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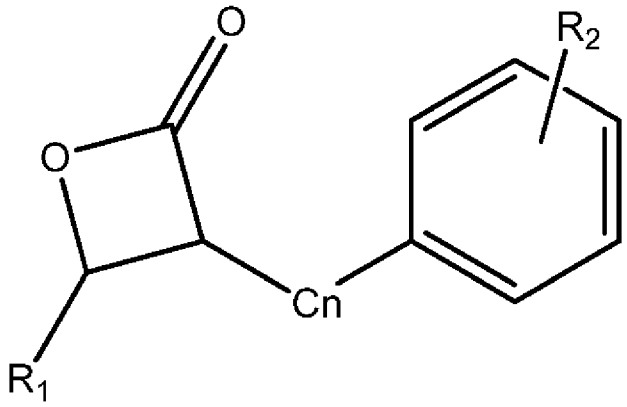
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(54) Title: ANTIBACTERIAL BETA-LACTONES, AND METHODS OF IDENTIFICATION, MANUFACTURE AND USE

(57) Abstract: The invention encompasses antibacterial beta-lactones, methods of making, pharmaceutical preparations thereof, and methods of utilization of such compounds for the treatment of bacterial infections.



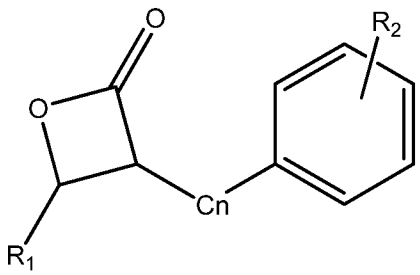
Structure A

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ANTIBACTERIAL BETA-LACTONES

Field of the Invention

An antibacterial therapeutic



Structure A

wherein R_1 is selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, alkoxyalkyl, substituted alkoxyalkyl, NH_2, NHR, NR_2, mono- or polyhydroxy-substituted alkyl, aryl, substituted aryl, heteroaryl, and substituted heteroaryl and the like; and wherein,

R_2 is contemplated as a single substitution of a hydrogen at any position on the benzene ring where the substituted moiety is selected from the group consisting of alkyl, substituted alkyl, alkynyl, substituted alkyl, vinyl, nitro, halo (*e.g.*, includes bromine, chlorine, fluorine and iodine), cyano, aryl, hetero aryl, alkoxy and the like; and

C_n is carbon and n is a number of from 1 to 5. The invention further encompasses a method of making Structure A, a pharmaceutical preparation of Structure A and method of therapeutically treating a subject by administering Structure A, with particular reference to treating an infection.

Background of the Invention

Proteases have been reportedly targeted for the therapeutic treatment of a large family of diseases, including type 2 diabetes, multiple myeloma, HIV, and hepatitis C virus infections. Given the demonstrated physiological significance of

this enzyme family in bacterial physiology, proteases have attracted broad interest as potential antibacterial targets. Of special interest are the highly conserved bacterial degradative proteases. Despite the essential physiological roles that these degradative proteases have and their conservation among bacterial species, there are currently no approved antibiotics that target this enzyme class. Disruption of these proteases may diminish cell viability or suppress virulence. Targeting virulence pathways with small molecules could prevent colonization of the host and enable removal of the bacteria by the host immune system. Blocking virulence represents a strategy for the development of novel antibacterial compounds.

The highly conserved, ATP-dependent ClpP (CaseinoLytic Protease) is a specific therapeutic target. ClpP is reported to be essential for virulence in many pathogenic bacteria, including *Staphylococcus aureus* and *Listeria monocytogenes*. Thus inhibiting ClpP activity in these organisms could suppress pathogenesis. For example, a *clpP* null strain of the pathogen *Staphylococcus aureus* exhibits strongly decreased production of virulence associated hemolysins, lipases, nucleases, and proteases. While ClpP is often required for virulence, it is not essential for viability in most bacteria. However, in the Actinomycetes, a functional ClpP protease is required for viability. This class includes many clinically relevant pathogens such as *Mycobacterium tuberculosis, Mycobacterium leprae, Streptomyces*, and *Corynebacterium*. Inhibition of the ClpP protease in these organisms should represent a toxic event, and thus provides an excellent drugable target.

The acyl depsipeptides (ADEPs) are reportedly the only modulators of the essential mycobacterial ClpP peptidase, but *M. tuberculosis* has efflux pumps that confer resistance.

Note is made of the following publications. The teachings of these publications and all references cited herein are incorporated by reference in their entirety.

Compton, C. L., Schmitz, K. R., Sauer, R. T. Sello, J. K. (2013) "Antibacterial of and Resistance to Small Molecule Inhibitors of the ClpP Peptidase". *ACS Chemical Biology*, Epub Ahead of Print. PMID: 24047344

Böttcher T, Sieber S. (2008) β-lactones as specific inhibitors of ClpP attenuate the production of extracellular virulence factors of *Staphylococcus aureus*. *J. Am. Chem. Soc*. 130, 14400-14401.

Böttcher T, Sieber S. (2009) β-lactones decrease the intracellular virulence of *Listeria monocytogenes* in macrophages. *ChemMedChem*. 4, 1260-1263.

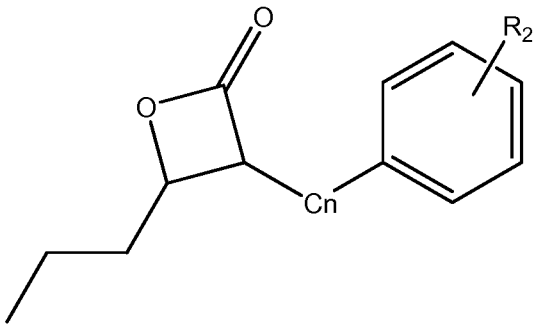
Böttcher T, Sieber S. (2009) Structurally refined β-lactones as potent inhibitors of devastating bacterial virulence factors. *ChemBioChem*. 10, 663-666.

Danheiser R, Nowick J. (1991) A practical and efficient method for the synthesis of β-lactones. *J. Org. Chem*. 56, 1176-1185.

Summary of the Invention

Disclosed are β-lactones believed to be covalent inhibitors of the ClpP protease. Particularly noted is inhibition of such protease in *Escherichia coli, Streptomyces coelicolor, Mycobacterium smegmatis,* and *Mycobacterium tuberculosis*. In addition to the ClpP inhibitors, disclosed herein is a high throughput system to identify new classes of bacterial ClpP activity modulators.

This invention includes a composition of Structure 1



Structure 1

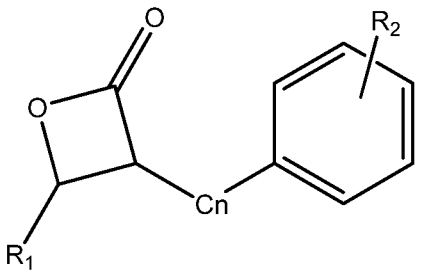
wherein R_2 is a single substitution of a hydrogen at any position on the benzene ring where the substituted moiety is selected from the group consisting of alkyl, substituted alkyl, alkynyl, substituted alkyl, vinyl, nitro, halo (*e.g.*, includes bromine, chlorine, fluorine and iodine), cyano, aryl, hetero aryl, alkoxy; and

C_n is carbon and n is a number of from 1 to 5.

In some embodiments, the composition is substantially in *cis* form or substantially in *trans* form or a mixture thereof.

Particular embodiments comprise the composition said cis trans mixture wherein the mixture is from about 1:99 *cis* to *trans* about 99:1 cis to trans (w/w) with particular reference to about 50:50.

The invention further includes the composition of Structure A



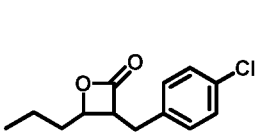
Structure A

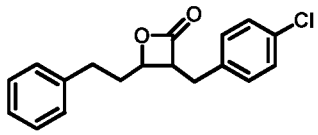
wherein R_1 is selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, alkoxyalkyl, substituted alkoxyalkyl, NH_2, NHR, NR_2, mono- or polyhydroxy-substituted alkyl, aryl, substituted aryl, heteroaryl, and substituted heteroaryl;

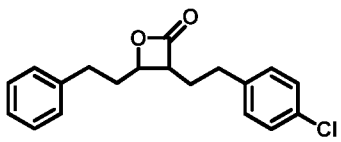
R_2 is a single substitution of a hydrogen at any position on the benzene ring where the substituted moiety is selected from the group consisting of alkyl, substituted alkyl, alkynyl, substituted alkyl, vinyl, nitro, halo, cyano, aryl, hetero aryl, alkoxy; and,.

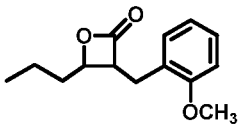
C_n is carbon and n is a number of from 1 to 5.

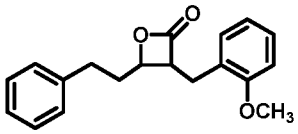
Specific forms of the composition of Structure A noted are

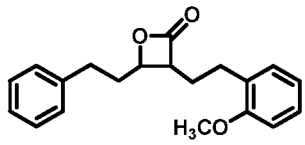

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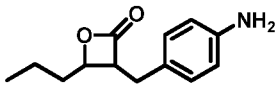

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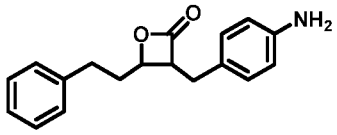

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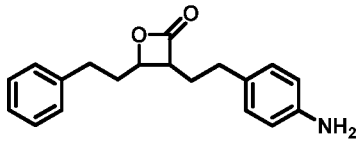

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or


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In some embodiments, the composition of Structure A is substantially in *cis* form or substantially in *trans* form or a mixture thereof.

Particular embodiments comprise the composition said cis trans mixture wherein the mixture is from about 1:99 *cis* to *trans* about 99:1 *cis* to *trans* (w/w) with particular reference to about 50:50.

Yet further included is a pharmaceutical dosage form comprising a therapeutically effective amount of Structure 1 or Structure A in a pharmaceutically acceptable carrier with particular reference to a topical carrier.

Also included is a method of treating a dermatological infection by the step of topically applying a therapeutically amount of the composition of Structure 1 or Structure A. In particular embodiments of the method the infection treated is selected from the group comprising *Staphylococcus aureus, Listeria monocytogenes, Mycobacterium tuberculosis, Mycobacterium leprae, Streptomyces*, and *Corynebacterium.*

The disclosed method of treating a dermatological infection includes the step of topically applying a therapeutically amount of the composition of Structure

1 or Structure A. Particular reference is made to such method in treating infection wherein said infection treated is from the group comprising *Staphylococcus aureus, Listeria monocytogenes, Mycobacterium tuberculosis, Mycobacterium leprae, Streptomyces*, and *Corynebacterium*.

Detailed Description of the Invention

Attention is first directed to the disclosed high-throughput cell based assay for discovering modulators of the ClpP protease. The disclosed cell-based assay is useful in the discovery and evaluation of small molecules that modulate ClpP activity.

High-Throughput Cell Based Assay *Streptomyces* bacteria are reported to have redundant copies of the ClpP protease, encoded by genetic loci clpP1clpP2 and *clpP3clpP4*. *clpP1clpP2* is constitutively transcribed and gives rise to the ClpP1P2 protease. The second bicistronic operon *clpP3/4* is not transcribed. Transcription of *clpP3clpP4* is reportedly dependent on the transcriptional regulator, PopR. Under normal physiological conditions, ClpP1P2 degrades PopR. If the activity of ClpP1P2 is perturbed, PopR is no longer degraded and accumulates in the cell. Eventually, PopR reaches a concentration in which it binds the PopR dependent promotors and initiates transcription. Small molecules that modulate the activity of ClpP are believed likely to interfere with the ability of ClpP to degrade PopR. Accumulation of PopR in these situations results in concomitant up regulation of transcripts associated with PopR dependent promoters. Indeed, we have found that β-lactones inhibitors of ClpP1P2 induce the transcription of *clpP3clpP4* in wild-type *S. coelicolor*.

A high throughput screen to identify other classes of inhibitors of the ClpP protease is useful. Using a chemical genetics approach, we have constructed *S. coelicolor* strains that harbor reporter plasmids in which either the *neo* gene (kanamycin resistance) or the *lux* gene operon (bioluminescence) are under the

control of the PopR-dependent promoter of *clpP3clpP4*. For the *neo* reporter (kanamycin resistance), viability of the strain is linked to the transcription of the *neo* gene, which is under the control of a PopR dependent promotor. Normally ClpP1P2 is degrading PopR, so there is no transcription from the PopR dependent promotors and thus no viability in the presence of Kanamycin. However, when the gene encoding ClpP1P2 have loss of function mutations or the enzyme's activity is inhibited by small molecules, the organism is unable to degrade PopR and is subsequently viable in the presence of kanamycin. Similarly, In the case of the *lux* reporter (bioluminescence), bioluminescence of the strain is under the same control as the *neo* reporter. Thus only strains with perturbations in ClpP1P2 activity will show any bioluminescence or resistance to kanamycin.

These reporter strains are useful to identify compounds that interfere with the degradation of PopR by ClpP1P2. Without being bound by any particular theory it is believed that the compounds identified in this assay act by one of three mechanisms: (1) they inhibit the activity of the protease (*e.g.*, β-lactones); (2) they alter the substrate specificity of ClpP (*e.g.*, antibiotic acyldepsipeptides or ADEPs); or (3) they inhibit its activity by binding to its accessory ATPase (*e.g.*, cyclomarin A1).

This reporter strain is believed superior to *in vitro* reporters in that this assay accounts for permeability, efflux, as well as degradation by native enzymes, such as esterases or proteases.

EXAMPLE 1

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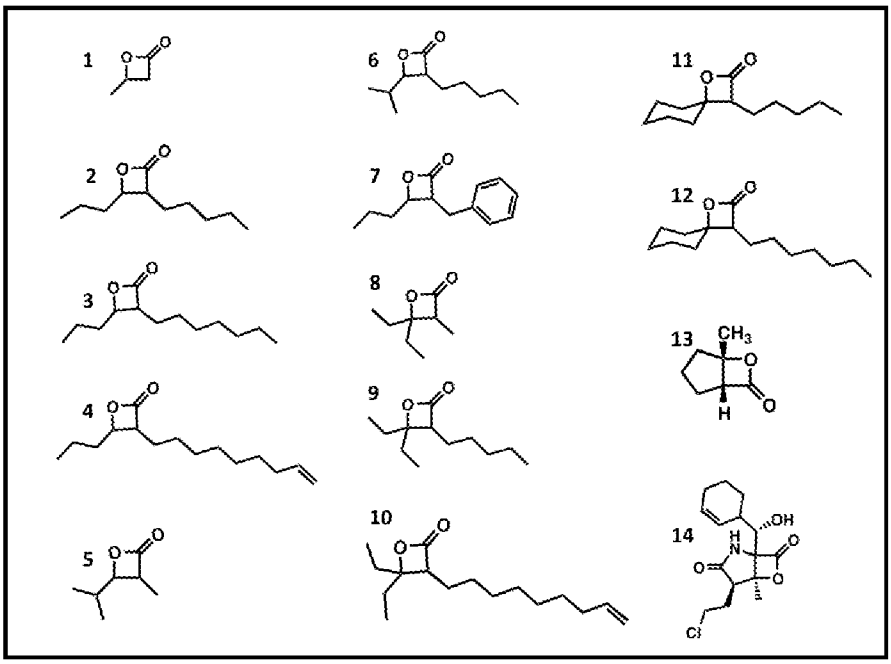
The utility of this reporter strain has been validated with a myriad of small molecule modulators, including 20+ β-lactones and 10 ADEPS. Experiments, including positive and negative controls, were performed and the assay correctly identified all activity modulators. Additionally, a concentration dependence of the reporter has been established. As the concentration of β-lactones is increased, so is the density of cell growth with regards to the neo reporter strain. Using this

reporter strain we can identify the efficacy of the small molecule for perturbing the ClpP protease activity.

Procedure for using Neo Reporter strain

A micromolar concentration a Structure A or Structure 1 molecule is prepared in DMSO. This DMSO stock is added to molten DNAgar containing 10µg/mL kanamycin to obtain the desired concentration of small molecule. The agar is plated and allowed to solidify. After solidification, $1x10^6$ spores of wild-type *S. coelicolor* M145 harboring the *neo* reporter vector are spread out on top of the media. Optical density is measured at 48hrs. Known β-lactones inhibitors of ClpP (including Structure) resulted in viability of the strain on the above media conditions. Beta-lactones that do not inhibit ClpP did not enable the reporter strain to grown in media supplemented with kanamycin.

A library of 12 different compounds with a common β -lactone core (compounds 2-13) was synthesized. Two commercially available compounds (β -lactones 1 and 14) were purchased. The bioactivities of all 14 compounds were tested using the *S. coelicolor* strain with the *neo* reporter gene. Four compounds (compounds 2, 3, 4, and 7) enabled the reporter strain to grow on media supplemented with kanamycin. These four compounds were presumed to be inhibitors of ClpP1P2.

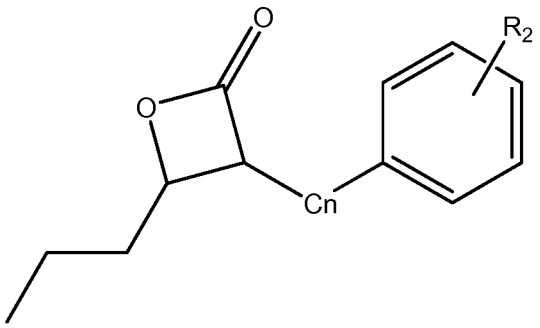


To determine if the results in the *S. coelicolor* based assay had relevance to *Mycobacteria*, all fourteen compounds were tested for toxicity to *M. smegmatis* MC2155, a surrogate of *M. tuberculosis*. Compounds 2, 3, 4, and 7 were bioactive compounds identified in the *S. coelicolor* assay and were the compounds that suppressed the growth of *M. smegmatis*. Three of these inhibitory compounds were *trans*-disubstituted β -lactones with unbranched alkyl substituents on both the α- and β -carbons (β -lactones 2-4). For these molecules, there was a positive correlation between the length of the alkyl group on the α -carbon and antibacterial activity. However, the more potent compound (β -lactone 7; MIC = 10μg/mL) had an aromatic benzyl substituent on the α -carbon and an unbranched *n*-propyl group on the β -carbon. The two most active compounds by our test method (4 and 7) from the activity assays with *M. smegmatis* were subsequently tested for toxicity to the human pathogen, *M. tuberculosis* H37Rv. Strikingly, both molecules also inhibited growth of *M. tuberculosis* H37Rv, with β -lactone **7** being the most active against *M. smegmatis* and *M. tuberculosis* (MIC = 10 and 28 μg/mL, respectively). The compounds were subsequently shown to be inhibitors of ClpP *in vitro*. In Mycobacteria, ClpP inhibition is associated with

killing. Previous reports of ClpP inhibition generally described suppression of virulence in pathogenic bacteria (*S. aureus* and *L. monocytogenes*).

Preparation of Antibacterial B-Lactones

Herein we indentify β-lactone covalent inhibitors of the ClpP protease in *Escherichia coli, Streptomyces coelicolor*, as well as *Mycobacterium smegmatis*. These compounds are typically toxic to M. smegmatis below 5μM.



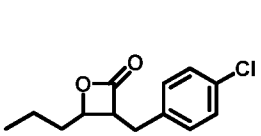
Structure 1

The compound of Structure 1 exists in either *cis* or *trans* forms with respect to relative configurations at the alpha and beta carbons of the beta-lactone. With a high performance chromatography instrument, the diastereomers can be separated. Alternatively, a stereospecific synthesis can be used to obtain the pure *cis* or *trans* material. The synthetic methods have been reported by Danheiser and co-workers. Danheiser R, Nowick J. (1991) "A practical and efficient method for the synthesis of β-lactones," *The Journal of Organic Chemistry*. 56(3):1176-85.

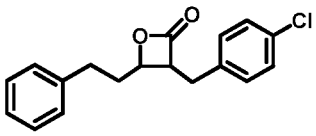
Preparation of Structure 1

A compound of Structure 1 was prepared by the following means. First, a thiophenol ester was generated from the corresponding acid chloride following the below procedure. After the thiol ester was generated, it was used in a reaction with an appropriate aldehyde to generate Structure 1.

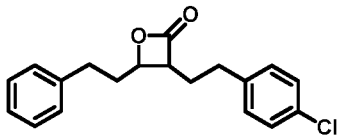
Particular attention is drawn to the following β-lactones as inhibitors of the ClpP peptidase including the peptidase as found in bacteria.



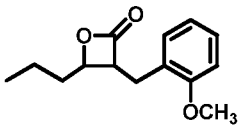
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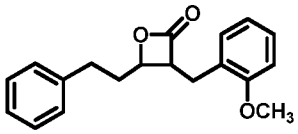
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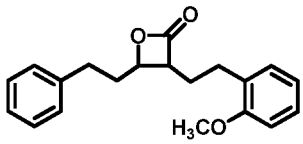
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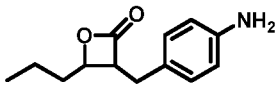
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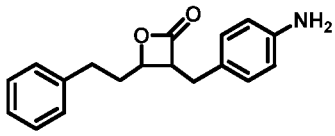
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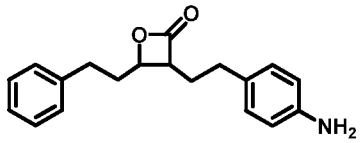
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These compounds and analogs thereof can be prepared in a straightforward fashion using methodology as disclosed in Danheiser *et al.* as noted above. In this method, thiol esters are condensed with aldehydes in a base-catalyzed aldol-type reaction. The aldol product of the condensation undergoes a spontaneous cyclization yielding the desired β-lactones. Additional methodology is disclosed in Compton *et al. ACS Chemical Biology,* as noted above. Analogs of this invention are selected from the exemplified examples or pharmaceutically acceptable salts formed by the reaction of acid and base, such as hydrochloric acid, fumaric acid, maleic acid, succinic acid, acetic acid, citric acid, tartaric acid, carbonic acid, phosphoric acid, oxalic acid, sodium carbonate, sodium hydride, potassium hydroxide, ammonium hydroxide, etc.

General Procedure for the Preparation of Thiol Esters.

A 100-mL, round-bottomed flask equipped with a rubber septa was evacuated and put under a positive pressure of Nitrogen. Then, charged with 50

mL of methylene chloride, thiophenol (1 eq, 50 mmol), and pyridine (1 eq, 50 mmol) and then cooled in an ice bath while the appropriate acid chloride (1 eq, 50 mmol) was added by syringe over 5 min. The resulting suspension of white solid was stirred for an additional *5* min at 0 °C and at room temperature for 30 min and then poured into 100 mL of dH_20. The aqueous phase was separated and extracted with 25 mL of methylene chloride, and the combined organic phases were dried over Na_2SO_4, filtered, and concentrated. Flash Chromatography on silica with a mobile phase of 15:1 Hex: EtOAc afforded the desired thiol ester as a colorless oil. Thiol esters were prepared by means of this procedure exhibited an 86-98% yield.

General Procedure for the Preparation of β-Lactones from Thiol Esters and Aldehydes: Preparation of trans 4-butyl 3-pentyloxetan-2-one.

A 100-mL, round-bottomed flask equipped with a rubber septa was evacuated and put under a positive pressure of Nitrogen. The flask was charged with 50mL of Dry THF and diisopropylamine (1.1eq, 11 mmol), and then cooled in an dry ice and acetone bath (-78°C) while *n*-butyllithium solution (2.25 M in hexanes, 1.035eq, 10.35 mmol) was added via syringe over 2 min. After 30 min, *S*-phenyl 3-phenylpropanethioate (1eq, 10.0 mmol) was added drop wise via syringe over 5 min. After 30 min, a solution of butyryl aldehyde (1eq, 10.0 mmol) in 12.5 mL of THF was added dropwise over 20 min via a syringe cooled externally with dry ice. The reaction mixture was stirred at -78 °C for 60 min and then allowed to warm to 0°C over 120 min. Saturated NH_4Cl solution (50mL) was then added, and the resulting mixture was partitioned between 50 mL of water and 50 mL of diethyl ether. The organic phase was extracted with three 50-mL portions of 1N HCL, three 50-mL portions of sat. Na_2CO_3 solution and two 50-mL portions of saturated NaCl solution, dried over $NaSO_4$, filtered, and concentrated to afford a pale yellow oil. Flash column chromatography on silica gel (gradient elution with ethyl acetate: hexanes) gave single diastereomeric β-Lactones.

EXAMPLE 3.
Therapeutic Use of Structure 1

The above compounds or derivatives are used to treat bacterial infections caused by number of pathogenic bacteria, including but not limited to *Mycobacterium*, *Streptomyces*, *Listeria*, *Corynebacterium*, *Staphylococcus*, and *Streptococcus*. The compounds could be applied as a topical cream, capsule or tablet, or as an injection. Treatment with the compounds can directly cause selective toxicity or avirulence in these pathogenic strains.

A 49 year-old overweight male presents a wound on the skin that is infected with *Mycobacterium leprae*, *Streptococcus aureus*, etc. The subject is treated with a topical dosage of cis Structure 1 at 3mg/kg of ointment (White Petrolatum and Mineral Oil). The infection is resolved within 12 hours.

In another embodiment the ointment of structure 1 contains 0.35% Structure 1 by weight.

Therapeutically effect ranges of Structure 1 are (i) for cis, (ii) for trans and (iii) for a mixture -- from about 0.001 mg/kg to about 100mg/kg. The compounds are administered as an antibacterial ointment. Further formulations are known in the art with particular reference to Marriott, *et al.*, Pharmaceutical Compounding and Dispensing, (Pharmaceutical Pr; 2 edition (2010)), ISBN-10: 0853699127.

The pharmacologically active compositions of this invention can be processed in accordance with conventional methods of Galenic pharmacy to

produce medicinal agents for administration to subjects, e.g., mammals including humans.

The compositions of this invention individually or in combination are employed in admixture with conventional excipients, i.e., pharmaceutically acceptable organic or inorganic carrier substances suitable for parenteral, enteral (e.g., oral or inhalation) or topical application which do not deleteriously react with the active compositions. Note is made of ointment bases including

- Hydrocarbon bases, e.g. hard paraffin, soft paraffin, microcrystalline wax and ceresine;
- Absorption bases, e.g. wool fat, beeswax;
- Water soluble bases, e.g. macrogols 200, 300, 400;
- Emulsifying bases, e.g. emulsifying wax, cetrimide; and,
- Vegetable oils, e.g. olive oil, coconut oil, sesame oil, almond oil and peanut oil.

Suitable pharmaceutically acceptable carriers include but are not limited to water, salt solutions, alcohols, gum arabic, vegetable oils, benzyl alcohols, polyethylene glycols, gelatin, carbohydrates such as lactose, amylose or starch, magnesium stearate, talc, titanium dioxide, silicic acid, viscous paraffin, perfume oil, fatty acid esters, hydroxy methylcellulose, polyvinyl pyrrolidone, etc. The pharmaceutical preparations can be sterilized and if desired mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic substances and the like which do not deleteriously react with the active compositions. They can also be combined where desired with other active agents, e.g., vitamins.

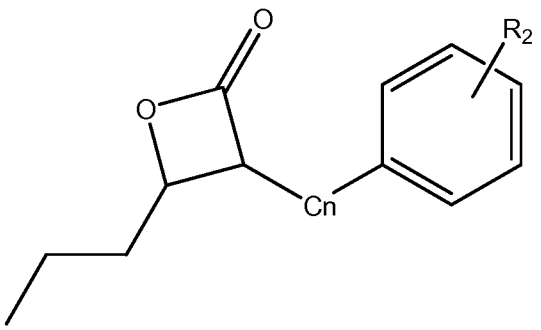
In some embodiments of the present invention, dosage forms include instructions for the use of such compositions.

For parenteral application, particularly suitable are injectable, sterile solutions, preferably oily or aqueous solutions, as well as suspensions, emulsions, or implants, including suppositories. Ampules, vials, and injector cartridges are convenient unit dosages.

Also for parenteral application, particularly suitable are tablets, dragees, liquids, drops, suppositories, or capsules. A syrup, elixir, or the like can be used wherein a sweetened vehicle is employed. Sublingual and buccal forms are also noted.

Sustained or directed release compositions can be formulated, e.g., liposomes or those wherein the active component is protected with differentially degradable coatings, e.g., by microencapsulation, multiple coatings, etc. It is also possible to freeze-dry the new compositions and use the lyophilizates obtained, for example, for the preparation of products for injection.

Claims



Structure 1

1. The composition

R_2 is a single substitution of a hydrogen at any position on the benzene ring where the substituted moiety is selected from the group consisting of alkyl, substituted alkyl, alkynyl, substituted alkyl, vinyl, nitro, halo (*e.g.*, includes bromine, chlorine, fluorine and iodine), cyano, aryl, hetero aryl, alkoxy;

C_n is carbon and n is a number of from 1 to 5, and analogs thereof.

2. The composition of Claim 1 substantially in *cis* form.

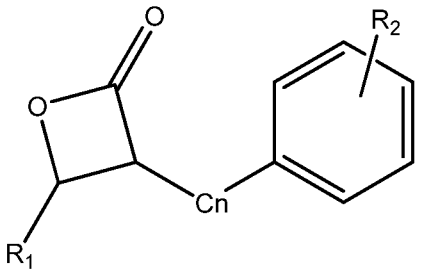
3. The Composition of Claim 1 substantially in *trans* form.

4. The composition of Claim 1 in a mixture of *cis* and *trans* form.

5. The composition of Claim 4 wherein said mixture is from about 1:99 *cis* to *trans* about 99:1 *cis* to *trans* (w/w).

6. The composition of Claim 5 wherein said *cis* to *trans* is about 50:50.

7. The composition



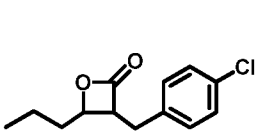
Structure A

wherein R_1 is selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, alkoxyalkyl, substituted alkoxyalkyl, NH_2, NHR, NR_2, mono- or polyhydroxy-substituted alkyl, aryl, substituted aryl, heteroaryl, and substituted heteroaryl;

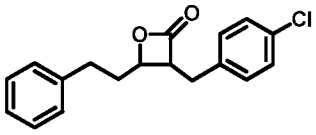
R_2 is c a single substitution of a hydrogen at any position on the benzene ring where the substituted moiety is selected from the group consisting of alkyl, substituted alkyl, alkynyl, substituted alkyl, vinyl, nitro, halo, cyano, aryl, hetero aryl, alkoxy; and,.

C_n is carbon and n is a number of from 1 to 5.

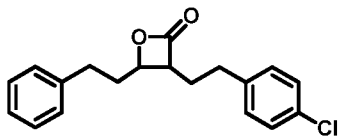
8. The composition of Claim 7 wherein said composition is selected from the from the group comprising



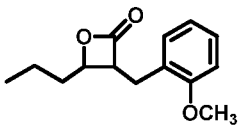
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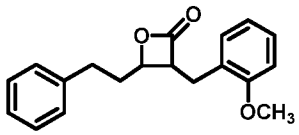
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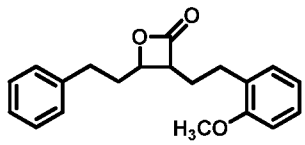
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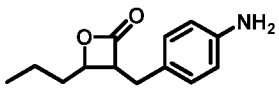
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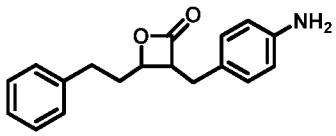
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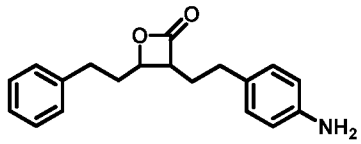


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or



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and analogues thereof.

9. The composition of Claim 7 substantially in *cis* form.

10. The Composition of Claim 7 substantially in *trans* form.

11. The composition of Claim 7 in a mixture of *cis* and *trans* form.

12. The composition of Claim 11 wherein said mixture is from about 1:99 *cis* to *trans* about 99:1 *cis* to *trans* (w/w).

13. The composition of Claim 12 wherein said *cis* to *trans* is about 50:50.

14. A pharmaceutical dosage form comprising a therapeutically effective amount of Structure 1 in a pharmaceutically acceptable topical carrier.

15. A pharmaceutical dosage form comprising a therapeutically effective amount of Structure A in a pharmaceutically acceptable topical carrier.

16. A method of treating a dermatological infection by the step of topically applying a therapeutically amount of the composition of Claim 1.

17. The method of Claim 16 wherein said infection is selected from the group comprising *Staphylococcus aureus, Listeria monocytogenes, Mycobacterium tuberculosis, Mycobacterium leprae, Streptomyces*, and *Corynebacterium.*

18. A method of treating a dermatological infection by the step of topically applying a therapeutically amount of the composition of Claim 7.

19. The method of Claim 18 wherein said infection is selected from the group comprising *Staphylococcus aureus, Listeria monocytogenes, Mycobacterium tuberculosis, Mycobacterium leprae, Streptomyces*, and *Corynebacterium.*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/52611

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/337; A61P 31/04; C07D 305/02 (2014.01)
CPC - A61K 31/337; C07D 305/02
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A61K 31/337; A61P 31/04; C07D 305/02 (2014.01)
CPC: A61K 31/337; C07D 305/02 USPC: 514/449; 549/263

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MicroPatent (US Granted, US Applications, EP-A, EP-B, WO, JP, DE-G, DE-A, DE-T, DE-U, GB-A, FR-A); ProQuest; Google Advanced Search; IP.com; KEYWORDS: beta lacton*, bacter* infect*, treat*, gram* positiv*, gram* negativ*, pharmaceut* composition*, accept* carrier*, salin*, administ* topic*, Staphyl* aur*, 2 oxetanon*, cis beta lacton*, trans beta lacton*, stereochem*, enantiomer* exces*

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ARONICA, LA et al., Synthesis of functionalised beta-lactones via silylcarbocyclisation/ desilylation reactions of propargyl alcohols, Tetrahedron 66, pages 265-273, 2010; <DOI: 10.1016/j.tet.2009.10.112>; page 269, Scheme 7, Table 5, Entry 5	7
X	LALL, MS et al., Serine and Threonine beta-Lactones: A New Class of Hepatitis A Virus 3C Cysteine Proteinase Inhibitors, J. Org. Chem. 67, pages 1536-1547, 2002; <DOI: 10.1021/jo0109016>; page 1540, Scheme 4, compound 27	7
Y	US 2011/0196027 A1 (SIEBER, SA et al.) 11 August 2011; paragraphs [0012], [0014]-[0019], [0030]-[0031], [0038], [0050], [0080], [0087], [0113], [0115]-[0116], [0119]-[0120], [0126]	1-19
Y	DAINES, RA et al., First X-ray Cocrystal Structure of a Bacterial FabH Condensing Enzyme and a Small Molecule Inhibitor Achieved Using Rational Design and Homology Modeling, J. Med. Chem. 46(1), pages 5-8, 2003; page 5, column 2, paragraph 2; page 6, column 2, paragraph 1; page 7, column 1, paragraph 1 – column 2, paragraph 2; page 8, column 1, paragraphs 1, 3	1-19
Y	US 2010/0173982 A1 (SMITH, JW et al.) 08 July 2010; paragraphs [0003], [0072], [0281], [0289], [0302]	2-6, 9-13
P, Y	COMPTON, CL et al., Antibacterial Activity of and Resistance to Small Molecule Inhibitors of the ClpP Peptidase, ACS Chem. Biol. 8, pages 2669-2677, 18 September 2013; entire document	1-19

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
08 October 2014 (08.10.2014)	26 NOV 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)