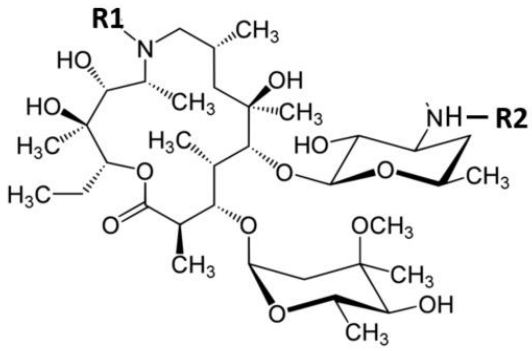
[0006] What is needed, then, is the identification of compounds already approved for one or more treatments that also have senolytic activity.

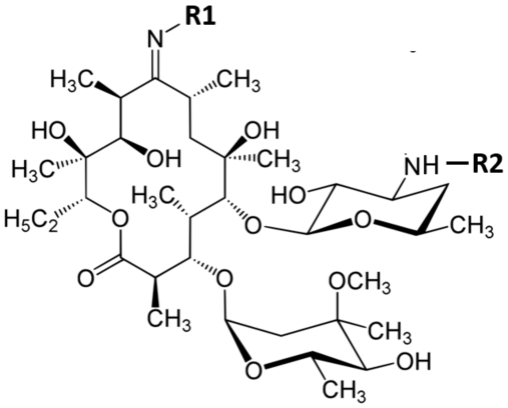
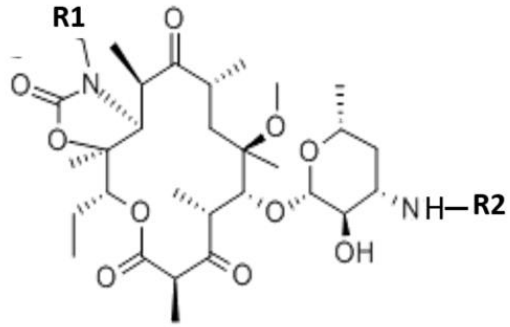
SUMMARY

[0007] This disclosure relates compounds having senolytic activity that may be used as senolytic drugs. Many FDA-approved drugs possess varying degrees of senolytic activity. Identifying such compounds and improving their selectivity towards inhibiting senescent cells would dramatically accelerate their availability for anti-aging drug trials. Described herein are the identification of such compounds, identified following the use of controlled DNA-damage as a tool to induce senescence in human fibroblasts. BrdU-treatment, which has a long history of being used as a DNA-damaging agent, may be used as an efficient platform for screening compounds for senolytic activity. More specifically, BrdU-treatment allows for reproducibly inducing senescence in cultured cells, with high efficiency.

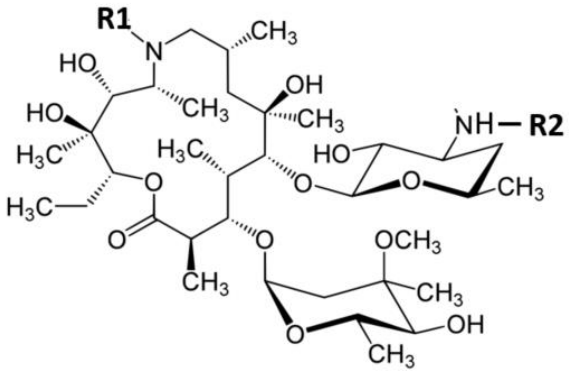
[0008] Using the BrdU-treatment as a platform for identifying senolytic activity, the inventors identified two macrolide antibiotics of the erythromycin family, specifically azithromycin and roxithromycin, as clinically-approved drugs that have efficacy as senolytic drugs. In direct support of the high specificity of these complex interactions, the parent macrolide compound – erythromycin itself – has no senolytic activity in the screening assay disclosed herein. However, telithromycin (another macrolide), also shows senolytic activity, as do chemical analogs or derivatives of azithromycin, roxithromycin, and telithromycin. Note that this description uses the terms ‘derivative’ and ‘analog’ interchangeably. The screening methodology has been used to identify senolytic agents, such as those described herein. Further, senolytic agents may be modified with one or more targeting signals to improve senolytic activity, and may be used in conjunction with other therapeutic agents, such as mitochondrial biogenesis inhibitors, to more potently eradicate senescent cells.

[0009] The present approach may take various forms. In one aspect, the present disclosure refers to a senolytic composition comprising a pharmaceutically effective amount of a compound

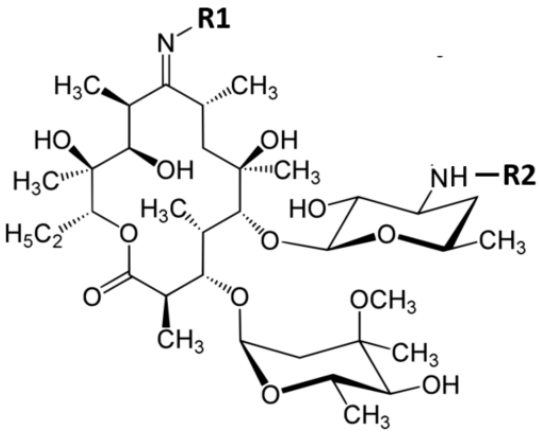
having a formula selected from ,

, and ;wherein R1 and R2 may be the same or different, and are selected from a membrane-targeting signal, a mitochondria-targeting signal, hydrogen, carboxyl, an alkane, a cyclic alkane, an alkane-based derivative, an alkene, a cyclic alkene, an alkene-based derivative, an alkyne, an alkyne-based derivative, a ketone, a ketone-based derivative, an aldehyde, an aldehyde-based derivative, a carboxylic acid, a carboxylic acid-based derivative, an ether, an ether-based derivative, an ester, an ester-based derivative, an amine, an amino-based derivative, an amide, an amide-based derivative, a monocyclic arene, a polycyclic arene, a heteroarene, an arene-based derivative, a heteroarene-based derivative, a phenol, a phenol-based derivative, a benzoic acid, and a benzoic acid-based derivative; and at least one of R1 and R2 is one of a membrane-targeting signal selected from a short-chain fatty acid and a medium-chain fatty acid. In another aspect, the present disclosure refers to the use of the senolytic composition as disclosed herein in the manufacture of a medicament for inducing death of senescent cells in a subject. In another aspect, the present disclosure refers to the use of the senolytic composition as disclosed herein in the manufacture of a medicament for delaying the onset of an age-related disease in a subject. In yet another aspect, the present disclosure refers to the composition as disclosed herein for use in delaying the onset of an age-related disease. Some examples may take the form of a senolytic composition having a

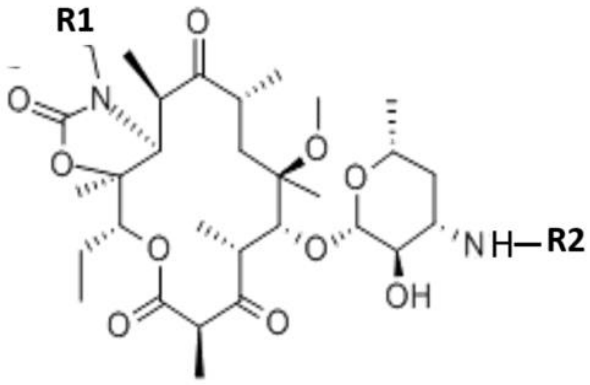
therapeutic amount of at least one senolytic agent selected from azithromycin, roxithromycin, telithromycin, an azithromycin analog, a roxithromycin analog, and a telithromycin analog. This disclosure uses the terms “derivative” and “analog” interchangeably, although the typical usage may be different. For example, examples may be in the form of a pharmaceutically effective amount of a formula selected from one of the following:



,



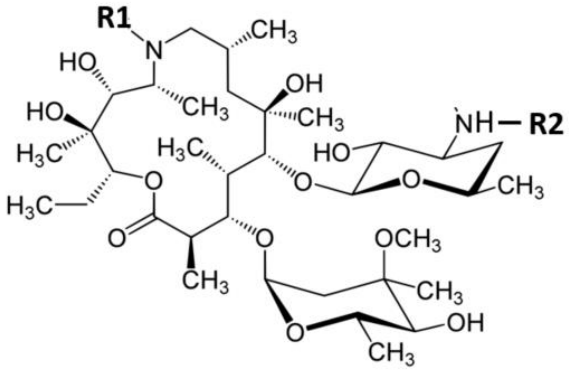
, and



. In such examples, R1 and R2 represent functional groups, may be the same group or different groups, provided that at least one of R1 and R2 is one of a membrane-targeting signal and a mitochondria-targeting signal. Otherwise, R1 and R2 are selected from hydrogen, carboxyl, an alkane, a cyclic alkane, an alkane-based derivative, an alkene, a cyclic alkene, an alkene-based derivative, an alkyne, an alkyne-based derivative, a ketone, a ketone-based derivative, an aldehyde, an aldehyde-based derivative, a carboxylic acid, a carboxylic acid-based derivative, an ether, an ether-based derivative, an ester, an ester-based derivative, an amine, an amine-based derivative, an amide, an amide-based derivative, a monocyclic arene, a polycyclic arene, a heteroarene, an arene-based derivative, a heteroarene-based derivative, a phenol, a phenol-based derivative, a benzoic acid, and a benzoic acid-based derivative. In some examples, at least one of R1 and R2 is a membrane-targeting signal selected

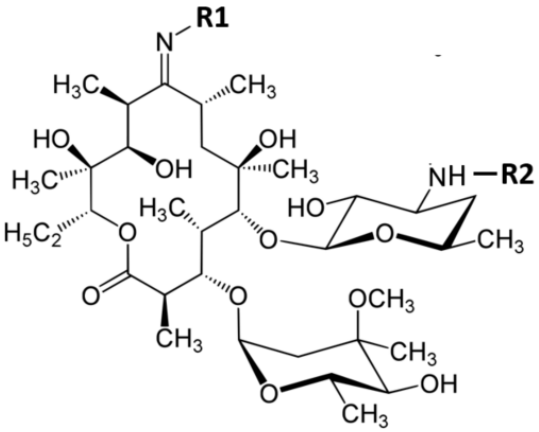
from palmitic acid, stearic acid, myristic acid, oleic acid, a short-chain fatty acid, and a medium-chain fatty acid. The senolytic composition of claim 1, wherein at least one of R1 and R2 is a mitochondria-targeting signal selected from tri-phenyl-phosphonium (TPP), a TPP-derivative, guanidinium, a guanidinium derivative, and 10-N-nonyl acridine orange. In some examples of the senolytic composition, at least one of R1 and R2 is a TPP-derivative selected from 2-butene-1,4-bis-TPP; 2-chlorobenzyl-TPP; 3-methylbenzyl-TPP; 2,4-dichlorobenzyl-TPP; 1-naphthylmethyl-TPP; p-xylylenebis-TPP; a derivative of 2-butene-1,4-bis-TPP; a derivative of 2-chlorobenzyl-TPP; a derivative of 3-methylbenzyl-TPP; a derivative of 2,4-dichlorobenzyl-TPP; a derivative of 1-naphthylmethyl-TPP; and a derivative of p-xylylenebis-TPP.

[0010] In some examples, the senolytic compound may be an azithromycin derivative or analog. For example, the senolytic composition may be a compound having the formula



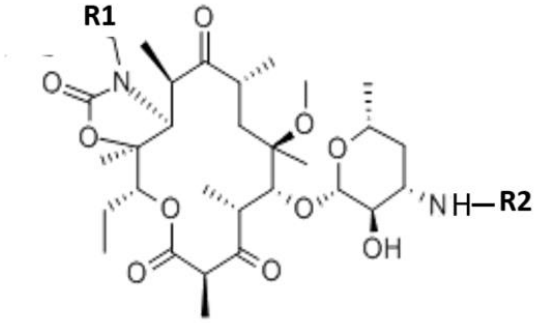
, wherein either R1 is methyl and R2 is one of a membrane-targeting signal and a mitochondria-targeting signal, or R1 is one of a membrane-targeting signal and a mitochondria-targeting signal and NH–R2 is $N(CH_3)_2$. Of course, the functional groups may be different, as described elsewhere herein. Under the typical usage of the terminology, a single substitution from the base compound (e.g., azithromycin) would be referred to as an analog, and one or more modifications made to a base compound may be considered a derivative. For simplicity, this disclosure uses the terms “analog” and “derivative” interchangeably.

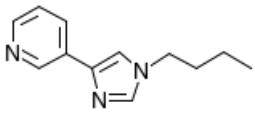
[0011] In some examples, the senolytic compound may be a roxithromycin derivative or analog. For example, the senolytic composition may be a compound having the formula



, wherein either R1 is $O–CH_2–O–(CH_2)_2–OCH_3$ and R2 is one of a membrane-targeting signal and a mitochondria-targeting signal, or R1 is one of a membrane-targeting signal and a mitochondria-targeting signal and NH–R2 is $N(CH_3)_2$. Of course, the functional groups may be different, as described elsewhere herein.

[0012] In some examples, the senolytic compound may be a telithromycin derivative or analog. For example, the senolytic composition may be a compound having the formula



,wherein either R1 is 

and R2 is one of a membrane-targeting signal and a mitochondria-targeting signal, or R1 is one of a membrane-targeting signal and a mitochondria-targeting signal and NH–R2 is $N(CH_3)_2$.

[0013] In preferred examples of the present approach, the senolytic composition has at least one functional group targeting signal to improve the mitochondrial uptake and, as a result, the senolytic activity of the compound. Using the generic formula described above, at least one of R1 and R2 is either a membrane-targeting signal selected from palmitic acid, stearic acid, myristic acid, oleic acid, a short-chain fatty acid, and a medium-chain fatty acid, or a mitochondria-targeting signal selected from 2-butene-1,4-bis-TPP; 2-chlorobenzyl-TPP; 3-methylbenzyl-TPP; 2,4-dichlorobenzyl-TPP; 1-naphthylmethyl-TPP; p-xylylenebis-TPP; a derivative of 2-butene-1,4-bis-TPP; a derivative of 2-chlorobenzyl-TPP; a derivative of 3-methylbenzyl-TPP; a derivative of 2,4-

dichlorobenzyl-TPP; a derivative of 1-naphthylmethyl-TPP; and a derivative of p-xylylenebis-TPP.

[0014] In some examples, the senolytic composition may also include one or more additional therapeutic agents. For example, some examples may include a therapeutic amount of at least one of tetracycline, chlortetracycline, oxytetracycline, demeclocycline, methacycline, doxycycline, minocycline, and tigecycline. Some examples may include a therapeutic amount of at least one of pyrvinium, atovaquone, bedaquiline, irinotecan, sorafenib, niclosamide, stirpentol, chloroquine, and rapamycin. And some examples may include a therapeutic amount of at least one of a mitoriboscin, a mitoketoscin, a mitoflavoscin, 2-butene-1,4-bis-TPP; a derivative of 2-butene-1,4-bis-TPP; 2-chlorobenzyl-TPP; a derivative of 2-chlorobenzyl-TPP; 3-methylbenzyl-TPP; a derivative of 3-methylbenzyl-TPP; 2,4-dichlorobenzyl-TPP; a derivative of 2,4-dichlorobenzyl-TPP; 1-naphthylmethyl-TPP; a derivative of 1-naphthylmethyl-TPP; p-xylylenebis-TPP; and a derivative of p-xylylenebis-TPP. It should be appreciated that a TPP-derivative may be conjugated with a derivative or analog of a senolytic compound, and function as a targeting signal, and may be present as separate therapeutic compound. Some examples may include at least one of Vitamin C, berberine, caffeic acid phenyl ester, silibinin, brutieridin, and melitidin.

[0015] Examples taking the form of the senolytic composition may be made in various forms, including, for example, a cosmetic, a pill, a lotion, a shampoo, a cream, a soap, a skin cleaner, a shaving preparation, an after-shave, a gel, a stick, a paste, a spray, an aerosol, a powder, a liquid, an aqueous suspension, an aqueous solution, a foam, a transdermal patch, a tincture, and a vapor. These forms are intended to be non-limiting examples.

[0016] Examples of the present approach may take the form of a composition for use in senescence therapy. Such compositions include a therapeutic amount of a senolytic agent as discussed above. For example, the senolytic agent may be one of an azithromycin analog, a roxithromycin analog, and a telithromycin analog, having at least one of (i) a membrane-targeting signal selected from palmitic acid, stearic acid, myristic acid, oleic acid, a short-chain fatty acid, and a medium-chain fatty acid; and (ii) a mitochondria targeting signal selected from tri-phenyl-phosphonium (TPP), a TPP-derivative, guanidinium, guanidinium derivatives, and 10-N-nonyl acridine orange.

[0017] In some examples, the composition for use in senescence therapy may also include a therapeutic amount of at least one of tetracycline, chlortetracycline, oxytetracycline,

demeclocycline, methacycline, doxycycline, minocycline, and tigecycline. Some examples may include a therapeutic amount of at least one of pyrvinium, atovaquone, bedaquiline, irinotecan, sorafenib, niclosamide, stirpentol, chloroquine, and rapamycin. And some examples may include a therapeutic amount of at least one of a mitoriboscin, a mitoketoscin, a mitoflavoscin, 2-butene-1,4-bis-TPP; a derivative of 2-butene-1,4-bis-TPP; 2-chlorobenzyl-TPP; a derivative of 2-chlorobenzyl-TPP; 3-methylbenzyl-TPP; a derivative of 3-methylbenzyl-TPP; 2,4-dichlorobenzyl-TPP; a derivative of 2,4-dichlorobenzyl-TPP; 1-naphthylmethyl-TPP; a derivative of 1-naphthylmethyl-TPP; p-xylylenebis-TPP; and a derivative of p-xylylenebis-TPP. Additionally, some examples may include at least one of Vitamin C, berberine, caffeic acid phenyl ester, silibinin, brutieridin, and melitidin.

[0018] The present approach may take the form of a method for inducing death of senescent cells in a subject. In such examples, a therapeutic amount of a senolytic agent is administered to the subject. The senolytic agent may be at least one of azithromycin, roxithromycin, telithromycin, an azithromycin analog, a roxithromycin analog, and a telithromycin analog, as discussed above. Derivatives or analogs may include one or more targeting signals, such as a membrane-targeting and a mitochondria targeting signal. The method may include administering one or more additional therapeutic agents. For example, a therapeutic amount of at least one of tetracycline, chlortetracycline, oxytetracycline, demeclocycline, methacycline, doxycycline, minocycline, and tigecycline may be administered with the senolytic agent. As another example, therapeutic amount of at least one of pyrvinium, atovaquone, bedaquiline, irinotecan, sorafenib, niclosamide, stirpentol, chloroquine, and rapamycin may be administered. As a further example, a therapeutic amount of at least one of a mitoriboscin, a mitoketoscin, a mitoflavoscin, 2-butene-1,4-bis-TPP; a derivative of 2-butene-1,4-bis-TPP; 2-chlorobenzyl-TPP; a derivative of 2-chlorobenzyl-TPP; 3-methylbenzyl-TPP; a derivative of 3-methylbenzyl-TPP; 2,4-dichlorobenzyl-TPP; a derivative of 2,4-dichlorobenzyl-TPP; 1-naphthylmethyl-TPP; a derivative of 1-naphthylmethyl-TPP; p-xylylenebis-TPP; and a derivative of p-xylylenebis-TPP may be administered. As another example, a therapeutic amount of at least one of Vitamin C, berberine, caffeic acid phenyl ester, silibinin, brutieridin, and melitidin may be administered. The therapeutic agent may be co-administered with the senolytic agent, or in some examples may be administered before or after the senolytic agent.

[0019] Some examples of the present approach may take the form of a method for delaying the onset of an age-related disease in a subject. The age-related disease may be at least one of atherosclerosis, arthritis, cancer, cardiovascular disease, cataract, dementia, diabetes, hair loss, hypertension, inflammatory disease, kidney disease, muscular atrophy, neurological disease, osteoarthritis, osteoporosis, pulmonary disease, vertebral disc degeneration, and alopecia. For example, the age-related disease may be a neurological disease, such as mild cognitive impairment, motor neuron dysfunction, Alzheimer's disease, Parkinson's disease, and macular degeneration. In such examples, a therapeutic amount of a senolytic agent, as described, herein may be administered to the subject. In some examples, the senolytic agent may be administered with another therapeutic agent, as described herein. The senolytic agent may be administered at the onset, i.e., at or shortly after the diagnosis of an age-related disease. Alternatively, the senolytic agent may be administered routinely after diagnosis, and the frequency and dosage may be determined using techniques known in the art. In some examples, the senolytic agent may be administered prior to onset, particularly where an age-related disease is expected or likely to occur in a subject (e.g., due to genetic markers or other biological markers).

[0020] The present approach may also take the form of a composition for use in delaying the onset of an age-related disease. For example, and without limitation, the present approach may be used to delay the onset of atherosclerosis, arthritis, cancer, cardiovascular disease, cataract, dementia, diabetes, hair loss, hypertension, inflammatory disease, kidney disease, muscular atrophy, neurological disease, osteoarthritis, osteoporosis, pulmonary disease, vertebral disc degeneration, and alopecia. In such examples, a therapeutic amount of a senolytic agent comprising at least one of azithromycin, roxithromycin, telithromycin, an azithromycin analog, a roxithromycin analog, and a telithromycin analog, may be administered, as described herein. In some examples, the senolytic agent may be administered with another therapeutic agent, as described herein.

[0021] The present approach may also take the form of a method for screening compounds for senolytic activity. In such examples, a cell population is exposed to a DNA damaging agent for a first duration to generate a senescent cell population. An example DNA damaging agent is bromodeoxyuridine (BrdU), though other DNA damaging agents may be used

without departing from the present approach. At least a portion of the senescent cell population is treated with a candidate compound for a second duration to form a treated cell population. Candidate compounds are the compounds being screened for senolytic activity. The treated cell population is analyzed for at least one marker of senolytic activity. Example markers of senolytic activity include cell viability, aerobic glycolysis, autophagy, inhibitory activity, and cell proliferation reduction. For example, autophagic LC3 proteins may be quantitatively measured, as described herein. The Sulphorhodamine B assay and measuring cell-induced electrical impedance are examples of assays that may be used to analyze cell populations for senolytic activity. The first duration may vary, and in the examples described below, was about 8 days. The second duration may also vary, and in the examples discussed below was about 3 days to about 5 days. In some examples, the BrdU may be washed out before the second duration.

[0022] Further examples of the present approach may be recognized by those having ordinary skill in the art, having reviewed the following detailed description.

DESCRIPTION OF THE DRAWINGS

[0023] Fig. 1 illustrates a method for screening and identifying senolytic drugs according to an embodiment of the present approach.

[0024] Fig. 2 shows results of DNA synthesis reduction in MRC-5 fibroblasts.

[0025] Fig. 3A shows results from the SRB assay for azithromycin in MRC-5 fibroblasts. Figs. 3B and 3C show images of MRC-5 fibroblasts treated with 100 μM azithromycin, without and with BrdU pretreatment, respectively.

[0026] Fig. 4 shows results from the SRB assay for roxithromycin in MRC-5 fibroblasts.

[0027] Fig. 5 shows results of autophagy induction for 50 μM azithromycin in MRC-5 fibroblasts.

[0028] Figs. 6A and 6B show extracellular acidification rate results from metabolic flux analysis for MRC-5 cells after 72 hours of treatment with azithromycin.

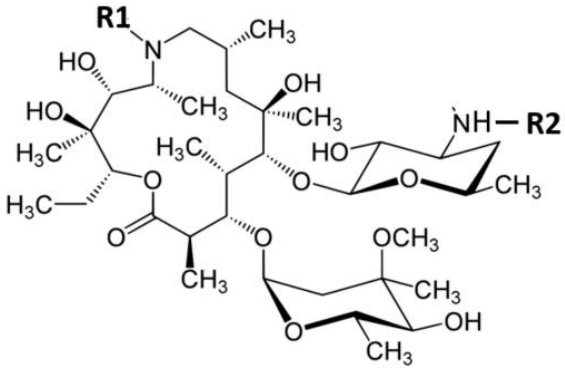
[0029] Figs. 7A and 7B show oxygen consumption rate results from metabolic flux analysis for MRC-5 cells after 72 hours of treatment with azithromycin.

[0030] Fig. 8 shows senolytic activity in senescent human skin cells pretreated with BrdU for 8 days to induce senescence, and then exposed to azithromycin for 5 days.

[0031] Fig. 9A shows a representative cell tracing from xCELLigence real-time cell health monitoring, for MRC-5 cell lines comparing BrdU pretreatment, BrdU pretreatment with

What is claimed is:

1. A senolytic composition comprising a pharmaceutically effective amount of a compound having a formula selected from



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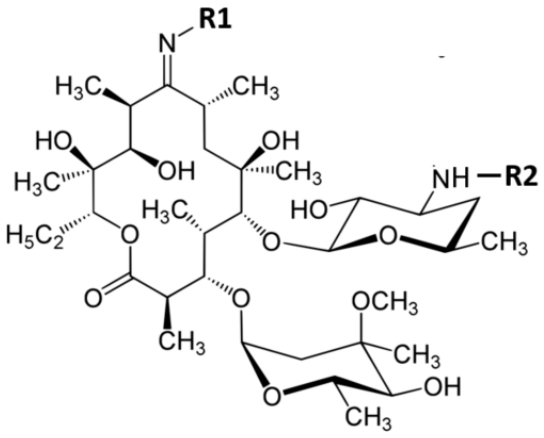
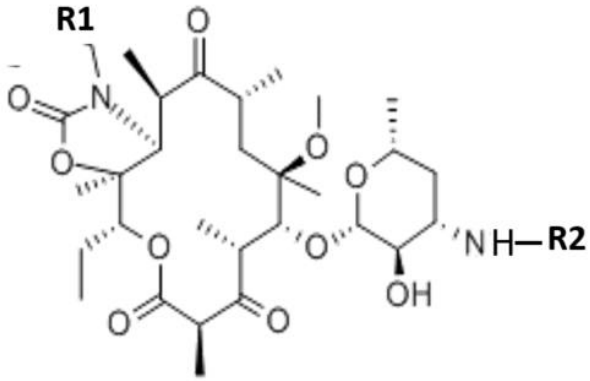


, and



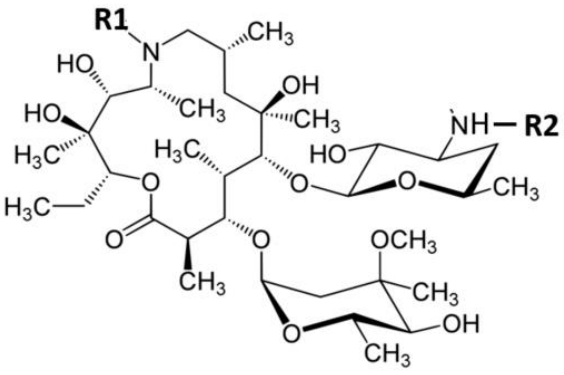
;

wherein R1 and R2 may be the same or different, and are selected from a membrane-targeting signal, a mitochondria-targeting signal, hydrogen, carboxyl, an alkane, a cyclic alkane, an alkane-based derivative, an alkene, a cyclic alkene, an alkene-based derivative, an alkyne, an alkyne-based derivative, a ketone, a ketone-based derivative, an aldehyde, an aldehyde-based derivative, a carboxylic acid, a carboxylic acid-based derivative, an ether, an ether-based derivative, an ester, an ester-based derivative, an amine, an amino-based derivative, an amide, an amide-based derivative, a monocyclic arene, a polycyclic arene, a heteroarene, an arene-based derivative, a heteroarene-based derivative, a phenol, a phenol-based derivative, a benzoic acid, and a benzoic acid-based derivative; and

at least one of R1 and R2 is one of a membrane-targeting signal selected from a short-chain fatty acid and a medium-chain fatty acid.

2. The senolytic composition of claim 1, wherein at least one of R1 and R2 is myristic acid.

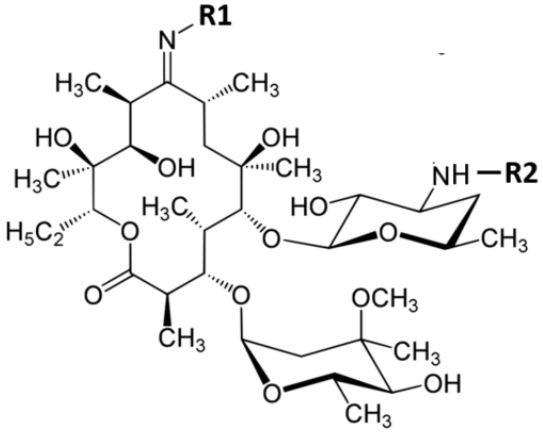
3. The senolytic composition of claim 1, wherein the compound has a formula



,

wherein either R1 is methyl and R2 is one of the membrane-targeting signal and a TPP-derivative, or R1 is one of the membrane-targeting signal and a TPP-derivative and NH–R2 is $N(CH_3)_2$.

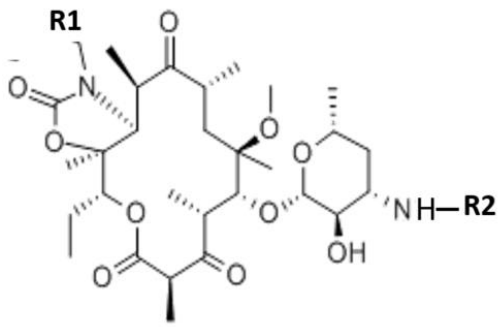
4. The senolytic composition of claim 1, wherein the compound has a formula



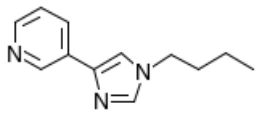
,

wherein either R1 is $O–CH_2–O–(CH_2)_2–OCH_3$ and R2 is one of the membrane-targeting signal and a TPP-derivative, or R1 is one of the membrane-targeting signal and a TPP-derivative and NH–R2 is $N(CH_3)_2$.

5. The senolytic composition of claim 1, wherein the compound has a formula



,

wherein either R1 is  and R2 is one of the membrane-targeting signal and a TPP-derivative, or R1 is one of the membrane-targeting signal and a TPP-derivative and NH–R2 is $N(CH_3)_2$.

6. The senolytic composition of any of claims 3-5, wherein either at least one of R1 and R2 is the membrane-targeting signal selected from a short-chain fatty acid and a medium-chain fatty acid.

7. Use of the senolytic composition of any of claims 1-6 in the manufacture of a medicament for inducing death of senescent cells in a subject.

8. The use of claim 7, wherein at least one of R1 and R2 is myristic acid.

9. The use of claim 7, further comprising a therapeutic amount of at least one of tetracycline, chlortetracycline, oxytetracycline, demeclocycline, methacycline, doxycycline, minocycline, and tigecycline.

10. The use of claim 7, further comprising a therapeutic amount of at least one of pyrvinium, atovaquone, bedaquiline, irinotecan, sorafenib, niclosamide, stirpentol, chloroquine, and rapamycin.

11. The use of claim 7, further comprising a therapeutic amount of at least one of a mitoriboscin, a mitoketoscin, a mitoflavoscin, 2-butene-1,4-bis-TPP; a derivative of 2-butene-1,4-bis-TPP; 2-chlorobenzyl-TPP; a derivative of 2-chlorobenzyl-TPP; 3-methylbenzyl-TPP; a derivative of 3-methylbenzyl-TPP; 2,4-dichlorobenzyl-TPP; a derivative of 2,4-dichlorobenzyl-TPP; 1-naphthylmethyl-TPP; a derivative of 1-naphthylmethyl-TPP; p-xylylenebis-TPP; and a derivative of p-xylylenebis-TPP.

12. The use of claim 7, further comprising at least one of Vitamin C, berberine, caffeic acid phenyl ester, silibinin, brutieridin, and melitidin.

13. Use of the senolytic composition of any of claims 1-6 in the manufacture of a medicament for delaying the onset of an age-related disease in a subject.

14. The use of claim 13, wherein the age-related disease comprises at least one of atherosclerosis, arthritis, cancer, cardiovascular disease, cataract, dementia, diabetes, hair loss, hypertension, inflammatory disease, kidney disease, muscular atrophy, neurological disease, osteoarthritis, osteoporosis, pulmonary disease, vertebral disc degeneration, and alopecia.

15. The use of claim 14, wherein the neurological disease comprises at least one of mild cognitive impairment, motor neuron dysfunction, Alzheimer's disease, Parkinson's disease, and macular degeneration.

16. The use of claim 13, wherein the senolytic composition comprises at least one of an oral composition, a topical composition, and an intravenous composition.

17. The use of any of claims 13-16, wherein the senolytic agent comprises the compound of claim 1.

18. The use of any of claims13-16, further comprising a therapeutic amount of at least one of tetracycline, chlortetracycline, oxytetracycline, demeclocycline, methacycline, doxycycline, minocycline, and tigecycline.

19. The use of any of claims 13-16, further comprising a therapeutic amount of at least one of pyrvinium, atovaquone, bedaquiline, irinotecan, sorafenib, niclosamide, stirpentol, chloroquine, and rapamycin.

20. The use of any of claims 13-16, further comprising a therapeutic amount of at least one of a mitoriboscin, a mitoketoscin, a mitoflavoscin, 2-butene-1,4-bis-TPP; a derivative of 2-butene-1,4-bis-TPP; 2-chlorobenzyl-TPP; a derivative of 2-chlorobenzyl-TPP; 3-methylbenzyl-TPP; a derivative of 3-methylbenzyl-TPP; 2,4-dichlorobenzyl-TPP; a derivative of 2,4-dichlorobenzyl-TPP; 1-naphthylmethyl-TPP; a derivative of 1-naphthylmethyl-TPP; p-xylylenebis-TPP; and a derivative of p-xylylenebis-TPP.

21. The use of any of claims 13-16, further comprising administering at least one of Vitamin C, berberine, caffeic acid phenyl ester, silibinin, brutieridin, and melitidin.

22. The composition of any of claims 1-6 for use in delaying the onset of an age-related disease.

23. The composition of claim 22, wherein the age-related disease comprises at least one of atherosclerosis, arthritis, cancer, cardiovascular disease, cataract, dementia, diabetes, hair

loss, hypertension, inflammatory disease, kidney disease, muscular atrophy, neurological disease, osteoarthritis, osteoporosis, pulmonary disease, vertebral disc degeneration, and alopecia.

24. The composition of any of claims 22 and 23, wherein the senolytic agent further comprises a therapeutic amount of at least one of tetracycline, chlortetracycline, oxytetracycline, demeclocycline, methacycline, doxycycline, minocycline, tigecycline, pyrvinium, atovaquone, bedaquiline, irinotecan, sorafenib, niclosamide, stirpentol, chloroquine, rapamycin, a mitoriboscin, a mitoketoscin, a mitoflavoscin, 2-butene-1,4-bis-TPP; a derivative of 2-butene-1,4-bis-TPP; 2-chlorobenzyl-TPP; a derivative of 2-chlorobenzyl-TPP; 3-methylbenzyl-TPP; a derivative of 3-methylbenzyl-TPP; 2,4-dichlorobenzyl-TPP; a derivative of 2,4-dichlorobenzyl-TPP; 1-naphthylmethyl-TPP; a derivative of 1-naphthylmethyl-TPP; p-xylylenebis-TPP; a derivative of p-xylylenebis-TPP; Vitamin C, berberine, caffeic acid phenyl ester, silibinin, brutieridin, and melitidin.

25. The composition of claim 22, wherein at least one of R1 and R2 is myristic acid.