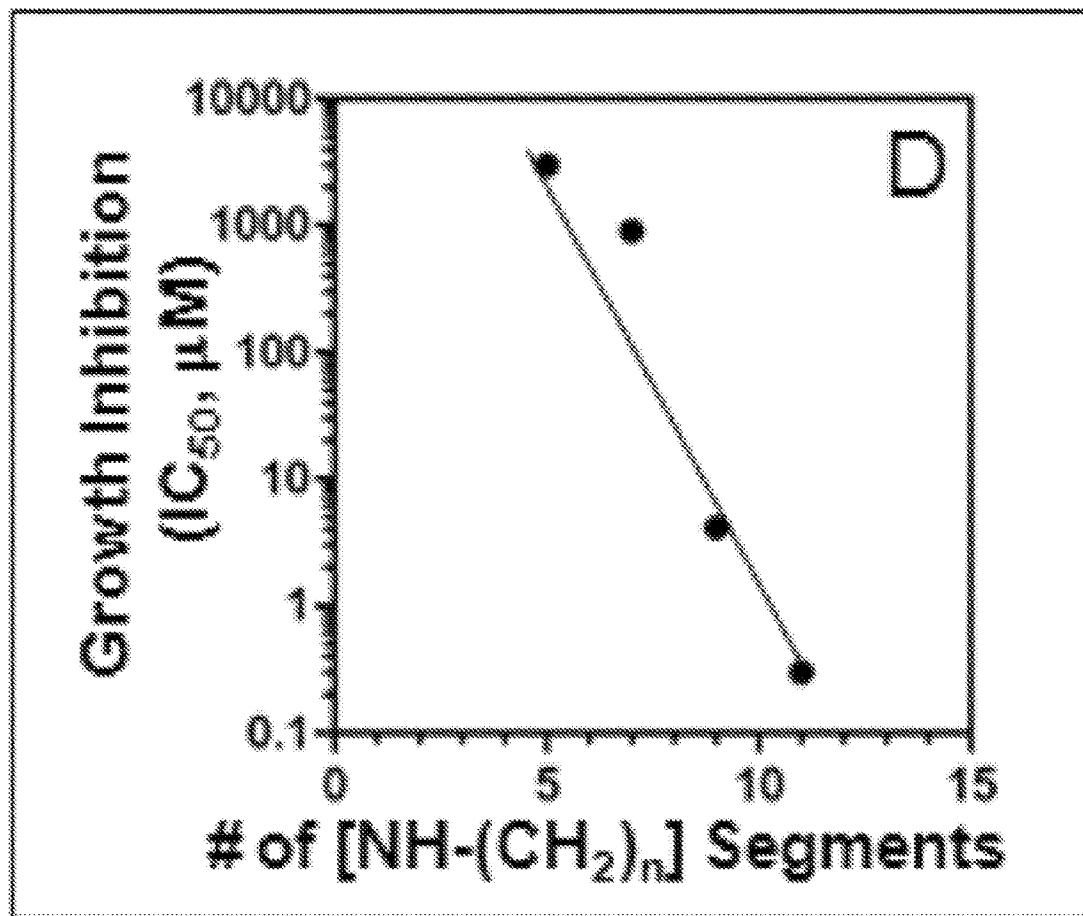


Figure 1 Continued

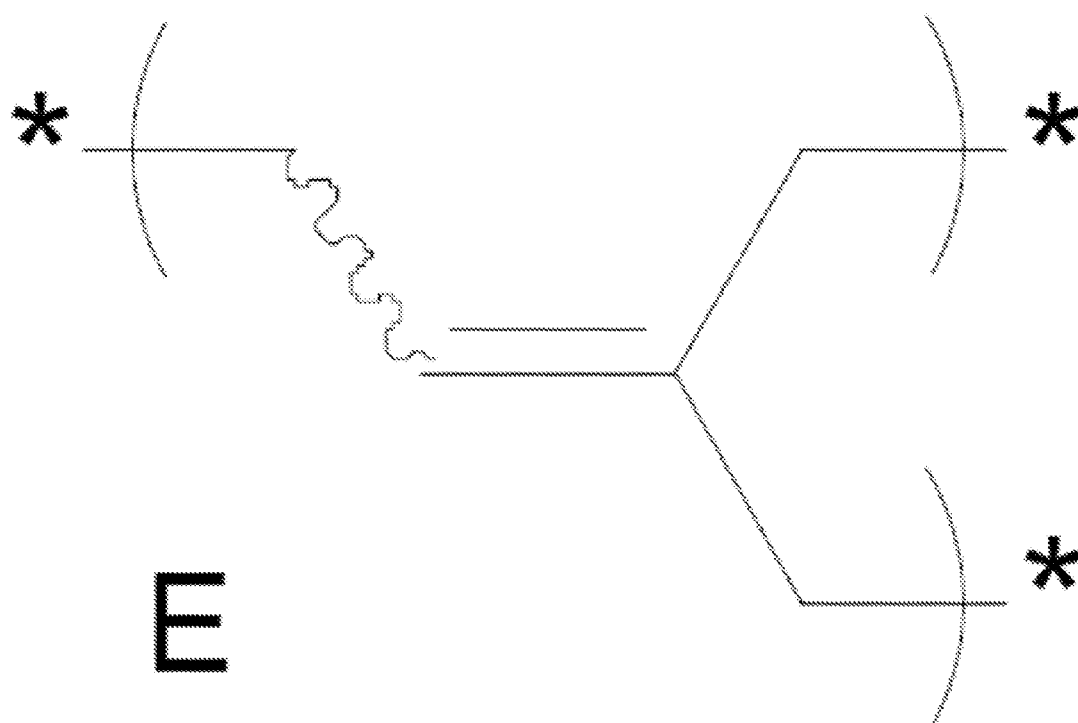


Figure 1 Continued

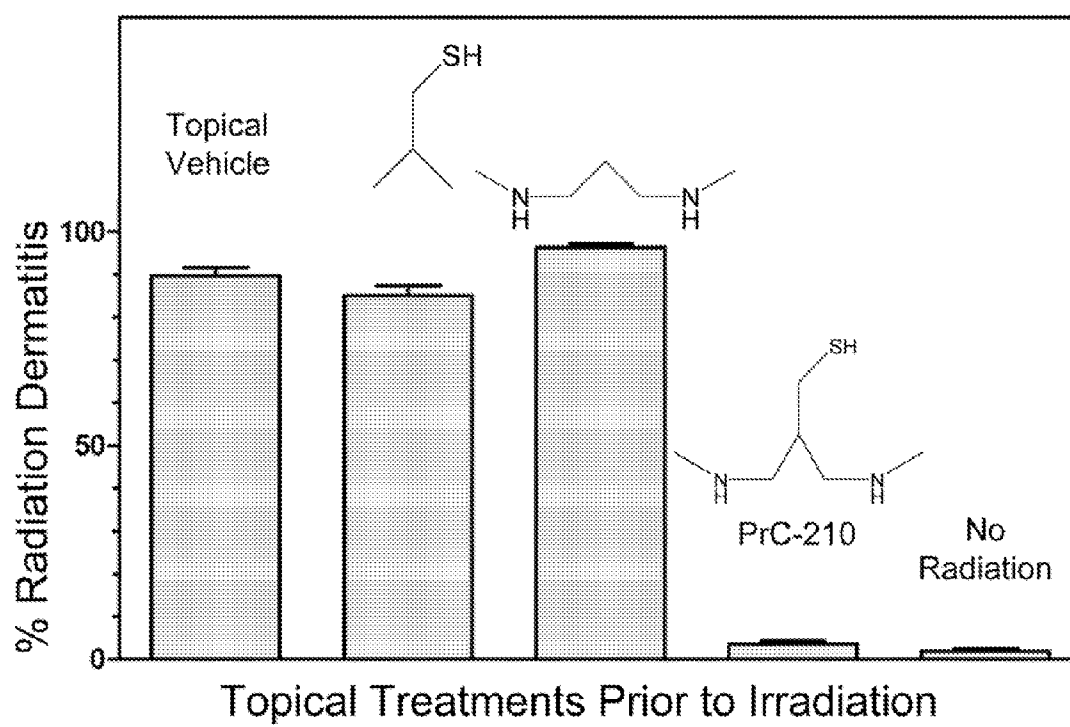


Figure 2

**SYNTHESIS AND GROWTH REGULATORY  
ACTIVITY OF A PROTOTYPE MEMBER OF A  
NEW FAMILY OF AMINOTHIOL  
RADIOPROTECTORS**

**[0001]** This application claims priority to U.S. Pat. Appl. Ser. No. 61/713,050, which is incorporated herein by reference in its entirety.

**FIELD OF INVENTION**

**[0002]** The synthesis, growth inhibition and radioprotective activity of the PrC-210 aminothiols, 3-(methyl-amino)-2-((methylamino)methyl)propane-1-thiol, and its polyamine and thiolated polyamine progenitors are reported. All of the molecules significantly inhibited growth of cultured normal human fibroblasts. The combination of an ROS-scavenging thiol group and a positively charged alkyl-amine backbone provided the most radioprotective aminethiol molecule.

**BACKGROUND OF THE INVENTION**

**[0003]** Radiotherapy-induced dermatitis is a common side effect seen in up to 85% of patients who receive a course of radiotherapy as part of their cancer therapy regimen. A topically administered radioprotector that could be applied prior to radiotherapy on each of the 30 irradiation days would reduce pain and long term scarring and would improve patient compliance in receiving all days of treatment. Skipped radiotherapy days have a discernible risk for the patient as measured by a decrease in 5 year survival rate for breast cancer.

**OBJECTS AND SUMMARY OF THE  
INVENTION**

**[0004]** There is also a need for new systemically administered radioprotectors that lack the side effects of nausea/vomiting and hypotension/fainting that have hampered the use of current generation aminethiol radioprotectors, most notably the five carbon aminothiophosphonate, amifostine. The aminethiol radioprotector design concepts of: (i) a flexible alkyl chain backbone, which carries a positive charge due to one or more amine groups, to achieve ionic interaction and concentration around negatively charged DNA in cells, and (ii) the presence of a free or capped thiol group to scavenge oxygen free radicals formed from ionizing radiation, have been used before in programs to build radioprotective molecules within both the U.S. and the former Soviet Union.

**[0005]** In the present investigation, we disclose a process in which: (i) the number of alkylamine segments in the aminethiol backbone is systematically increased to increase drug-DNA affinity and ionic interaction, resulting in increased growth inhibition that is associated with this enhanced drug-DNA interaction, and (ii) the placement or 'display' of a free thiol reactive oxygen species (ROS) scavenger at the end of a short alkyl side chain that displaces or 'displays' the scavenger moiety away from the DNA backbone to theoretically enable ROS scavenging before ROS attack on dG bases within cellular DNA. This work has resulted in a small family of new aminethiol molecules, the prototype of which, PrC-210, is described in initial detail here.

**[0006]** The synthesis of PrC-210, shown in Scheme 1, started with a double displacement of chloride from 1 using N-methyl mesitylene-sulfonamide (2) and sodium hydride, to form allylic sulfonamide 3. Hydroboration of 3 afforded

clean conversion to sulfonamide alcohol 4. Using standard conditions, 4 was converted to mesylate 5 which was immediately treated with potassium thioacetate to form 6. Following an established procedure, the mesitylene (Mts) protecting groups were removed with HBr/HOAc, in the presence of excess phenol. The deblocking procedure also hydrolyzed the thioacetate group. Work up resulted in a mixture of PrC-210 and the corresponding disulfide (dimer). The mixture was treated with 2-mercaptoethanol to cleave the disulfide and the product, PrC-210, was precipitated from EtOH as the HCL salt. Subsequent recrystallizations removed the sulfurous odor.

**[0007]** The synthesis of PrC-211, shown in Scheme 2, employed a modification of the route used for PrC-210. An attempt was made to form sulfonamide 10 directly by displacement of chloride from 1 using mesitylenesulfonamide, activated with sodium hydride. A complex mixture formed, from which 10 could not be isolated in pure form. Alternatively, 1 was treated with potassium phthalimide to form allylic phthalimide 8. Removal of the phthalate groups with hydrazine provided 9, which upon treatment with mesitylenesulfonyl chloride afforded the bis-sulfonamide 10 in good yield. Using the sequence from the PrC-210 preparation, hydroboration, mesylation, thioacetate displacement and deblocking, PrC-211 was obtained as the HCl salt and subsequently recrystallized.

**[0008]** The amine side chains, synthesized according to the route illustrated in Scheme 3, were constructed as sulfonamide-protected intermediates, each with a single point of attachment (N—H), at one terminus, for coupling to the olefinic core (FIG. 1E). Preparations for sulfonamides 17 and 20 have previously been described. A convenient alternative approach was found that employed a modification of a reported method, starting with N-(4-bromobutyl)phthalimide (14). Displacement with N-ethyl mesitylenesulfonamide and sodium hydride, afforded 15, which upon treatment with hydrazine, was converted to 16. Amidation with mesitylenesulfonyl chloride, under standard conditions, gave 17. Sulfonamide 17, a protected diamine, represents the smallest amine side chain unit in the series, which includes 20, 21, and 22. Sulfonamide 17 was systematically elaborated to the subsequent side chain units using the same methodology as described for 17.

**[0009]** Coupling of the various amine side chains to the olefinic core is shown in Scheme 4. The synthesis commenced with the known TBS-protected allylic alcohol 23. Mesylation, using standard conditions, provided the activated intermediate 24. Coupling with a sulfonamide side chain and sodium hydride provided 25. Removal of the TBS-protecting groups with HCl afforded diol 26. Treatment of 26 with 1 equiv of benzoyl chloride, in the presence of pyridine, afforded alcohol 27 as a mixture of isomers. Activation of the allylic alcohol 27, for amine side chain coupling, was attempted with methanesulfonyl chloride, resulting in a complex mixture. Alternatively, the alcohol was converted to bromide 28, with phosphorous tribromide, and immediately coupled with a second side chain unit to form 29. Hydrolysis of the benzoate group was carried out under standard conditions to afford the versatile polyamine alcohol intermediate 30, which was a common intermediate for the formation of both polyamines and polyamine thiols.

**[0010]** Conversion of the versatile intermediate 30 to polyamines and polyamine thiols is shown in Scheme 5. Treatment of 30 with methanesulfonyl chloride and triethy-



amine afforded the mesylate 31. Displacement with N-ethyl mesitylenesulfonamide, activated with sodium hydride, provided 32 which was deblocked with HBr/HOAc and phenol, to afford crude polyamine 33. Free-basing with potassium carbonate followed by HCl treatment gave 33 hydrochloride salt as a mixture of cis/trans isomers. Mesylate intermediate 31 was treated with potassium thioacetate to form 34 which was de-blocked, using the same method as for 32, to form 35. Free-basing and treatment with HCl provided 35 as the hydrochloride salt.

[0011] FIG. 1A provides a summary of the first set of polyamine structures that was synthesized using the strategies outlined in Schemes 3-5. We anticipated that these drug molecules would provide radio- and chemo-protection to human cells by inducing a cell cycle block in mammalian cells at the G1/S cell cycle border because of tight binding of the (+) charged polyamine backbones to the (-) charged DNA backbone. Such a block can provide time for DNA repair in radiation- or mutagen-treated cells before washout of the polyamine drug and restoration of cell cycle progression.

[0012] FIG. 1B shows a nearly identical set of polyamines now with the addition of a thiol group to each molecule. The goal in synthesizing these molecules was to achieve the same cell cycle inhibition anticipated for the FIG. 1A molecules, but now combined with the addition of a thiol group that could serve as a scavenger for the burst of short-lived ROS that is generated when mammalian tissue is irradiated. The aminothiols and polyamine structures in FIG. 1B are growth inhibitory when added to rapidly growing, normal human fibroblasts, and the potency of growth inhibition was directly related to the number of  $(\text{NH}-(\text{CH}_2)_n)$  segments present within the molecule (FIG. 1D).

[0013] Early in vitro studies of radioprotection with cultured cells demonstrated that the long, polyamine structures were so growth-inhibitory (FIG. 1C) that we could not add sufficient moles of the thiolated polyamines (e.g., PrC-117) to cell cultures that would enable the thiol groups to significantly scavenge and radioprotect when the tissue culture cells were irradiated. This provided the impetus to design and synthesize the PrC-200 series of small aminothiols, of which PrC-210 is the prototype.

[0014] To determine if PrC-210 could function as a topical radioprotector that could prevent radiation dermatitis when applied prior to a cancer patient's daily radiotherapy, a rat assay that realistically mimics human radiation dermatitis was created. This assay quantifies the severity of radiation dermatitis in rat skin 13 days after a single radiation dose of 17.33 Gy.

[0015] In this study, PrC-210 was applied to rat skin in an ethanol/water delivery vehicle four times in the 2 h before irradiation, and control rats received only topical vehicle before irradiation (FIG. 2). Additional drug/irradiation groups of three rats each received either the alkyl-thiol moiety or the alkyldiamine moiety from PrC-210 in the same delivery vehicle. Following the 17.33 Gy irradiation of a 1.5-3.0 cm patch of dorsal skin on the rats' backs, rats were scored 13 days later. The ability of the PrC-210 aminothiols to completely prevent radiation dermatitis was striking. Other radiation dermatitis test groups exploring efficacy of thiolated polyamines or other PrC-200 series aminothiols have shown that PrC-210 is the most potent and most effective topical aminothiols to date.

[0016] Additional embodiments will be apparent to persons skilled in the relevant art based on the teachings contained herein.

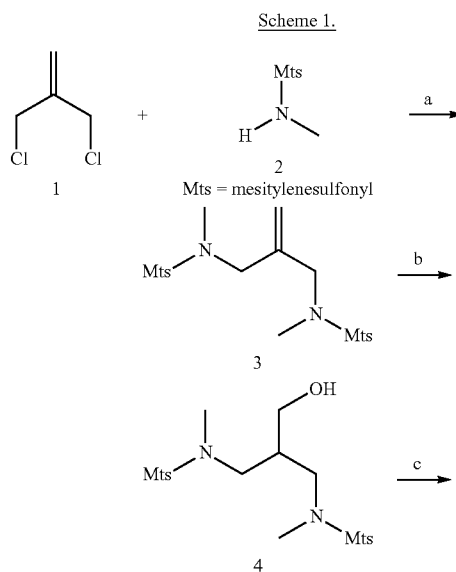
## BRIEF DESCRIPTION OF THE DRAWINGS

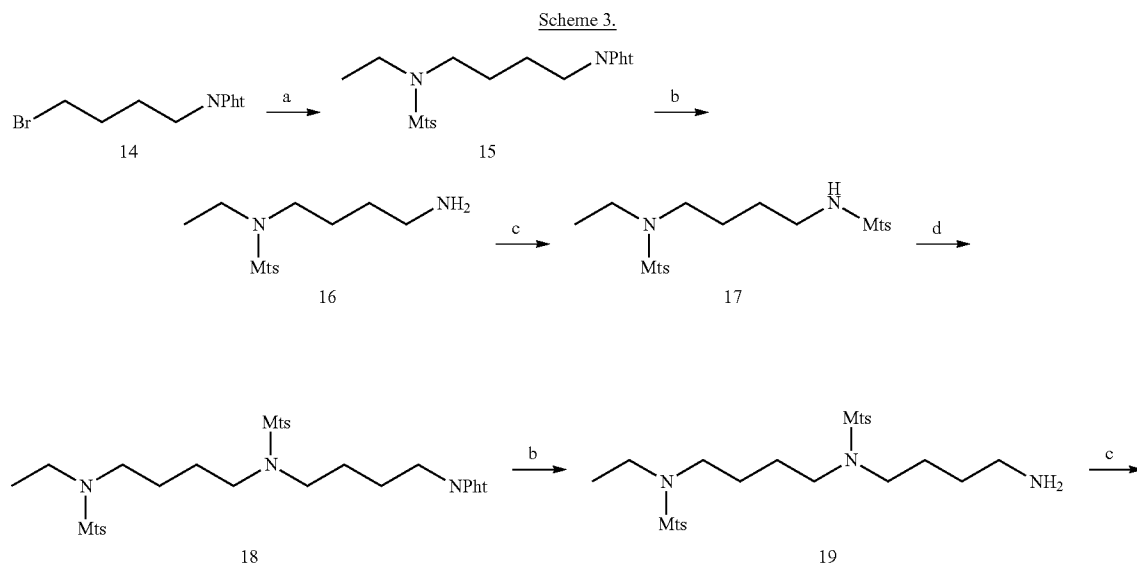
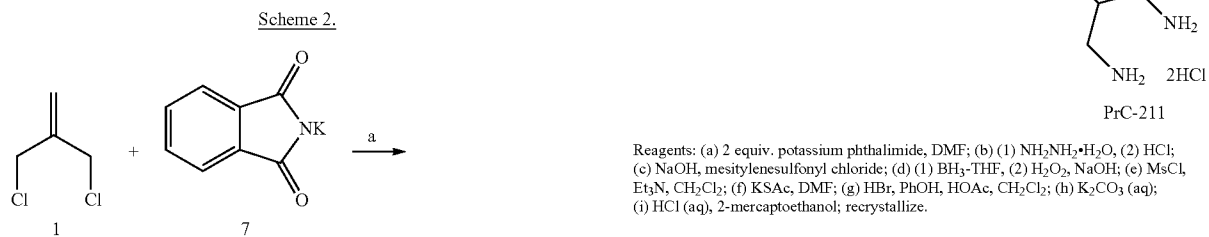
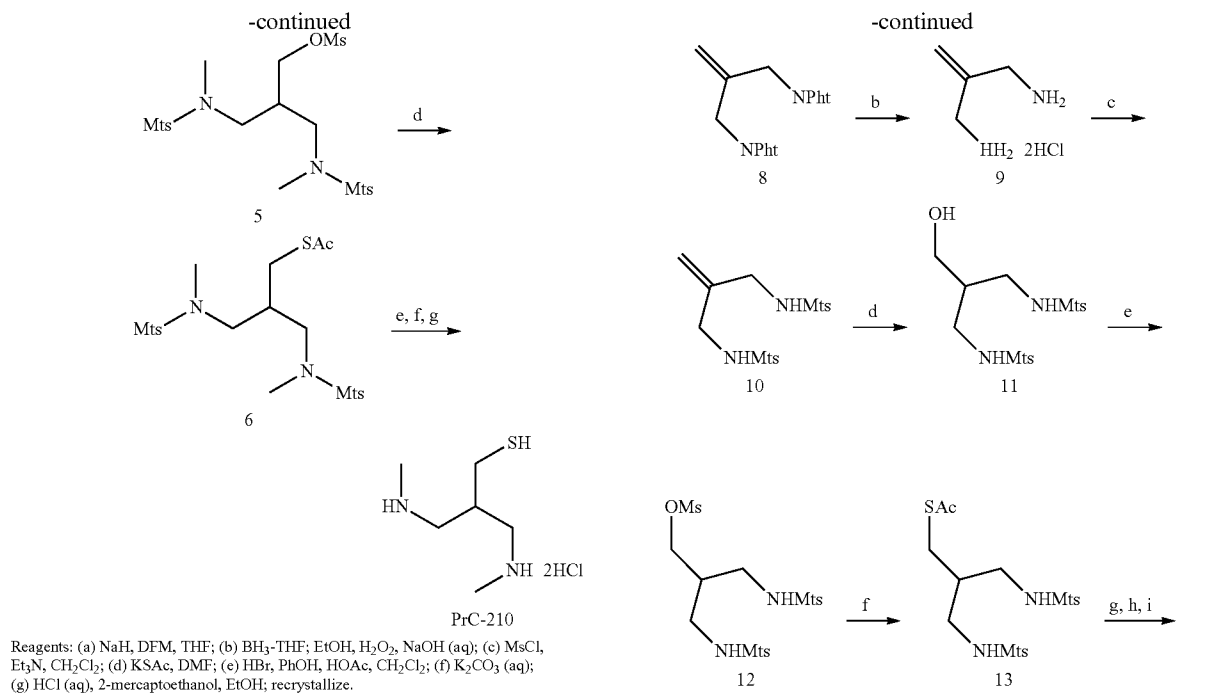
[0017] These and other features, aspects, and advantages of the present technology will become better understood with regard to the following drawings:

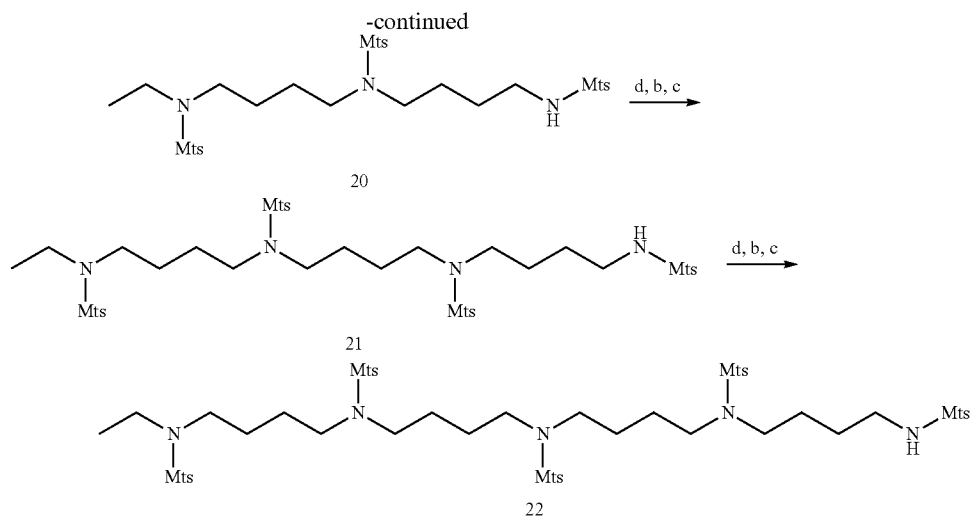
[0018] FIG. 1 shows growth inhibition of normal human cells in culture by polyamine and aminothiols molecules. Structures of (A) first generation synthetic polyamines. (B) First generation 'thiolated polyamines' and the next generation aminothiols, PrC-210. (C) Molecules were added at indicated concentrations to log phase, growing cultures of diploid human fibroblasts, and cell numbers were measured 4 days later. (D) Growth inhibition is directly related to the number of  $[\text{NH}-(\text{CH}_2)]$  segments present in the polyamine or aminothiols molecule (E) Olefinic core—common to polyamine and polyamine-thiol series.

[0019] FIG. 2 shows the prevention of radiation-induced dermatitis in rat model by prior topical application of the PrC-210 aminothiols or its component structures. Molecules were applied to skin in an alcohol/water delivery vehicle prior to receiving a 17.33 Gy radiation dose to the 1.5 cm $\times$ 3.0 cm skin site. Radiation dermatitis severity (i.e., % of the site covered by scab material) was scored 13 days following irradiation.

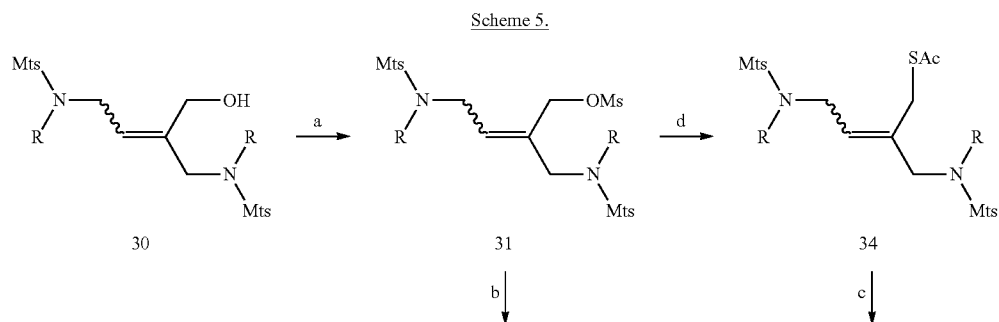
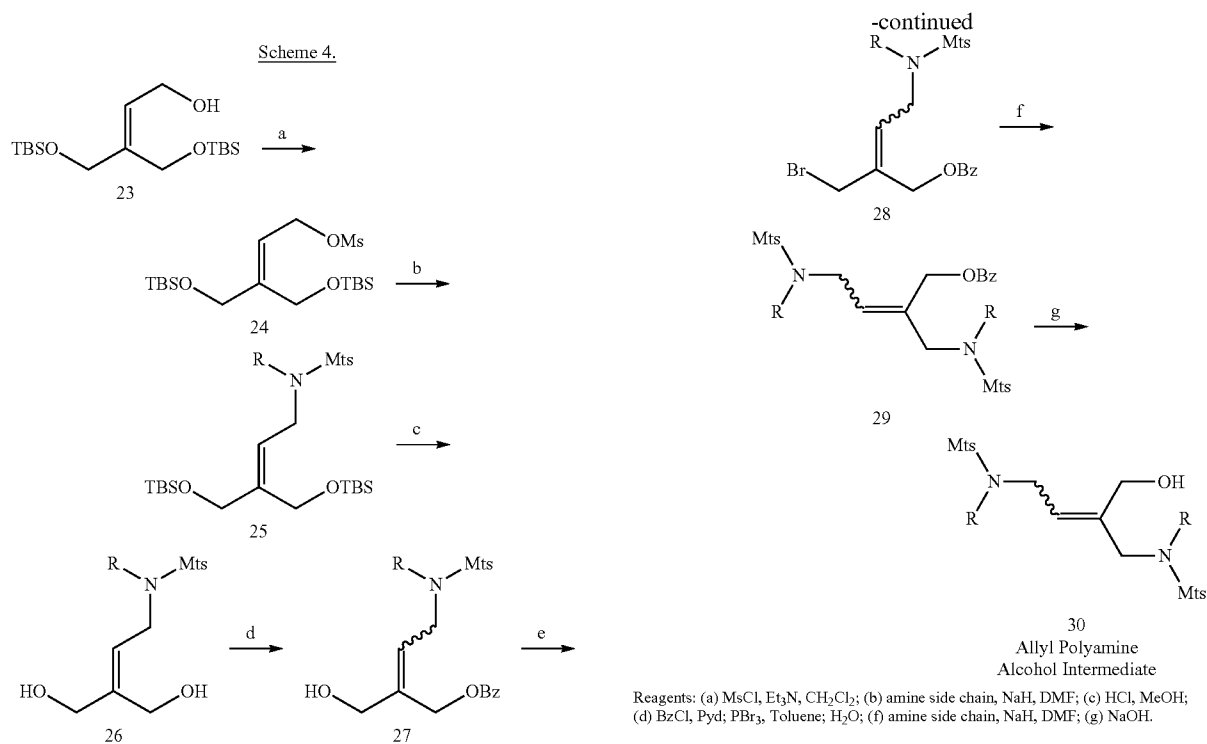
[0020] It is to be understood that the figures are not necessarily drawn to scale, nor are the objects in the figures necessarily drawn to scale in relationship to one another. The figures are depictions that are intended to bring clarity and understanding to various embodiments of apparatuses, systems, and methods disclosed herein. Wherever possible, the same reference numbers will be used throughout the drawings to refer to the same or like parts. Moreover, it should be appreciated that the drawings are not intended to limit the scope of the present teachings in any way.

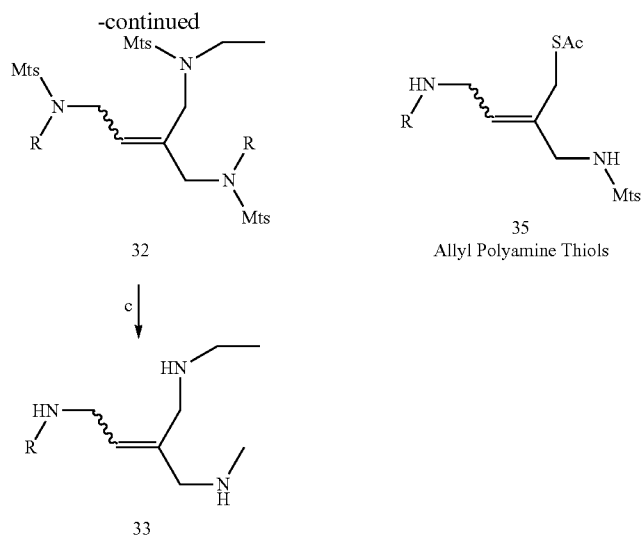






Reagents and conditions: (a) N-ethyl mesitylenesulfonamide, NaH, DMF; (b)  $N_2H_4 \cdot H_2O$ ; (c) mesitylenesulfonyl chloride,  $Et_3N$ ,  $CH_2Cl_2$ ; (d) 4-bromobutylphthalimide, NaH, DMF

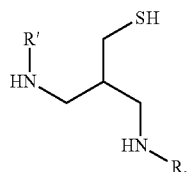




Reagents: (a)  $\text{MsCl}$ ,  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2$ ; (b) N-Et mesitylenesulfonamide,  $\text{NaH}$ ,  $\text{DMF}$ ; (c)  $\text{HBr}$ ,  $\text{PhOH}$ ,  $\text{HOAc}$ ,  $\text{CH}_2\text{Cl}_2$ ;  $\text{K}_2\text{CO}_3$  (aq);  $\text{HCl}$  (aq); (d)  $\text{KSac}$ ,  $\text{DMF}$ .

1. A method for protecting a subject from ionizing radiation, the method comprising:

administering systemically to the subject a radioprotector compound comprising a free thiol and a positively-charged backbone, wherein the radioprotector compound comprises a structure according to:



wherein R and R' are independently selected from H and  $\text{CH}_3$ , wherein systemic administration of the radioprotector compound to the subject protects the subject from ionizing radiation.

2-3. (canceled)

4. The method of claim 1 wherein administering systemically to the subject the radioprotector compound does not cause a side effect of nausea, vomiting, hypotension, or fainting in the subject.

5. The method of claim 1 further comprising blocking cell cycle progression at the G1/S cell cycle border.

6. The method of claim 1 wherein the radioprotector compound is sulfurous odor-free.

7. The method of claim 1 further comprising inhibiting the growth of a cell.

8. The method of claim 1 further comprising restoring cell cycle progression.

9. The method of claim 1 further comprising binding the positively-charged backbone to a DNA and displaying the free thiol away from the DNA.

10. The method of claim 1 further comprising scavenging reactive oxygen species.

11. The method of claim 1 wherein the subject is a mammal.

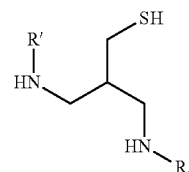
12. The method of claim 1 wherein the subject is a human.

13. The method of claim 1 wherein the subject is a human comprising a cell exposed to radiation for medical purposes.

14. The method of claim 1 wherein a cell or a tissue is protected from ionizing radiation.

15. A method of providing protection to a subject's DNA against a reactive oxygen species, the method comprising:

a) administering systemically to the subject a radioprotector compound comprising a free thiol and a positively-charged backbone, wherein the radioprotector compound comprises a structure according to:



wherein R and R' are independently selected from H and  $\text{CH}_3$ ;

b) binding the positively-charged backbone of the radioprotector compound to a DNA of the subject; and

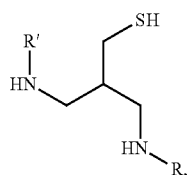
c) scavenging a reactive oxygen species with the free thiol, wherein the radioprotector compound provides protection to the subject's DNA against the reactive oxygen species.

16. (canceled)

17. The method of claim 15 further comprising inhibiting the growth of a cell.

18. A method of providing radioprotection to a subject, the method comprising:

a) providing a radioprotector compound that lacks the side effects of nausea/vomiting and hypotension/fainting, wherein the radioprotector compound comprises a structure according to:



wherein R and R' are independently selected from H and CH<sub>3</sub>;

- b) administering systemically the radioprotector compound to the subject, wherein the systemically administered radioprotector provides systemic radioprotection to the subject.

**19.** (canceled)

**20.** The method of claim **18** further comprising inhibiting the growth of a cell.

**21.** The method of claim **1**, wherein the radioprotector compound is in a form of an acid-addition salt.

**22.** The method of claim **15**, wherein the radioprotector compound is in a form of an acid-addition salt.

**23.** The method of claim **18**, wherein the radioprotector compound is in a form of an acid-addition salt.

\* \* \* \* \*