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(71) Applicants: MCMaster UNIVERSITY [CA/CA];
1280 Main Street West, Hamilton, Ontario L8S 4L8 (CA).
MASSACHUSETTS INSTITUTE OF TECHNOLOGY
[US/US]; 77 Massachusetts Avenue, Cambridge, Massa-
chusetts 02139 (US).

(72) Inventors: COLLINS, James J.; 118 Glen Avenue,
Newton Centre, Massachusetts 02459 (US). MAGOLAN,
Jakob; 34 Mountain Street, Grimsby, Ontario L3M 3K1
(CA). STOKES, Jonathan; 7 Kidd Ave, Grimsby, Ontario
L3M 3S4 (CA). FRAGIS, Meghan; 35 Westacott Crescent,
Ajax, Ontario L1T 4H7 (CA).

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(54) Title: NARROW-SPECTRUM ANTIBIOTIC COMPOUNDS AND USES THEREOF

(57) Abstract: The present disclosure relates to narrow-spectrum antibiotic compounds, compositions and uses thereof for inhibiting growth of an *Acinetobacter baumannii* bacterium and/or treating an *Acinetobacter baumannii* bacterial infection.

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NARROW-SPECTRUM ANTIBIOTIC COMPOUNDS AND USES THEREOF**RELATED APPLICATION**

[0001] This application claims priority to U.S. Provisional Patent Application Serial No. 63/463,751, filed May 3, 2023.

FIELD

[0002] The present disclosure relates to the field of antibiotics, and in particular, to narrow-spectrum antibiotic compounds against *Acinetobacter baumannii* and uses thereof.

BACKGROUND

[0003] *Acinetobacter baumannii* is a nosocomial Gram-negative pathogen that often displays multidrug-resistance due to its robust outer membrane and its ability to acquire and retain extracellular DNA¹ that frequently encodes antibiotic resistance genes. Moreover, it can survive for prolonged durations on surfaces and is resistant to desiccation. Discovering fundamentally novel antibiotics against *A. baumannii* has proven challenging through conventional screening approaches. Indeed, most new antibiotics that achieve clinical use are analogs of existing classes². However, while structural analogs of existing antibiotic classes may satisfy short-term clinical needs, their long-term efficacy is inherently limited due to the high prevalence of existing resistance determinants³. Ideally, new antibiotic discovery efforts should focus on identifying novel chemotypes with mechanisms of action that are unique relative to existing antibiotics. Such compounds are likely to have prolonged utility, given that the probability of pre-existing clinical resistance is low

[0004] Machine learning methods allow for the rapid exploration of vast chemical/sequence spaces *in silico*, increasing the probability of discovering desirable new chemotypes with antibacterial activity, particularly against challenging pathogens like *A. baumannii*. As a reference, typical high throughput screening programs are limited to testing a few million molecules for antibacterial activity at the largest scales⁴. Contrarily, contemporary algorithmic approaches can assess hundreds of millions to billions of molecules for antibacterial properties. For example, Stokes *et al*⁵ applied a message passing neural network trained on growth inhibition of lab strain *Escherichia coli* to discover novel broad spectrum small molecule antibacterial compounds. In a complementary application, Ma *et al*⁶ applied multiple natural language processing neural network models to predict broad spectrum antimicrobial peptides encoded in the human gut microbiome. These, and other important studies (detailed in ⁷) showcase the importance of machine learning approaches towards the discovery of structurally and functionally novel antibiotic candidates.

[0005] Beyond simply discovering structurally and functionally novel antibiotics, a largely unmet need exists for the application of narrow spectrum therapies that target specific bacterial species. Such antibiotics are beneficial for two reasons⁸: first, the rate at which resistance to narrow spectrum agents would disseminate is likely lower than conventional broad-spectrum agents, since narrow spectrum drugs do not impose a universal selective pressure that favors the wide propagation of resistance determinants. Second, narrow spectrum antibiotics would not disrupt the ecology of the microbiota during treatment. Indeed, dysbiosis has been associated with a wide array of poor health outcomes, including infectious diseases⁹, inflammatory bowel diseases¹⁰, metabolic diseases¹¹, neuropsychiatric disorders¹², and cancer¹³. For instance,

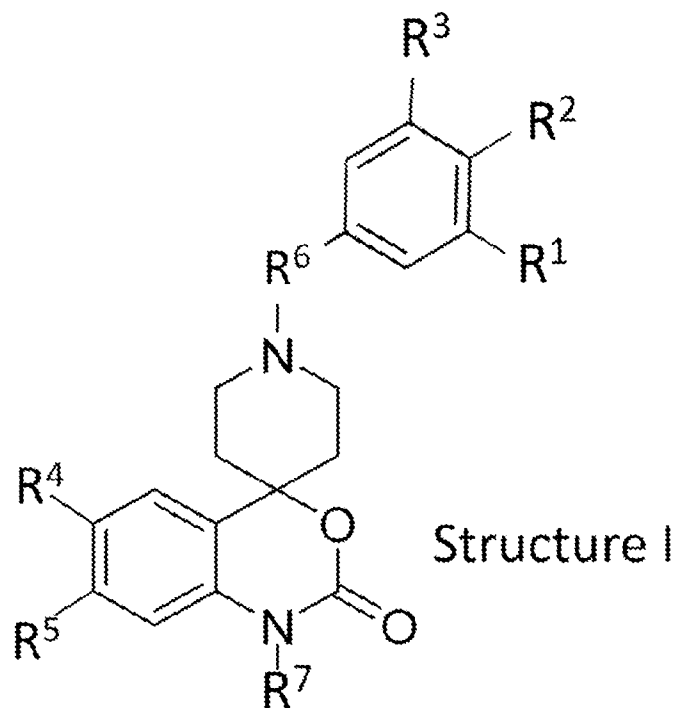
Clostridioides difficile infections are prime examples of opportunistic infections resulting from antibiotic-induced dysbiosis, causing upwards of 224,000 infections in hospitalized patients and 13,000 deaths in the US alone in 2017¹⁴.

[0006] An opportunity exists to apply contemporary machine learning methods to discover structurally and functionally novel antibiotics that specifically target challenging pathogens, with *A. baumannii* being a prime candidate.

SUMMARY

[0007] In the present disclosure, ~7,500 molecules were screened for those that inhibited the growth of *A. baumannii* *in vitro*. A neural network was trained with this growth inhibition dataset and *in silico* predictions were performed for structurally novel molecules with activity against *A. baumannii*. Through this approach, antibacterial compounds with narrow-spectrum activity against *A. baumannii* were discovered. Further investigations revealed that this compound perturbs lipoprotein trafficking through a mechanism involving lipoprotein release complex – inner membrane subunit (LolE). Moreover, it could control an *A. baumannii* infection in a murine wound model.

[0008] Accordingly, provided is a compound for inhibiting growth of an *Acinetobacter baumannii* bacterium, the compound having Structure I as follows:



or a pharmaceutically acceptable salt, solvate and/or prodrug thereof;

wherein:

$R^1 - R^3$ are independently selected from H, OH, CH_3 , C_2H_5 , NO_2 , CN, halogen, alkoxy (e.g., methoxy), or trifluoromethyl, with at least one and more typically two, and quite possibly all three of $R^1 - R^3$ being H;

R^4 and R^5 are independently selected from H, OH, halogen, or amine (e.g., primary amine), with none, more typically one, and possibly both of R^4 and R^5 being H;

R^6 is $C_1 - C_3$ alkyl or alkylene; and

R^7 is H or $C_1 - C_3$ alkyl or alkylene.

[0009] According to an additional or alternative aspect of the present disclosure, for the compound having structure I, R^1 and R^3 are H.

[0010] According to an additional or alternative aspect of the present disclosure, for the compound having structure I, R^2 is selected from H, OH, CH_3 , C_2H_5 , NO_2 , halogen, alkoxy, or trifluoromethyl or any sub-combination thereof.

[0011] According to an additional or alternative aspect of the present disclosure, for the compound having structure I, R^2 is selected from H, methoxy, halogen, or trifluoromethyl or any sub-combination thereof.

[0012] According to an additional or alternative aspect of the present disclosure, for the compound having structure I, R^4 is H.

[0013] According to an additional or alternative aspect of the present disclosure, for the compound having structure I, R^5 is selected from H, OH, halogen or primary amine, and more typically from H or halogen, with one or both of R^4 and R^5 being H.

[0014] According to an additional or alternative aspect of the present disclosure, for the compound having structure I, R^6 is C_1 - C_2 alkyl.

[0015] According to an additional or alternative aspect of the present disclosure, for the compound having structure I, R^6 is C_2 alkyl.

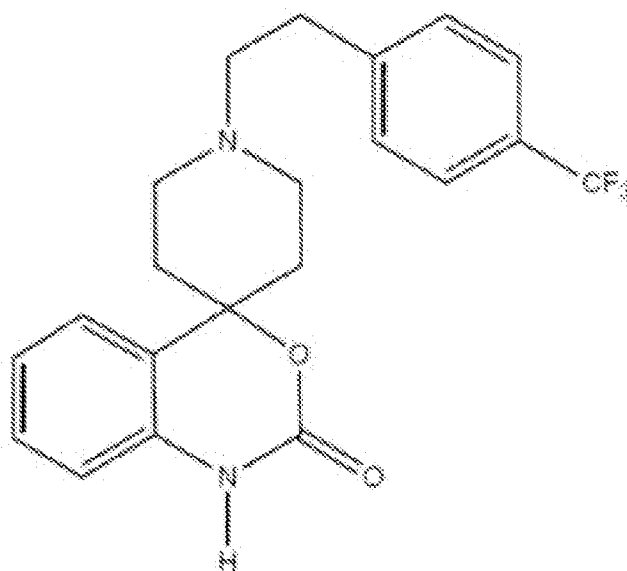
[0016] According to an additional or alternative aspect of the present disclosure, for the compound having structure I, R^7 is H or C_1 - C_2 alkyl.

[0017] According to an additional or alternative aspect of the present disclosure, for the compound having structure I, R^7 is H.

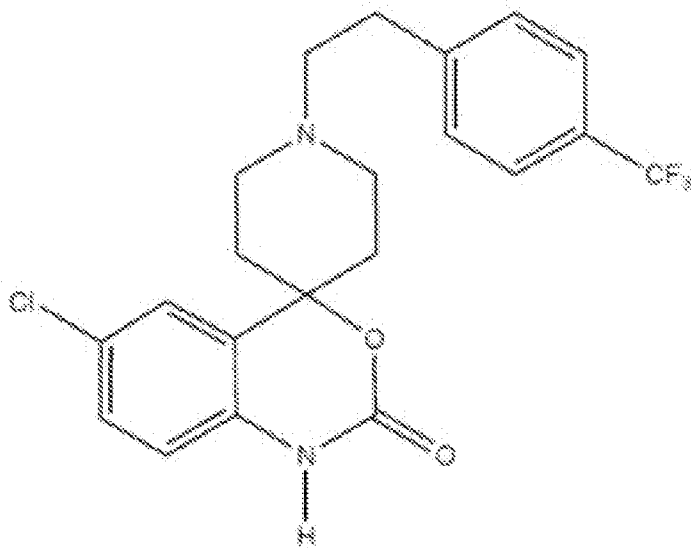
[0018] According to an additional or alternative aspect of the present disclosure, for the compound having structure I, at least one of one of $R^1 - R^5$ is a halogen selected from F, Br, or Cl.

[0019] According to an additional or alternative aspect of the present disclosure, for the compound having structure I, wherein at least one of one of $R^1 - R^5$ is a halogen selected from F or Cl.

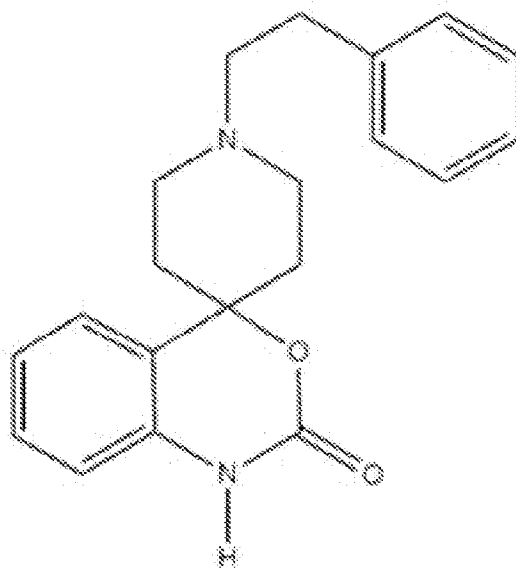
[0020] According to an additional or alternative aspect of the present disclosure, for the compound having structure I, the compound is selected from:



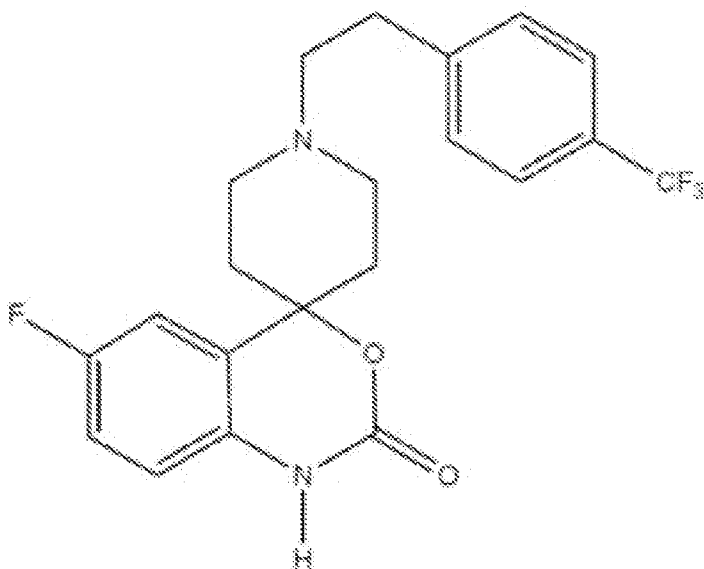
1'-(4-(trifluoromethyl)phenethyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



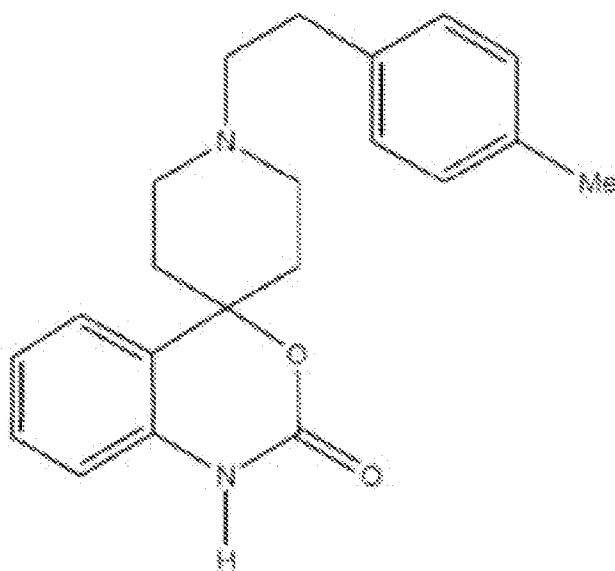
6-chloro-1'-(4-(trifluoromethyl)phenethyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



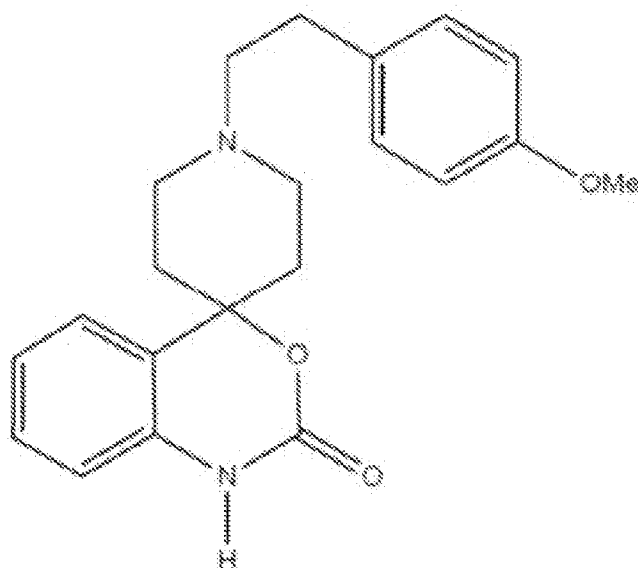
1'-phenethylspiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



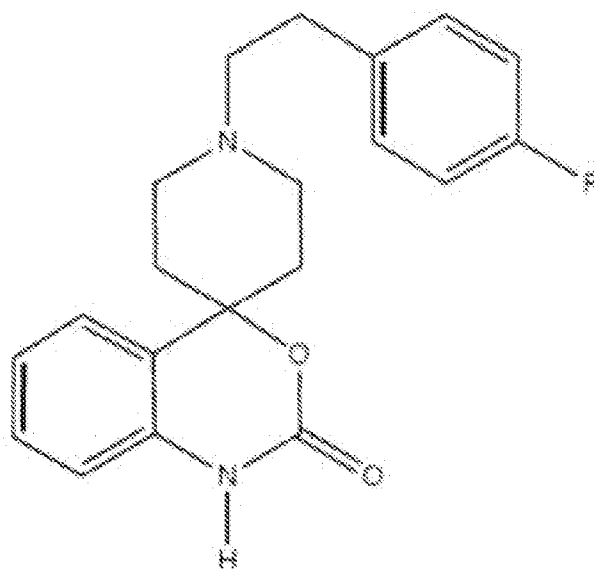
6-fluoro-1'-(4-(trifluoromethyl)phenethyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one.



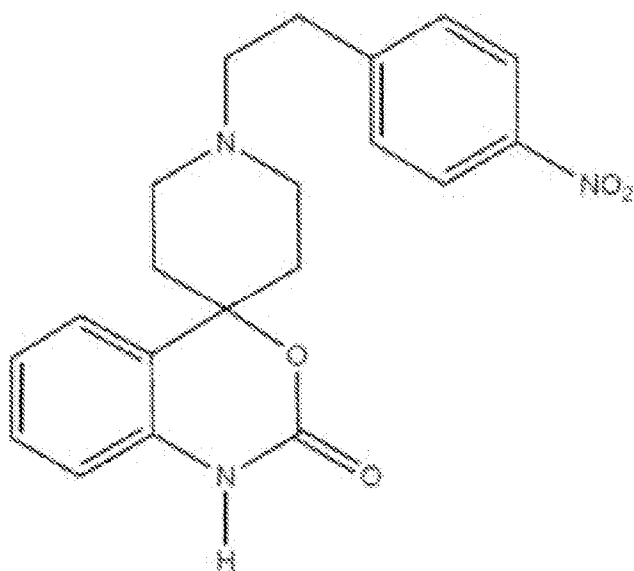
1'-(4-methylphenethyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



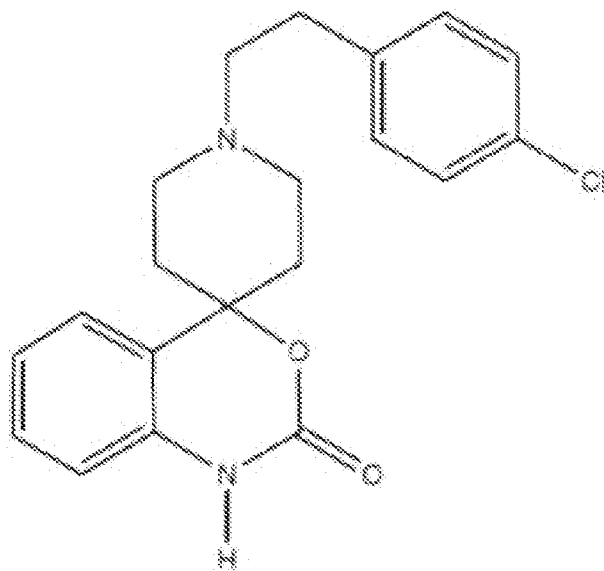
1'-(4-methoxyphenethyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



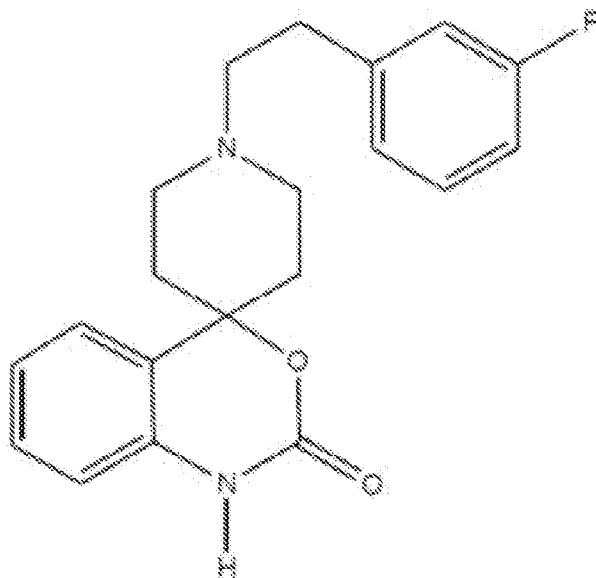
1'-(4-methoxyphenethyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



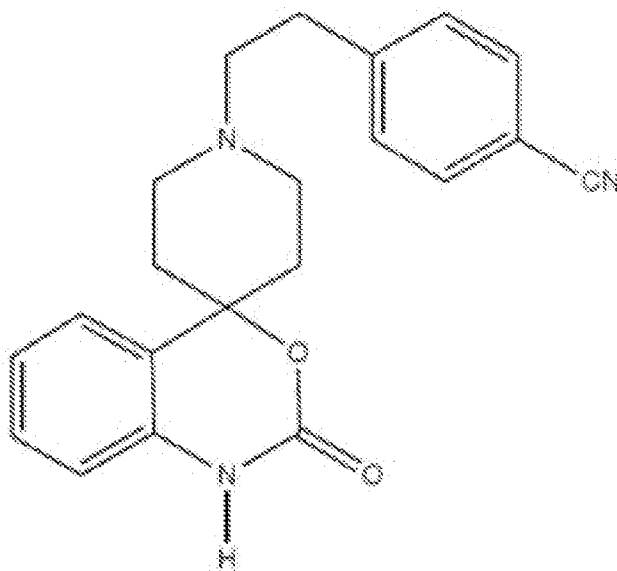
1'-(4-nitrophenethyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



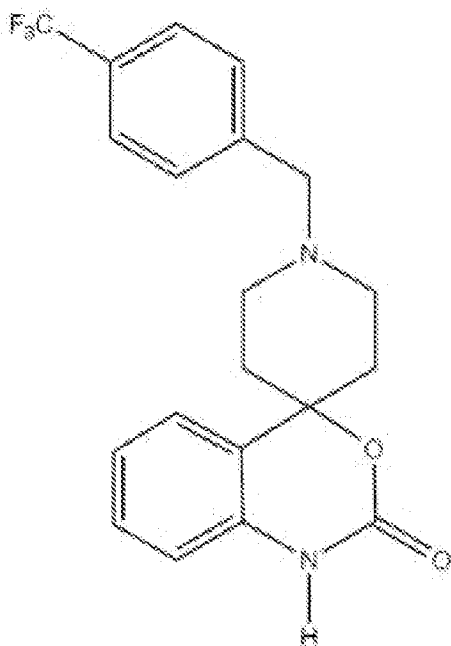
1'-(4-chlorophenethyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



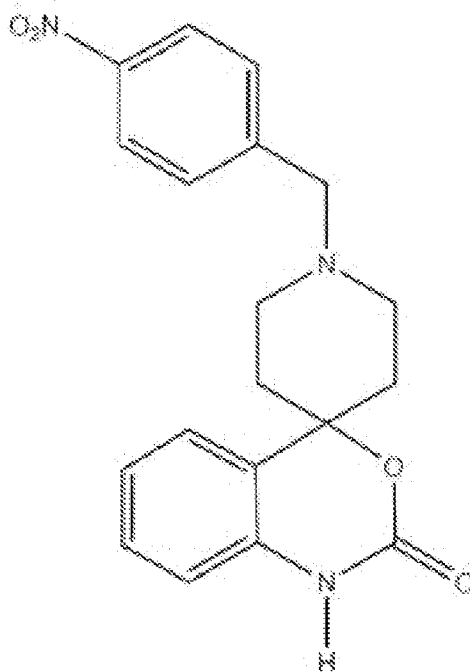
1'-(3-fluorophenethyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



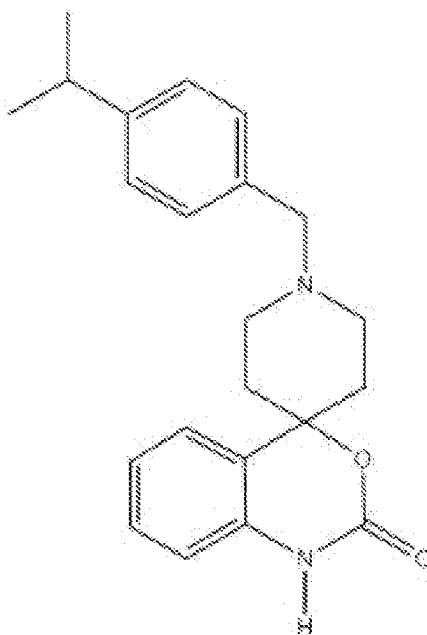
4-(2-(2-oxo-1,2-dihydrospiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-1'-yl)ethyl)benzonitrile,



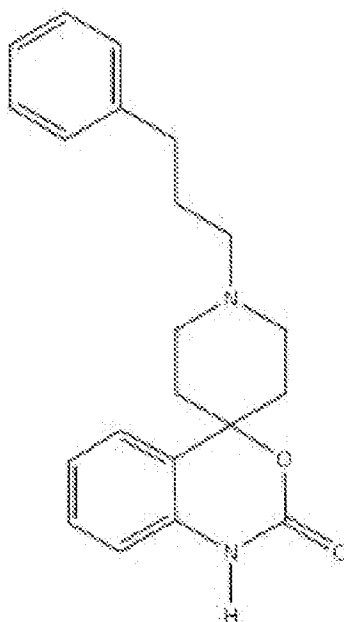
1'-(4-(trifluoromethyl)benzyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



1'-(4-nitrobenzyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



1'-(4-isopropylbenzyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one
, and/or



1'-(3-phenylpropyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,

or a pharmaceutically acceptable salt, solvate and/or prodrug thereof.

[0021] According to an additional or alternative aspect of the present disclosure, for the compound having structure I or any other compound disclosed herein, the compound is combined with a pharmaceutically acceptable carrier or vehicle to form a pharmaceutical composition.

[0022] According to an additional or alternative aspect of the present disclosure, for the compound having structure I or any compound disclosed herein, there is provided a method for inhibiting growth of an *Acinetobacter baumannii* bacterium, comprising contacting the bacterium with an effective amount of such compound.

[0023] According to an additional or alternative aspect of the present disclosure, for the compound having structure I or any compound disclosed herein, there is provided a method of treating an *Acinetobacter baumannii* bacterial infection comprising administering an effective amount of such compound to a subject in need thereof.

[0024] According to an additional or alternative aspect of the present disclosure, for the compound having structure I or any compound disclosed herein, there is provided a use of such compound to inhibit growth of an *Acinetobacter baumannii* bacterium.

[0025] According to an additional or alternative aspect of the present disclosure, for the compound having structure I or any compound disclosed herein, there is provided a use of such compound to treat or prevent an *Acinetobacter baumannii* bacterial infection.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] Certain embodiments of the disclosure will be described in greater detail with reference to the attached drawings, a brief description of which follows:

[0027] FIG. 1A is a schematic of aspects of compound discovery using machine learning to aid in guiding discovery;

[0028] FIG. 1B is a graph of growth inhibition of *A. baumannii* in accordance with an aspect of the present disclosure;

[0029] FIG. 1C is a graph of activity predictions using machine learning according to an aspect of the present disclosure;

[0030] FIG. 1D is a plot of data useful in a machine learning model according to an aspect of the present disclosure;

[0031] FIG. 1E is a graph of growth inhibition of *A. baumannii* in accordance with an aspect of the present disclosure;

[0032] FIG. 1F is a graph of activity against *A. baumannii* in accordance with an aspect of the present disclosure;

[0033] FIG. 1G is another graph of activity against *A. baumannii* in accordance with an aspect of the present disclosure;

[0034] FIG. 2A is a graph of growth inhibition of *A. baumannii* in accordance with an aspect of the present disclosure;

[0035] FIG. 2B is a graph of growth inhibition of *A. baumannii* in accordance with an aspect of the present disclosure;

[0036] FIG. 2C is a graph of growth inhibition of *P. aeruginosa* in accordance with an aspect of the present disclosure;

[0037] FIG. 2D is a graph of growth inhibition of *S. aureus* in accordance with an aspect of the present disclosure;

[0038] FIG. 2E is a map of growth inhibition in human skin and gut in accordance with an aspect of the present disclosure;

[0039] FIG. 3A is graph of growth inhibition of multiple microbes in accordance with an aspect of the present disclosure;

[0040] FIG. 3B is a depiction of the structure of microbes in accordance with an aspect of the present disclosure;

[0041] FIG. 3C is a graph of sequencing in accordance with an aspect of the present disclosure;

[0042] FIG. 3D is a graph of growth inhibition of *A. baumannii* in accordance with an aspect of the present disclosure;

[0043] FIG. 3E is a quantification of expression in accordance with an aspect of the present disclosure;

[0044] FIG. 3F depicts fluorescence micrographs of *A. baumannii* in accordance with an aspect of the present disclosure;

[0045] FIG. 4A is a graph of results of dorsal wound infection model in accordance with an aspect of the present disclosure;

[0046] FIG. 4B provides representative images of the dorsal surface in accordance with an aspect of the present disclosure;

[0047] FIG. 5A is a plot of screening data in accordance with an aspect of the present disclosure;

[0048] FIG. 5B depicts graphs of growth inhibition data in accordance with an aspect of the present disclosure;

[0049] FIG. 5C is a graph of growth inhibition of *A. baumannii* in accordance with an aspect of the present disclosure;

[0050] FIG. 5D is a graph of growth kinetics of *A. baumannii* cells in accordance with the present disclosure;

[0051] FIG. 6A is a graph of growth inhibition of *A. baumannii* in accordance with an aspect of the present disclosure;

[0052] FIG. 6B is a graph of growth inhibition of *B. breve* in accordance with an aspect of the present disclosure;

[0053] FIG. 6C is a graph of growth inhibition of *B. longum* in accordance with an aspect of the present disclosure;

[0054] FIG. 6D is a graph of growth inhibition of *E. lenta* in accordance with an aspect of the present disclosure;

[0055] FIGs. 7A-7H are graph of growth inhibition of *A. baumannii* by multiple different antibiotics in accordance with an aspect the present disclosure;

[0056] FIG. 7I and 7J are structural predictions in accordance with an aspect the present disclosure;

[0057] FIG. 7K provides graphs of RNA sequencing of *A. baumannii* in accordance with an aspect of the present disclosure;

[0058] FIG. 7L is a graph of growth inhibition of *A. baumannii* in accordance with an aspect of the present disclosure;

[0059] FIG. 7M provides graphs of quantifications in accordance with an aspect of the present disclosure.

DETAILED DESCRIPTION**I. DEFINITIONS**

[0060] Unless otherwise indicated, the definitions and embodiments described in this and other sections are intended to be applicable to all embodiments and aspects of the present disclosure herein described for which they are suitable as would be understood by a person skilled in the art. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be limiting.

[0061] In understanding the scope of the present disclosure, the term “comprising” and its derivatives, as used herein, are intended to be open ended terms that specify the presence of the stated features, elements, components, groups, integers, and/or steps, but do not exclude the presence of other unstated features, elements, components, groups, integers and/or steps. The foregoing also applies to words having similar meanings such as the terms, “including”, “having” and their derivatives. The term “consisting” and its derivatives, as used herein, are intended to be closed terms that specify the presence of the stated features, elements, components, groups, integers, and/or steps, but exclude the presence of other unstated features, elements, components, groups, integers and/or steps. The term “consisting essentially of”, as used herein, is intended to specify the presence of the stated features, elements, components, groups, integers, and/or steps as well as those that do not materially affect the basic and novel characteristic(s) of features, elements, components, groups, integers, and/or steps.

[0062] Terms of degree such as “substantially”, “about” and “approximately” as used herein mean a reasonable amount of deviation of the modified term such that the

end result is not significantly changed. These terms of degree should be construed as including a deviation of at least 5% of the modified term if this deviation would not negate the meaning of the word it modifies. The modifier “about” used in connection with a quantity is inclusive of the stated value and has the meaning dictated by the context (e.g., it includes the degree of error associated with measurement of the particular quantity). In addition, all ranges disclosed herein are inclusive of the endpoints and also any intermediate range points, whether explicitly stated or not, and the endpoints are independently combinable with each other.

[0063] As used in this disclosure, the singular forms “a”, “an” and “the” include plural references unless the content clearly dictates otherwise. For example, an embodiment including “an antibiotic” should be understood to present certain aspects with one antibiotic or two or more additional antibiotics.

[0064] In embodiments comprising an “additional” or “second” component, such as an additional or second antibiotic, the second component as used herein is chemically different from the other components or first component. A “third” component is different from the other, first, and second components, and further enumerated or “additional” components are similarly different.

[0065] The term “and/or” as used herein means that the listed items are present, or used, individually or in combination. In effect, this term means that “at least one of” or “one or more” of the listed items is used or present.

[0066] The term “or” as used herein is intended to include “and” unless the context clearly indicates otherwise

[0067] The abbreviation, “e.g.” is derived from the Latin *exempli gratia* and is used herein to indicate a non-limiting example. Thus, the abbreviation “e.g.” is synonymous with the term “for example.”

[0068] The term “suitable” as used herein means that the selection of the particular compound or conditions would depend on the specific synthetic manipulation to be performed or composition to be prepared, the identity of the molecule(s) to be transformed and/or the specific use for the compound, but the selection would be well within the skill of a person trained in the art.

[0069] The term “cell” as used herein refers to a single cell or a plurality of cells and includes a cell either in a cell culture or in a subject.

[0070] The term “treating” or “treatment” and the like as used herein, and as is well understood in the art, refers to an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results include, but are not limited to alleviation or amelioration of one or more symptoms or conditions, arresting development of disease, diminishment of extent of disease, stabilized (i.e. not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, including regression of the disease, diminishment of the reoccurrence of disease, and remission (whether partial or total), whether detectable or undetectable. “Treating” and “treatment” may also refer to prolonging survival as compared to expected survival if not receiving treatment. “Treating” and “treatment” as used herein also include prophylactic treatment. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of affecting a partial or complete cure for a disease and/or symptoms of the disease. For example, a subject with early

cancer can be treated to prevent progression, or alternatively a subject in remission can be treated to prevent recurrence. Prophylactic treatment includes preventing the disease or a symptom of a disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it (e.g., including diseases that may be associated with or caused by a primary disease).

[0071] Treating may refer to any indicia of success in the treatment or amelioration or prevention of an infection, including any objective or subjective parameter such as abatement; remission; diminishing of symptoms; or making the disease condition more tolerable to the patient; slowing in the rate of degeneration or decline; or making the final point of degeneration less debilitating. The treatment or amelioration of symptoms is based on one or more objective or subjective parameters; including the results of an examination by a physician. Accordingly, the term "treating" includes the administration of the methods of the present disclosure to prevent, delay, alleviate, arrest or inhibit development of the symptoms or conditions associated with diseases.

[0072] The term "subject" as used herein includes all members of the animal kingdom including mammals such as a mouse, a rat, a dog and a human. Thus, the methods and uses of the present disclosure are applicable to both human therapy and veterinary applications.

[0073] The term "pharmaceutically acceptable" as used herein means compatible with the treatment of subjects, for example humans.

[0074] The term "pharmaceutically acceptable carrier" as used herein means a non-toxic solvent, dispersant, excipient, adjuvant or other material which is mixed with

the active ingredient in order to permit the formation of a pharmaceutical composition, i.e., a dosage form capable of administration to a subject.

[0075] The term “pharmaceutically acceptable salt” as used herein means either an acid addition salt or a base addition salt which is suitable for, or compatible with the treatment of subjects.

[0076] The term “solvate” as used herein means a compound, or a salt and/or prodrug of a compound, wherein molecules of a suitable solvent are incorporated in the crystal lattice. A suitable solvent is physiologically tolerable at the dosage administered.

[0077] The term “prodrug” as used herein means a compound, or salt and/or solvate of a compound, that, after administration, is converted into an active drug.

[0078] The term “administering” or “administration” and the like as used herein refers to the placement of a compound, one of more compounds or a pharmaceutical composition thereof, as disclosed herein into a cell, either in cell culture or in a subject, by a method or route which results in at least partial delivery to a desired site. The compounds and compositions disclosed herein can be administered by any appropriate route which results in an effective treatment in the subject. Possible routes of administration of the compounds and pharmaceutical compositions disclosed herein include, but are not limited to, topical, intravenous, intraperitoneal, intramuscular, subcutaneous, transdermal, oral, buccal, sublingual, intranasal, or rectal routes of administration, or a combination thereof.

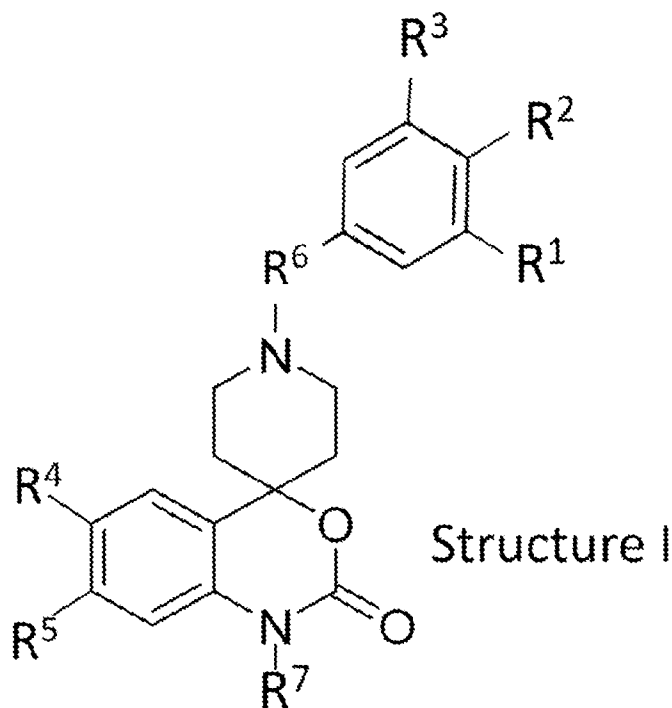
[0079] As used herein, the term “effective amount” or “therapeutically effective amount” means an amount of a compound, or one or more compounds, of the disclosure

that is effective, at dosages and for periods of time necessary to achieve the desired result.

II. Compounds, Compositions, Methods and Uses of the Disclosure

[0080] Herein, ~7,500 molecules were screened for those that inhibited the growth of *A. baumannii* *in vitro*. A message passing neural network was trained with this growth inhibition dataset and performed predictions on the Drug Repurposing Hub¹⁵ for structurally novel molecules with activity against *A. baumannii*. Through this approach, antibacterial compounds with narrow-spectrum activity against *A. baumannii* were discovered, which could overcome intrinsic and acquired resistance mechanisms in clinical isolates. Further mechanistic investigations revealed that these compounds perturb lipoprotein trafficking through a mechanism involving LolE, a functionally conserved protein that contributes to shuttling lipoproteins from the inner membrane to the outer membrane^{16,17}. Moreover, these compounds were able to control an *A. baumannii* infection in a murine wound model.

[0081] Accordingly, the present disclosure provides compounds (i.e., antimicrobial compounds) for inhibiting growth of *Acinetobacter baumannii*. In one embodiment, the compounds are defined according to Structure I as follows:



[0082] Compounds having Structure I have been found to have particularly desirable activity against *A. baumannii* but are not so limited unless specifically stated.

[0083] Substructure I allows for multiple variations in an embodiment I as follows:

$R^1 - R^3$ are independently selected from H, OH, CH_3 , C_2H_5 , NO_2 , CN, halogen, alkoxy (e.g., methoxy), or trifluoromethyl, with at least one and more typically two, and quite possibly all three of $R^1 - R^3$ being H; and/or

R^4 and R^5 are independently selected from H, OH, halogen or amine (e.g., primary amine), with none or more typically one or even possibly both of R^4 and R^5 being H; and/or

R^6 is $C_1 - C_3$ alkyl or alkylene; and/or

R^7 is H or $C_1 - C_3$ alkyl or alkylene.

[0084] Substructure I further allows for multiple variations and/or further definition in embodiment I with any one or any combination of the following:

R^1 and R^3 are H;

R^2 is selected from H, OH, CH_3 , C_2H_5 , NO_2 , halogen, alkoxy (e.g., methoxy), or trifluoromethyl, and more typically from H, methoxy, halogen or trifluoromethyl, or any sub-combination thereof;

R^4 is H;

R^5 is selected from H, OH, halogen or amine (e.g., primary amine), and more typically from H or halogen, with none or more typically one or both of R^4 and R^5 being H; and/or

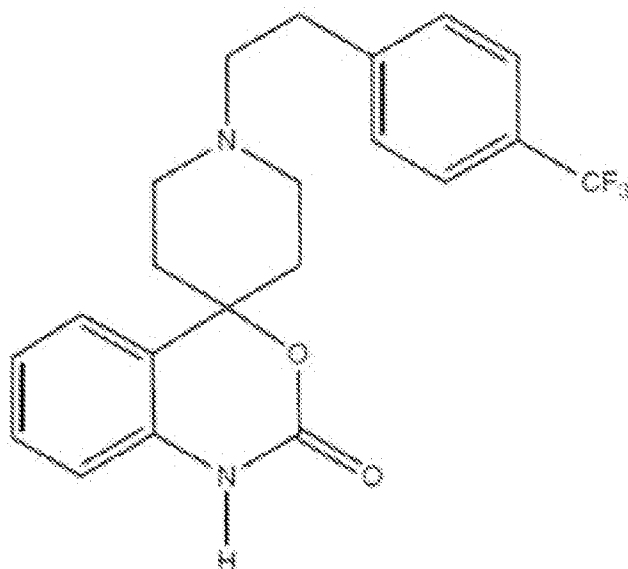
R^6 is C_1 - C_2 alkyl and typically C_2 alkyl; and/or

R^7 is H or C_1 - C_2 alkyl and typically H.

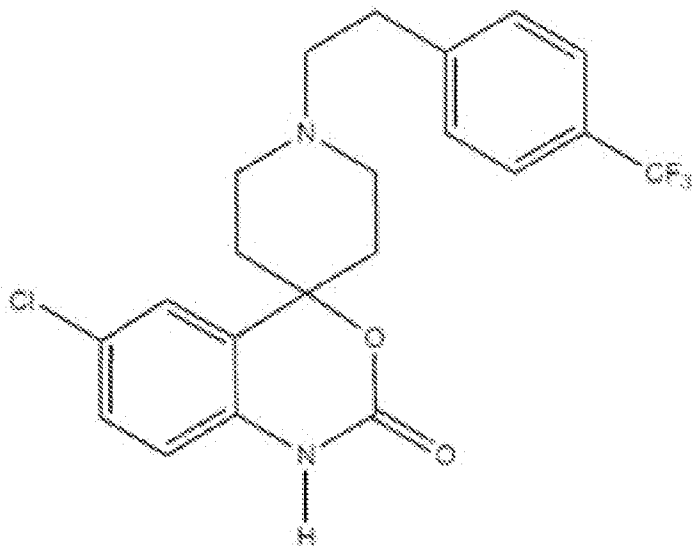
[0085] Within Substructure I, when any one or combination of $R^1 - R^5$ is halogen, the halogen is typically selected from F, Br, or Cl. Thus, any one or combination of $R^1 - R^5$ can be specifically defined as F, Br, or Cl. In examples, R^2 is or is selected from F or Cl and/or R^4 is or is selected from F or Cl.

[0086] It is understood that substructure I as well as compounds disclosed herein include all stereoisomers thereof, unless otherwise specifically stated.

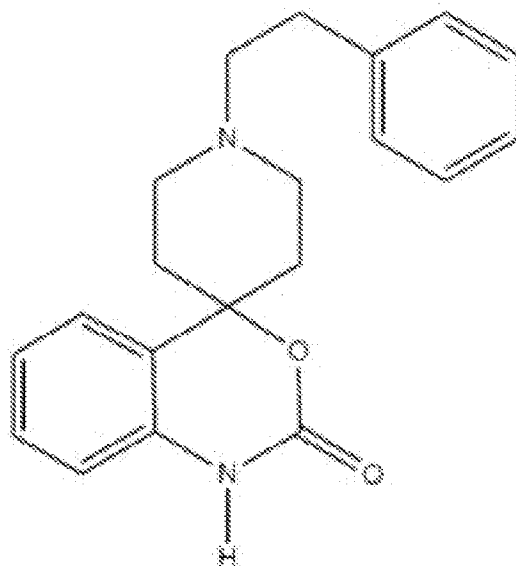
[0087] Accordingly, provided in the present disclosure is a compound for inhibiting growth of an *Acinetobacter baumannii* bacterium, particularly a compound of substructure I, selected from:



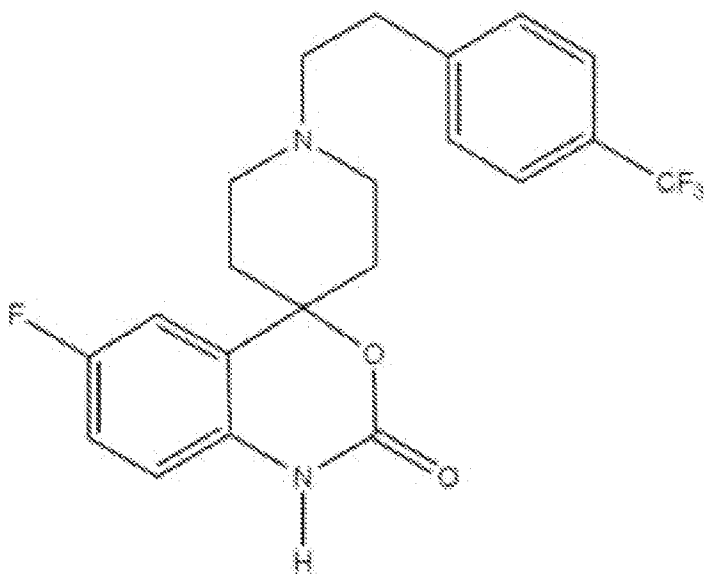
1'-(4-(trifluoromethyl)phenethyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



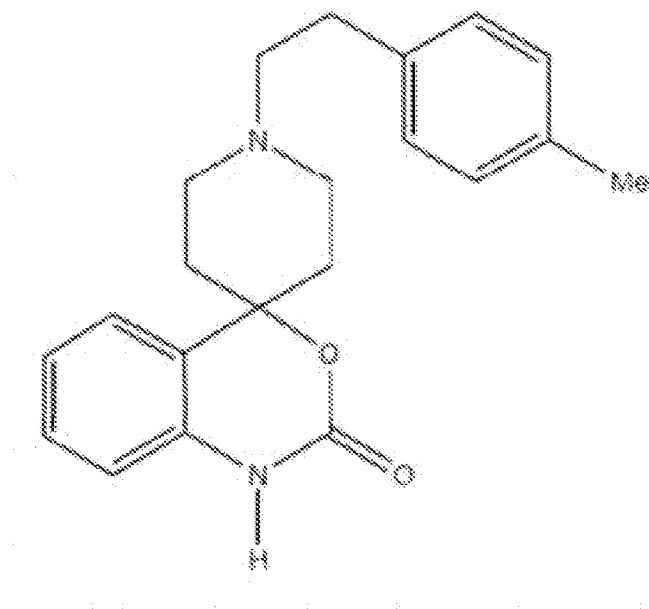
6-chloro-1'-(4-(trifluoromethyl)phenethyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



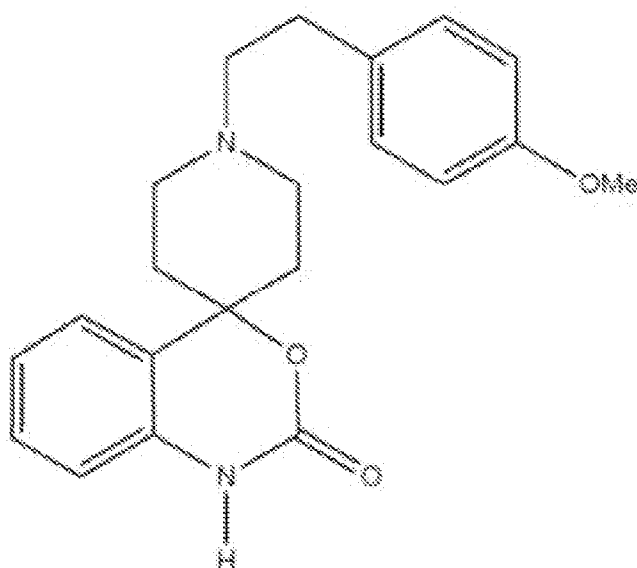
1'-phenethylspiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



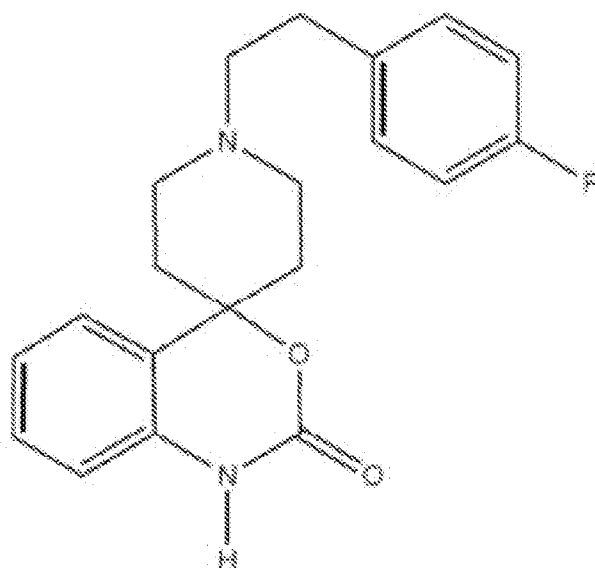
6-fluoro-1'-(4-(trifluoromethyl)phenethyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one.



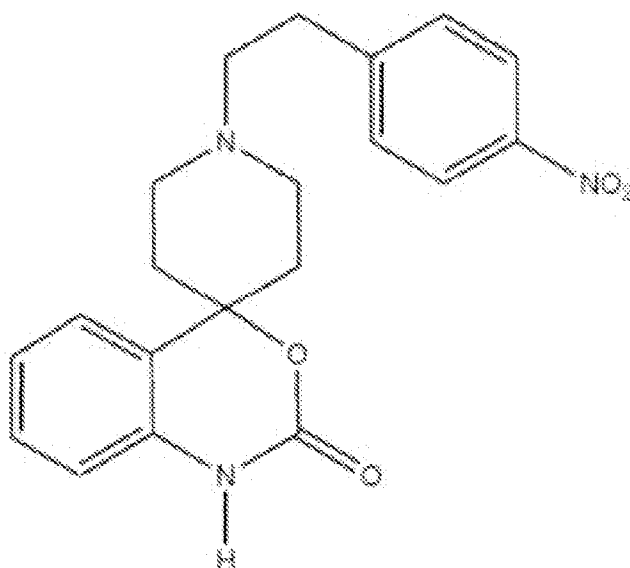
1'-(4-methylphenethyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one.



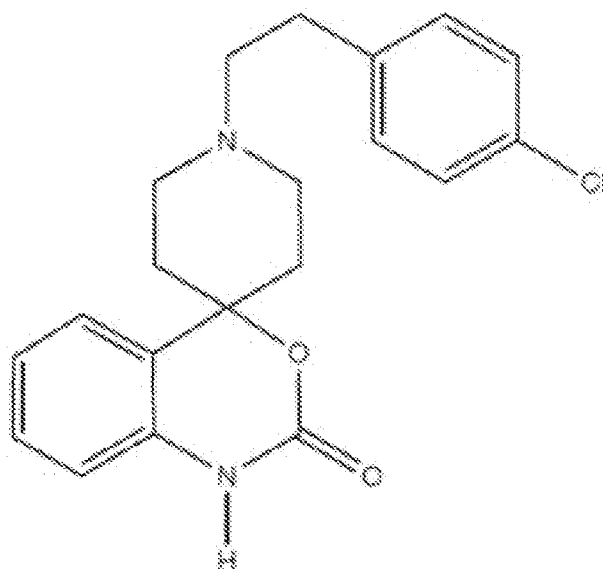
1'-(4-methoxyphenethyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one.



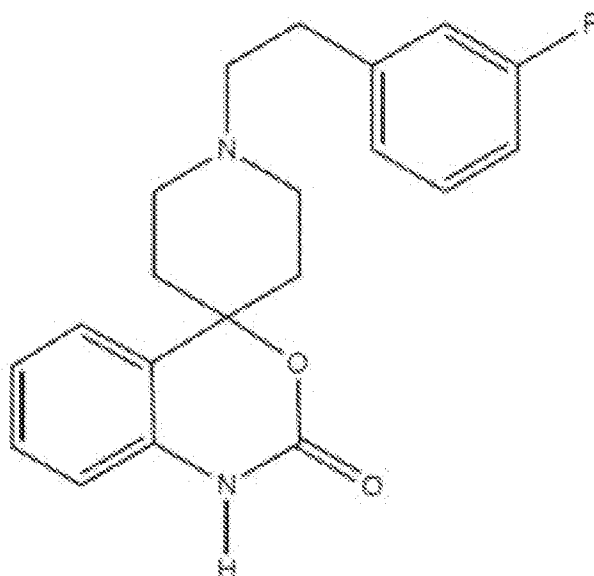
1'-(4-methoxyphenethyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



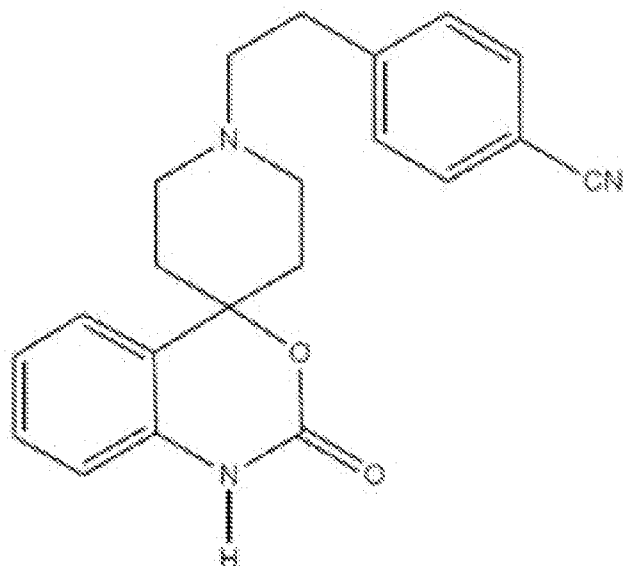
1'-(4-nitrophenethyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



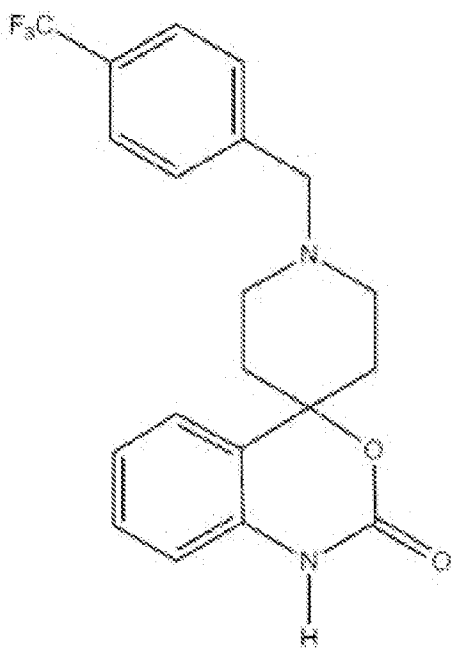
1'-(4-chlorophenethyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



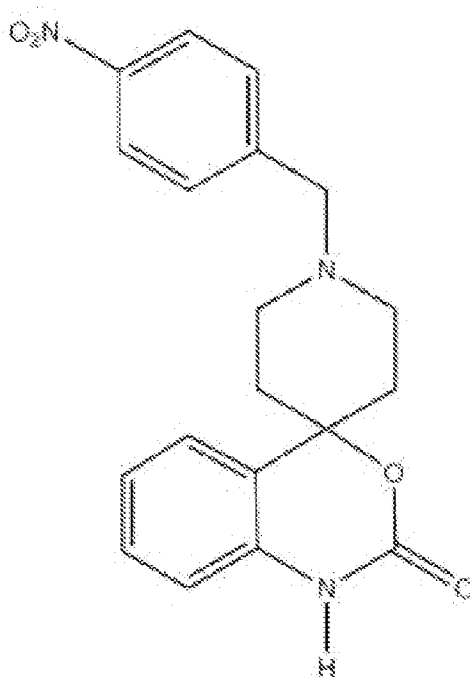
1'-(3-fluorophenethyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



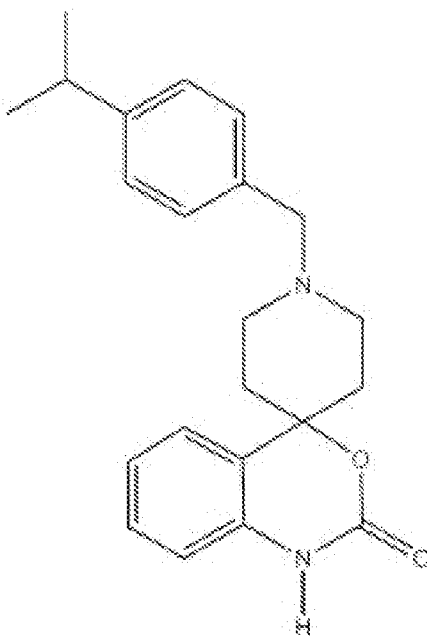
4-(2-(2-oxo-1,2-dihydrospiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-1'-yl)ethyl)benzonitrile,



1'-(4-(trifluoromethyl)benzyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,

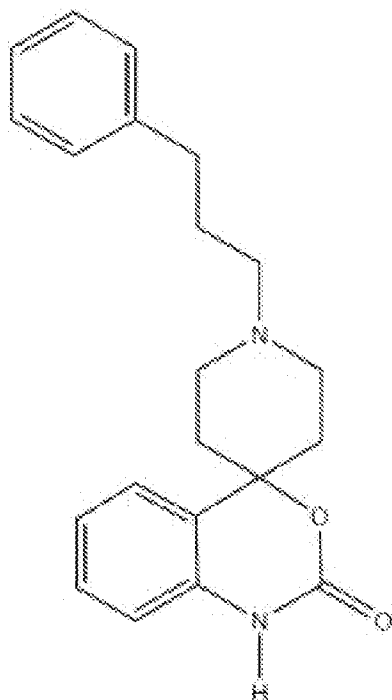


1'-(4-nitrobenzyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



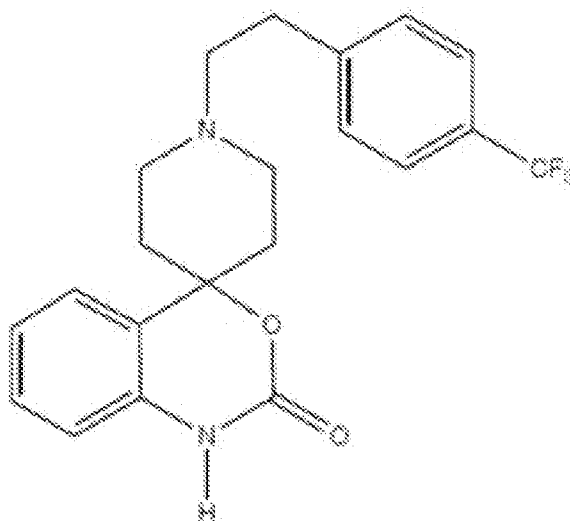
1'-(4-isopropylbenzyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one

, and/or



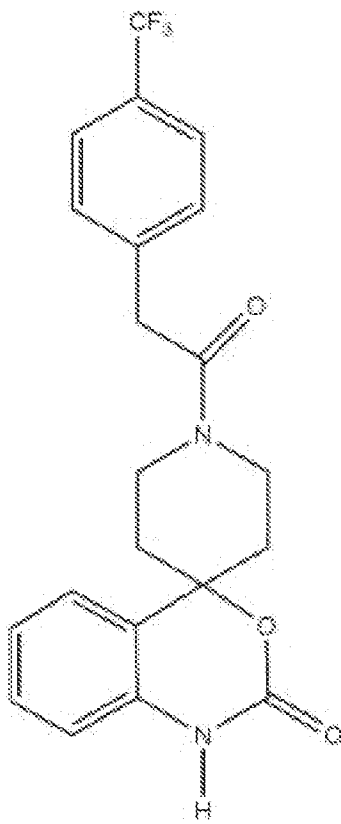
1'-(3-phenylpropyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,
or a pharmaceutically acceptable salt, solvate and/or prodrug thereof.

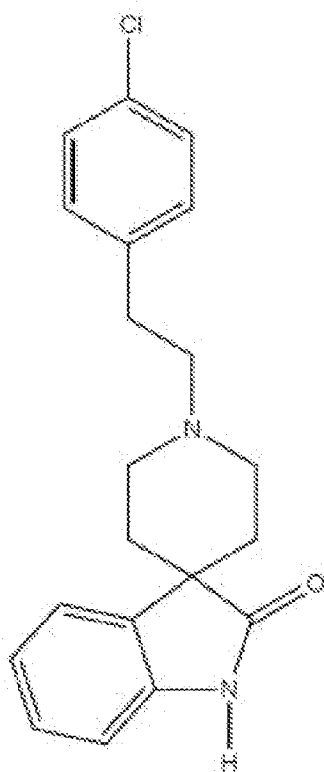
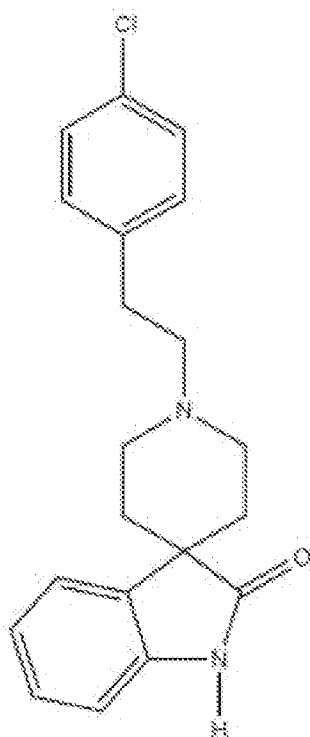
[0088] In some preferred embodiments, the compound is as follows:

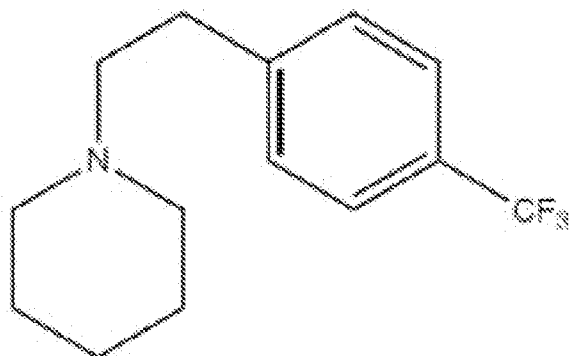


also referred to as abaucin.

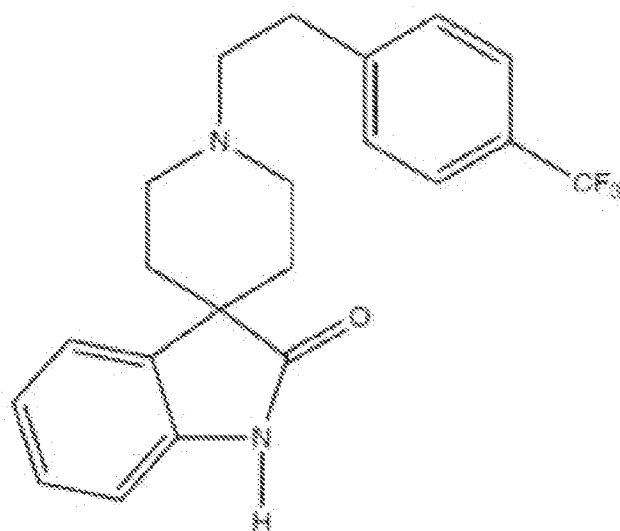
[0089] Additional compounds provided in the present disclosure for inhibiting growth of an *Acinetobacter baumannii* bacterium selected from:







, and/or



or a pharmaceutically acceptable salt, solvate and/or prodrug thereof.

[0090] It is contemplated that these additional or alternative compounds can be used in any of the ways and can be combined with other ingredients in the same manner as any of the compounds of Structure I.

[0091] When provided in salt form, the compounds of the present disclosure can be in any salt form possible unless otherwise stated. One desirable salt form for the compounds is a hydrochloride (HCl) salt form.

[0092] The compounds of the present disclosure typically exhibit a desirable minimum inhibitory concentration (MIC) against *A. Baumannii*, particularly *A.*

baumannii ATCC 17978, as calculated in accordance with the present disclosure. Such MIC is typically no greater than 64 µg/ml, more typically no greater than 32 µg/ml, even more typically no greater than 16 µg/ml or even possibly no greater than 8 µg/ml or 2 µg/ml.

[0093] The compounds of the present disclosure may be suitably formulated in a conventional manner into compositions using one or more carriers. Accordingly, the present disclosure includes a composition comprising one or more compounds of the disclosure and a carrier. The compounds of the disclosure may be suitably formulated into pharmaceutical compositions for administration to subjects in a biologically compatible form suitable for administration *in vivo*. Accordingly, the present disclosure also includes a composition comprising one of more compounds disclosed herein, or a pharmaceutically acceptable salt, solvate and/or prodrug thereof, and a pharmaceutically acceptable carrier or vehicle. Conventional procedures and ingredients for the selection and preparation of suitable compositions are described, for example, in Remington's Pharmaceutical Sciences (2000 - 20th edition) and in The United States Pharmacopeia: The National Formulary (USP 24 NF19) published in 1999.

[0094] In some embodiments, suitable pharmaceutically acceptable carriers include, but are not limited to, inert solid fillers or diluents and sterile aqueous or organic solutions. Pharmaceutically acceptable carriers are well known to those skilled in the art and include, but are not limited to, aqueous and non-aqueous solutions. Pharmaceutically acceptable carriers can be aqueous or non- aqueous solutions, suspensions and emulsions. Examples of non-aqueous solvents suitable for use in the present disclosure include, but are not limited to, propylene glycol, polyethylene glycol,

vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers suitable for use in the present disclosure include, but are not limited to, water, ethanol, alcoholic/aqueous solutions, glycerol, emulsions or suspensions, including saline and buffered media.

[0095] Compounds of the disclosure may also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxypropylmethacrylamide-phenol, polyhydroxy-ethylaspartamide-phenol, or polyethyleneoxide-polylysine substituted with palmitoyl residues. Furthermore, compounds of the disclosure may be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyglycolic acid, copolymers of polylactic and polyglycolic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacrylates and crosslinked or amphipathic block copolymers of hydrogels.

[0096] In some embodiments, depending on the mode of administration, the pharmaceutical composition will comprise from about 0.05 wt% to about 99 wt% or about 0.10 wt% to about 70 wt%, of the active ingredient (one or more compounds of the disclosure), and from about 1 wt% to about 99.95 wt% or about 30 wt% to about 99.90 wt% of one or more pharmaceutically acceptable carriers, all percentages by weight being based on the total composition.

[0097] Salts of the compounds of the disclosure are generally formed by dissolving the neutral compound in an inert organic solvent and adding either the desired acid or base and isolating the resulting salt by either filtration or other known means.

[0098] The formation of solvates of the compounds of the disclosure will vary depending on the compound and the solvate. In general, solvates are formed by dissolving the compound in the appropriate solvent and isolating the solvate by cooling or using an antisolvent. The solvate is typically dried or azeotroped under ambient conditions. The selection of suitable conditions to form a particular solvate can be made by a person skilled in the art. Examples of suitable solvents are ethanol, water and the like. When water is the solvent, the molecule is referred to as a “hydrate”.

[0099] Prodrugs of the compounds of the present disclosure may be, for example, conventional esters formed with available hydroxy, thiol, amino or carboxyl groups. For example, available hydroxy or amino groups may be acylated using an activated acid in the presence of a base, and optionally, in inert solvent (e.g. an acid chloride in pyridine). Some common esters which have been utilized as prodrugs are phenyl esters, aliphatic (C₁-C₂₄) esters, acyloxymethyl esters, carbamates and amino acid esters.

[00100] Throughout the processes described herein it is to be understood that, where appropriate, suitable protecting groups will be added to, and subsequently removed from, the various reactants and intermediates in a manner that will be readily understood by one skilled in the art. Conventional procedures for using such protecting groups as well as examples of suitable protecting groups are described, for example, in “*Protective Groups in Organic Synthesis*”, T.W. Green, P.G.M. Wuts, Wiley-Interscience, New York, (1999). It is also to be understood that a transformation of a group or substituent into another group or substituent by chemical manipulation can be conducted on any intermediate or final product on the synthetic path toward the final product, in which the possible type of transformation is limited only by inherent

incompatibility of other functionalities carried by the molecule at that stage to the conditions or reagents employed in the transformation. Such inherent incompatibilities, and ways to circumvent them by carrying out appropriate transformations and synthetic steps in a suitable order, will be readily understood to one skilled in the art. Examples of transformations are given herein, and it is to be understood that the described transformations are not limited only to the generic groups or substituents for which the transformations are exemplified. References and descriptions of other suitable transformations are given in “Comprehensive Organic Transformations – A Guide to Functional Group Preparations” R.C. Larock, VHC Publishers, Inc. (1989). References and descriptions of other suitable reactions are described in textbooks of organic chemistry, for example, “*Advanced Organic Chemistry*”, March, 4th ed. McGraw Hill (1992) or, “*Organic Synthesis*”, Smith, McGraw Hill, (1994). Techniques for purification of intermediates and final products include, for example, straight and reversed phase chromatography on column or rotating plate, recrystallisation, distillation and liquid-liquid or solid-liquid extraction, which will be readily understood by one skilled in the art.

[00101] In another aspect of the disclosure, provided is a method for inhibiting growth of an *Acinetobacter baumannii* bacterium, comprising contacting the bacterium with an effective amount of a compound disclosed herein or a pharmaceutically acceptable salt, solvate and/or prodrug thereof.

[00102] In a further aspect of the disclosure, provided is a method of treating an *Acinetobacter baumannii* bacterial infection comprising administering an effective amount of a compound disclosed herein or a pharmaceutically acceptable salt, solvate and/or prodrug thereof, to a subject in need thereof.

[00103] In some embodiments, the subject is a mammal. In some embodiments, the subject is a human.

[00104] Effective amounts may vary according to factors such as the disease state, age, sex and/or weight of the subject. The amount of a given compound that will correspond to such an amount will vary depending upon various factors, such as the given drug or compound, the pharmaceutical formulation, the route of administration, the type of condition, disease or disorder, the identity of the subject being treated, and the like, but can nevertheless be routinely determined by one skilled in the art. The effective amount is one that following treatment therewith manifests as an improvement in or reduction of any disease symptom.

[00105] *In vivo* administration of a compound and/or composition of the disclosure can be accomplished by any suitable method and technique presently or prospectively known to those skilled in the art. For example, a compound of the disclosure may be formulated in a physiologically or pharmaceutically acceptable form and administered by any suitable route known in the art including, for example, oral and parenteral routes of administration. As used herein, the term parenteral may include intravenous (such as by injection), intra-arterial, intraperitoneal, subcutaneous, intramuscular, intrathecal transepithelial, nasal, intrapulmonary (for example, by use of an aerosol), rectal and topical (including the use of a patch or other transdermal delivery device) modes of administration. Administration of a compound of the disclosure may comprise a single administration, or a plurality of administrations at continuous or distinct intervals over a selected period of time.

[00106] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous

preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists.

[00107] A compound of the disclosure may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard- or soft-shell gelatin capsules, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the compound may be incorporated with excipient and used in the form of ingestible tablets, buccal tablets, troches, capsules, caplets, pellets, granules, lozenges, chewing gum, powders, syrups, elixirs, wafers, aqueous solutions and suspensions, and the like. In the case of tablets, carriers that are used include lactose, corn starch, sodium citrate and salts of phosphoric acid. Pharmaceutically acceptable excipients include binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well known in the art. In the case of tablets, capsules, caplets, pellets or granules for oral administration, pH sensitive enteric coatings, such as Eudragits™ designed to control the release of active ingredients are optionally used. Oral dosage forms also include modified release, for example immediate release and timed-release, formulations. Examples of modified-release formulations include, for example, sustained-release (SR), extended-release (ER, XR, or XL), time-release or timed-release, controlled-release (CR), or continuous-release (CR or Contin), employed, for example, in the form of a coated tablet, an osmotic delivery device, a coated capsule, a microencapsulated microsphere, an agglomerated particle, e.g., as of

molecular sieving type particles, or, a fine hollow permeable fiber bundle, or chopped hollow permeable fibers, agglomerated or held in a fibrous packet. Timed-release compositions can be formulated, e.g. liposomes or those wherein the active compound is protected with differentially degradable coatings, such as by microencapsulation, multiple coatings, etc. Liposome delivery systems include, for example, small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines. For oral administration in a capsule form, useful carriers or diluents include lactose and dried corn starch.

[00108] Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they are suitably presented as a dry product for constitution with water or other suitable vehicle before use. When aqueous suspensions and/or emulsions are administered orally, the compound of the disclosure is suitably suspended or dissolved in an oily phase that is combined with emulsifying and/or suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added. Such liquid preparations for oral administration may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, methyl cellulose or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters or ethyl alcohol); and preservatives (e.g., methyl or propyl p-hydroxybenzoates or sorbic acid). Useful diluents include lactose and high molecular weight polyethylene glycols.

[00109] It is also possible to freeze-dry the compounds of the disclosure and use the lyophilizates obtained, for example, for the preparation of products for injection.

[00110] The compounds of the disclosure may be formulated for parenteral administration by injection, including using conventional catheterization techniques or infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as sterile suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulating agents such as suspending, stabilizing and/or dispersing agents. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. Alternatively, compounds of the disclosure are suitably in a sterile powder form for reconstitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

[00111] A compound of the disclosure may be administered as a topical composition, such as, but not limited to, a solution, gel, cream, lotion, liquid suspension, aerosol, nebulized spray, ointment, drops or patch. In some embodiments, a compound and/or composition of the disclosure is administered topically. In some embodiments, a compound and/or composition of the disclosure is administered as a topical cream.

[00112] Compositions for nasal administration may conveniently be formulated as aerosols, drops, gels and powders. For intranasal administration or administration by inhalation, the compounds of the disclosure are conveniently delivered in the form of a solution, dry powder formulation or suspension from a pump spray container that is squeezed or pumped by the patient or as an aerosol spray presentation from a pressurized container or a nebulizer. Aerosol formulations typically comprise a solution or fine suspension of the active substance in a physiologically acceptable aqueous or non-aqueous solvent and are usually presented in single or multidose quantities in sterile form in a sealed container, which can take the form of a cartridge or refill for use

with an atomising device. Alternatively, the sealed container may be a unitary dispensing device such as a single dose nasal inhaler or an aerosol dispenser fitted with a metering valve which is intended for disposal after use. Where the dosage form comprises an aerosol dispenser, it will contain a propellant which can be a compressed gas such as compressed air or an organic propellant such as fluorochlorohydrocarbon. Suitable propellants include but are not limited to dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, heptafluoroalkanes, carbon dioxide or another suitable gas. In the case of a pressurized aerosol, the dosage unit is suitably determined by providing a valve to deliver a metered amount. The pressurized container or nebulizer may contain a solution or suspension of the active compound. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated containing a powder mix of a compound of the disclosure and a suitable powder base such as lactose or starch. The aerosol dosage forms can also take the form of a pump-atomizer.

[00113] In another aspect of the disclosure, provided is the use of a compound disclosed herein to inhibit growth of an *Acinetobacter baumannii* bacterium.

[00114] In a further aspect of the disclosure, provided is the use of a compound disclosed herein or a pharmaceutically acceptable salt pharmaceutically acceptable salt, solvate and/or prodrug thereof, to treat or prevent an *Acinetobacter baumannii* bacterial infection.

[00115] **DRAWINGS**

[00116] **FIGs. 1A-1G** show machine learning-guided discovery of compounds (e.g., abaucin) in exemplary embodiments of the disclosure: (FIG. 1A) ~7,500

molecules were screened for those that inhibited the growth of *A. baumannii* (left funnel), trained a directed message passing deep neural network with this growth inhibition dataset, and performed predictions (top funnel) on the Drug Repurposing Hub for structurally novel molecules with activity against *A. baumannii* (bottom funnel); (FIG. 1B) growth inhibition of *A. baumannii* ATCC 17978 by a collection of 7,684 small molecules at 50 μ M – shown is the mean of two biological replicates; lower dots represent actives, with the hit cut-off defined as one standard deviation below the mean of the dataset; (FIG. 1C) rank-ordered prediction scores of molecules within the Drug Repurposing Hub by the trained model: molecules with prediction scores >0.2 were considered as preliminary candidates for experimental validation; (FIG. 1D) a t-distributed stochastic neighbour embedding (tSNE) plot showing the chemical relationship between the training dataset, the prediction set, and abaucin; (FIG. 1E) growth inhibition of *A. baumannii* by abaucin in LB medium conducted in biological duplicate; error bars represent absolute range of measurements and the structure of abaucin is shown; (FIG. 1F) killing of *A. baumannii* by abaucin in nutrient-replete conditions (LB) at varying concentrations after incubation for 1.5 hr, 3 hr, 4.5 hr, and 6 hr conducted in biological duplicate; error bars represent absolute range of measurements; (FIG. 1G) killing of *A. baumannii* by abaucin in nutrient-deplete conditions (PBS) at varying concentrations after incubation for 1.5 hr, 3 hr, 4.5 hr, and 6 hr conducted in biological duplicate; error bars represent absolute range of measurements.

[00117] **FIGs. 2A-2E** show abaucin has a narrow phylogenetic spectrum of antibacterial activity in exemplary embodiments of the disclosure: (FIG. 2A) growth inhibition of 41 multidrug-resistant clinical *A. baumannii* isolates by abaucin - drug

concentrations are shown as fold-MIC based on the MIC of abaucin against *A. baumannii* ATCC 17978 and each dot represents a different clinical strain (mean OD of two biologically independent replicates), while black bars show the mean growth inhibition of the entire panel at each abaucin concentration; (FIG. 2B) is the same parameters as (FIG. 2A), except using 24 carbapenem-resistant Enterobacteriaceae clinical isolates; (FIG. 2C) is the same parameters as (FIG. 2A), except using 24 multidrug-resistant *P. aeruginosa* clinical isolates; (FIG. 2D) is the same parameters as (FIG. 2A), except using 14 *S. aureus* clinical isolates; (FIG. 2E) heat map showing mean growth inhibition of a panel of human gut commensal species (left) and human skin commensal species (right) by abaucin at 0x, 1x, 10x, 20x MIC (left to right), ampicillin (A) at 128 µg/ml, and ciprofloxacin (C) at 2 µg/ml – darker grey indicates more growth inhibition as analyzed by optical density (Actino is Actinobacteriota, Bact is Bacteroidota, Fermi is Firmicutes, Proteo is Proteobacteria, Verruco is Verrucomicrobiota) conducted in biological duplicate.

[00118] **FIGs. 3A-3F** shows abaucin inhibits lipoprotein trafficking in *A. baumannii* in exemplary embodiments of the disclosure: (FIG. 3A) growth inhibition of wildtype *A. baumannii*, two independent abaucin-resistant mutants with the A362T mutation in LolE (2,901,674 C→T), a mutant with the Y394F mutation in LolE (2,901,577 T→A), and a mutant with an intergenic mutation (2,902,955 G→A) upstream of LolE conducted in biological duplicate and error bars represent absolute range of measurements (all strains were grown in LB medium); (FIG. 3B) structure of the LolCDE complex from *E. coli* (*EcLolC* at 300; *EcLolD* at 302; *EcLolE* at 304), with the predicted structure of *A. baumannii* LolE overlayed (*AbLolE* at 306) – the highlighted region shows the position of the A362T mutation of *AbLolE* and the

homologous position in *E. coli* (I365) and the grey ribbon shows the position of the nascent acyl chain of the bound lipoprotein; (FIG. 3C) RNA sequencing of wildtype *A. baumannii* treated with 5x MIC ABAUCIN for 3 hr shown as the mean of biological duplicates – transcript abundance in drug-treated samples is normalized to no-drug control cultures grown in identical conditions – vertical black lines show statistical significance cut-off values; (FIG. 3D) growth inhibition of *A. baumannii* harboring an empty CRISPRi vector, or three distinct sgRNAs targeting *lolE* conducted in biological duplicate and error bars represent absolute range of measurements (all strains were grown in LB medium with induction using aTc to knock down *lolE*); (FIG. 3E) qPCR quantifying the expression of *lolE* relative to the housekeeping gene *rpoD* in all four abaucin resistant mutants, normalized to wildtype *A. baumannii* conducted in biological duplicate with technical triplicates; bar height represents mean normalized expression; (FIG. 3F) fluorescence micrographs of wildtype *A. baumannii* treated with abaucin: from left to right, cells were treated with no drug, 1 µg/ml abaucin, 1.5 µg/ml abaucin, or 2 µg/ml abaucin prior to imaging.

[00119] **FIGs. 4A-4B** shows abaucin can suppress *A. baumannii* infection in a wound model in exemplary embodiments of the disclosure: (FIG. 4A) in a dorsal wound infection model, mice were infected with *A. baumannii* ATCC 17978 (~6.5 x 10⁶ CFU) and after 1 hr of infection, mice were treated with vehicle (n=6) or 4% abaucin (n=6) over 24 hr; bacterial load from wound tissue after treatment (25 hr post-infection) was determined by selective plating; pre-Tx represents the bacterial load at time of initial treatment (n=5); black lines represent mean ± standard deviation of the bacterial load for each group; ns means not statistically significant; ** means p<0.005 using unpaired t test with Welch's correction; (FIG. 4B) representative images of the dorsal surface of

mice prior to infection (t=0), after 24 hr of treatment with vehicle, and after 24 hr of treatment with ABAUCIN (inflammation seen in the vehicle control is shown with arrows).

[00120] **FIGs. 5A-5D** show model training data and predictions in exemplary embodiments of the disclosure: (FIG. 5A) replicate plot showing primary screening data of 7,684 small molecules for those that inhibited the growth of *A. baumannii* ATCC 17978 in LB medium at 50 μ M. (FIG. 5B) rank-ordered growth inhibition data of the prioritized 240 molecules from the prediction set that were selected for empirical validation (top); rank-ordered growth inhibition data of the 240 predicted molecules with the lowest prediction score (middle); rank-ordered growth inhibition data of the 240 predicted molecules with the highest prediction score that were not found in the training dataset (bottom) conducted in biological duplicate; error bars represent absolute range of measurements and dashed horizontal line represents the stringent hit cut-off of >80% growth inhibition at 50 μ M; (FIG. 5C) growth inhibition of *A. baumannii* by abaucin and serdemetan in LB medium conducted in biological duplicate; error bars represent absolute range of measurements; the structure of serdemetan is shown; (FIG. 5D) growth kinetics of *A. baumannii* cells after treatment with abaucin at varying concentrations for 6 hours conducted in biological duplicate; error bars represent absolute range of measurements.

[00121] **FIGs. 6A-6D** show antibacterial activity of abaucin against human commensal species in exemplary embodiments of the disclosure: (FIG. 6A) growth inhibition of *A. baumannii* ATCC 17978 by ampicillin and ciprofloxacin in LB medium conducted in biological duplicate; error bars represent absolute range of measurements; (FIG. 6B) growth inhibition of *B. breve* by abaucin conducted in biological duplicate;

error bars represent absolute range of measurements; (FIG. 6C) Growth inhibition of *B. longum* by abaucin conducted in biological duplicate; error bars represent absolute range of measurements; (FIG. 6D) non-validated growth inhibition of *E. lenta* by abaucin conducted in biological duplicate; error bars represent absolute range of measurements.

[00122] **FIGs. 7A-7M** shows abaucin mechanism of action in exemplary embodiments of the disclosure: (FIGs. 7A-7H) growth inhibition of wildtype *A. baumannii* (WT) and the four independent abaucin-resistant mutants by a collection of diverse antibiotics – from left to right for each plot, the mutants are: A362T isolate 1, Y394F, intergenic, and A362T isolate 2; experiments were conducted in biological duplicate; (FIG. 7I) structural prediction of wildtype *A. baumannii* LolE using RoseTTAFold (bottom), with the structural error estimate of each amino acid (top); position 362 is highlighted at 700 and resides in a disordered region of the protein; (FIG. 7J) same as (FIG 7I), except with the Y362T abaucin-resistant mutant of LolE at 702; (FIG. 7K) RNA sequencing of wildtype *A. baumannii* treated with 5x MIC abaucin for 4.5 hr (top) or 6 hr (bottom) shown as the mean of biological duplicates; transcript abundance is normalized to no-drug control cultures grown in identical conditions; vertical black lines show statistical significance cut-off values; (FIG. 7L) growth inhibition of *A. baumannii* harboring an empty CRISPRi vector, or three distinct sgRNAs targeting *lolE* conducted in biological duplicate; error bars represent absolute range of measurements (all strains were grown in LB medium without induction); (FIG. 7M) qPCR quantifying the expression of *lolE* relative to the housekeeping gene *gltA* (left) and *gyrB* (right) in all four abaucin resistant mutants, normalized to wildtype *A.*

baumannii conducted in biological duplicate with technical triplicates; bar height represents mean expression.

[00123] EXAMPLES

[00124] The following non-limiting examples are illustrative of the present disclosure. Specific elements of the examples are for descriptive purposes only and are not intended to limit the scope of the invention. Those skilled in the art could develop equivalent methods and utilize comparable materials that are within the scope of the invention.

Example 1.

[00125] Methods

[00126] Training data acquisition. *A. baumannii* ATCC 17978 was grown in 2 ml LB medium overnight at 37°C with shaking. Cells were diluted 1/10,000 into fresh LB and 99 µl of cells was added to each well of a 96-well flat-bottom plate (Corning). Next, 1 µl of a 5 mM stock of each molecule from a collection of 7,684 small molecules (FDA-approved drugs and molecules from screening collections from the Broad Institute) was added, in duplicate, using an Agilent Bravo liquid handling system. The final screening concentration was 50 µM. Plates were then incubated in sealed plastic bags at 37°C for 16 hr and subsequently read at 600 nm using a SpectraMax M3 plate reader to quantify cell growth. Plate data were normalized based on the interquartile mean of each plate prior to binarization into “active” and “non-active” categories for model training. Active molecules were defined as those that resulted in growth at least 1σ below the mean growth of the entire dataset.

[00127] **Model training and predictions.** A directed message passing neural network (Chemprop), like other message passing neural networks, learns to predict molecular properties directly from the graph structure of molecules, where atoms are represented as nodes and bonds are represented as edges. For every molecule in the training dataset, the molecular graph corresponding to each compound's SMILES string was reconstructed and the set of atoms and bonds was determined using the open-source package RDKit¹⁹. Next, a feature vector was initialized for each atom and bond, as described previously⁵.

[00128] The model applies a series of message passing steps where it aggregates information from neighboring atoms and bonds to build a representation of local chemistry. On each step of message passing, every bond's featurization is updated by summing the featurization of neighboring atoms and bonds, applying a single neural network layer, adding the bond's previous featurization, and then applying ReLU activation. After a defined number of message-passing steps, the learned featurizations across the molecule are summed to produce a single featurization for the whole molecule. Lastly, this featurization is subjected to a feed-forward neural network that outputs a prediction of the property of interest – in this case, antibiotic activity against *A. baumannii*. To augment the architecture described here, two model enhancements were employed: molecule-level features and ensembling, as described herein.

[00129] **Molecule-level features** – The message passing approach is ideal for extracting features of local chemistry within a larger molecule. However, it can struggle to extract global molecular features for larger molecules when the number of message-passing steps performed is less than the longest path through the molecule. To address

this limitation, the molecular representation that is learned during message passing was concatenated with 200 additional molecule-level features computed using RDKit.

[00130] Ensembling – Ensembling was used to further improve model performance, where several copies of the same model architecture with different random initial weights are trained and their predictions are averaged. Here, an ensemble of 10 models, with each model trained on a unique split of the training dataset, was used.

[00131] After model building and training on the 7,684-molecule training dataset (with ~6.2% active examples), the model was applied to the updated Drug Repurposing Hub, consisting of 6,680 compounds, many of which occupy a unique chemical space relative to that on which the model was trained. First, the model was evaluated on the training set of 7,684 molecules without ensembling to understand the performance of a single model. Here, the dataset was randomly split into 80% training data, 10% validation data, and 10% test data. The model was then trained on these data for 30 epochs (where an epoch is defined as a single pass through all the training data) and evaluated on the validation data at the end of each epoch. Once training was complete, the model parameters that performed best on the validation data were used and the model was tested on the test data. 10-fold cross-validation was run by repeating this procedure with 10 different splits by systematically dividing training, validation, and test sets such that all molecules appeared at equal proportions across all sets over the course of training iterations. After a model performance that was acceptable for the prediction task was achieved, predictions were conducted on the Drug Repurposing Hub.

[00132] The model had the following hyperparameters: number of message-passing steps = 3; neural network hidden size = 300; number of feed-forward layers = 2; dropout probability = 0.

[00133] **Structural analysis.** Tanimoto similarity was used to quantify the chemical relationship between molecules in the training dataset and prediction dataset. The Tanimoto similarity between two molecules is a measure of the proportion of shared chemical substructures. To compute Tanimoto similarity, Morgan fingerprints were first made for each molecule using radius = 2 and 2,048-bit fingerprint vectors. Morgan fingerprints were computed using RDKit. Tanimoto similarity was then computed as the number of chemical substructures contained in both molecules divided by the total number of unique chemical substructures in either molecule. The Tanimoto similarity is a number between 0 and 1, where 0 represents no shared substructures and 1 represents all substructures are shared. For t-SNE analysis, plots were created using the implementation of t-distributed stochastic neighbour embedding by scikit-learn⁴³. Herein, RDKit was first used to compute Morgan fingerprints for each molecule as described above. Then t-SNE was used with the Jaccard distance metric to reduce the data from 2,048 dimensions to the two dimensions that are plotted. Jaccard distance is Tanimoto distance, which is defined as: Tanimoto distance = 1 – Tanimoto similarity. Therefore, the distance between the points in a two-dimensional t-SNE plot is a representation of the Tanimoto similarity of those molecules, where greater distance between molecules indicates lower Tanimoto similarity. Scikit-learn default values were used for all t-SNE parameters besides the distance metric.

[00134] **Pathogen growth inhibition.** Cells were grown overnight in 2 ml LB medium and diluted 1/10,000 into fresh LB. In 96-well flat-bottom plates (Corning),

cells were then introduced to compound at a final concentration of 50 μ M, or to compound at two-fold serial dilutions, in final volumes of 100 μ l. Plates were then incubated at 37°C until untreated control cultures reached stationary phase, at which time plates were read at 600 nm using a SpectraMax M3 plate reader. The incubation time required to reach stationary phase differed slightly between strains. For abaucin analog spectrum of coverage assays, the laboratory strains were *E. coli* BW25113, *S. aureus* RN4220, and *P. aeruginosa* PA01. Clinical isolate panels were curated from the CDC ARIsolate Bank and assayed in LB as described above.

[00135] Bacterial cell killing. Cells were grown overnight in 2 ml LB medium and diluted 1/10,000 into fresh LB. In 96-well flat-bottom plates (Corning), cells were grown to the required density (100 μ l final volume) at 37°C, at which time compound was added at the indicated concentration and cultures were incubated for the required duration. Cells were then pelleted in plates by centrifugation at 4000 x g for 15 min at 4°C and washed in ice-cold PBS. After washing, cells were 10-fold serially diluted in PBS and plated on LB to quantify cell viability. In experiments where cells were incubated with antibiotic in nutrient-deplete conditions, cells were grown to the required density in LB media at 37°C, washed in PBS, and subsequently resuspended in PBS prior to the addition of compound (100 μ l final volume). After cultures were incubated for the required duration at 37°C, cells were pelleted in plates by centrifugation at 4000 x g for 15 min at 4°C and washed in ice-cold PBS. After washing, cells were 10-fold serially diluted in PBS and plated on LB to quantify cell viability. For post-treatment regrowth experiments, cells were treated with abaucin and incubated in LB for 6 hours as described above (100 μ l final volume). Cells were then pelleted in plates by centrifugation at 4000 x g for 15 min at 4°C and washed in ice-cold PBS.

After washing, cells were resuspended in 100 μ l fresh LB, diluted 1/1,000 in fresh 100 μ l LB, and grown at 37°C in a Biotek Synergy H1 plate reader. Plates were read at 600 nm every 20 minutes for 16 hours.

[00136] Commensal species growth inhibition. For analysis of commensal species from the human gut, strains were grown from frozen stock on brain heart infusion (BHI) agar supplemented with 0.5 g/L L-cysteine, 10 mg/L hemin, and 1 mg/L vitamin K (BHI3). Single colonies were picked, transferred to 96-well plates containing liquid BHI3 and grown for 24 hr at 37°C. Antibiotics (ampicillin, ciprofloxacin, and abaucin) were arrayed in BHI3 broth at two-fold the final desired concentrations, and pre-reduced in an anaerobic environment overnight. Liquid bacterial cultures were standardized to two-fold the desired optical density ($OD_{600} = 0.02$). The two-fold concentrated cultures were then inoculated into 96-well plates (50 μ l) containing two-fold concentrated antibiotics (50 μ l). The final antibiotic concentrations were ampicillin = 128 μ g/ml; ciprofloxacin = 0.25 μ g/ml; abaucin = 2 μ g/ml, 20 μ g/ml, and 40 μ g/ml. The final culture volume was 100 μ l. After inoculation, plates were sealed with breathable membranes (Breathe-Easy) and incubated at 37°C without shaking for 24 hr. After incubation the plates were read at 600nm using Biotek Synergy H1 plate reader. All bacterial strains were grown and incubated in a Coy Laboratory Vinyl Anaerobic Chamber (5% CO₂, 2% H₂, 93% N₂). All media was pre-reduced under anaerobic conditions for at least 4 hr before use. For analysis of commensal skin species in aerobic conditions, strains were grown from frozen on BHI3 agar and incubated at 37°C for 24 hr. Single colonies were picked and transferred to 96-well plates containing liquid BHI3, which were then incubated at 37°C without shaking for 24 hr. Liquid cultures were then standardized to $OD_{600} = 0.02$ (two-fold the final optical density).

Antibiotics (ampicillin, ciprofloxacin, and abaucin) were arrayed in BHI3 broth at two-fold the final concentrations (the final antibiotic concentrations were ampicillin = 128 µg/ml; ciprofloxacin = 0.25 µg/ml; abaucin = 2 µg/ml, 20 µg/ml, and 40 µg/ml). The two-fold concentrated cultures (50 µl) were then inoculated into 96-well plates containing the two-fold concentrated antibiotics (50 µl) to achieve a final volume of 100 µl. Plates were sealed with breathable membranes (Breathe-Easy) and incubated at 37°C without shaking for 48 hr under 5% CO₂. After incubation, plates were read at 600 nm using a Biotek Synergy H1 plate reader.

[00137] Suppressor mutant evolution and sequencing. *A. baumannii* ATCC 17978 was grown in 2 ml LB medium overnight at 37°C with shaking. $\sim 1 \times 10^8$ CFU in 100 µl liquid LB was deposited onto solid LB plates supplemented with abaucin at varying concentrations ranging from 2 µg/ml to 10 µg/ml. Plates were incubated at 37°C and monitored every 24 hr for the emergence of abaucin suppressor mutants. Upon the emergence of colonies, these were purified by re-streaking onto solid LB and solid LB supplemented with abaucin at the same concentration from which the colonies were originally harvested. This was to assess mutational stability and purify the abaucin-resistant strains. Four independent abaucin-resistant strains were subsequently selected for whole genome sequencing. Chromosomal DNA from each mutant and wildtype *A. baumannii* ATCC 17978 was purified using a Gentra Puregene DNA isolation kit (Qiagen). DNA (chromosomal and plasmid) was sequenced on an Illumina MiSeq platform and reads were aligned to the reference *A. baumannii* ATCC 17978 genome (CP053098.1; CP053099.1; CP053100.1) using Breseq⁴⁴ to identify the mutations conferring resistance to abaucin. For frequency of resistance quantification, 1.1×10^8 CFU of *A. baumannii* ATCC 17978 in 100 µl liquid LB was deposited onto solid LB

plates supplemented with abaucin at the noted concentrations. After 24 hr, 48 hr, and 72 hr of incubation at 37°C, colonies were counted, and these values were divided by $[1.1 \times 10^8]$ to quantify frequency of resistance.

[00138] Transcriptomic analysis. *A. baumannii* ATCC 17978 was grown in 2 ml LB medium overnight at 37°C with shaking. Cells were diluted 1/10,000 into 50 ml fresh LB and grown to mid-log at 37°C with shaking, at which time cultures were supplemented with 5x MIC (10 µg/ml) abaucin or no drug and grown for an additional 3 hr, 4.5 hr, or 6 hr. After the required durations of incubation post-treatment, 2 ml samples were harvested and flash-frozen on liquid nitrogen. cDNA library construction and sequencing for these samples were performed by Genewiz. Paired-end sequence data in FASTQ file format were aligned to the transcripts of CP053098.1 (*A. baumannii* ATCC 17978 chromosome), CP053099.1 (*A. baumannii* ATCC 17978 plasmid 1), and CP053100.1 (*A. baumannii* ATCC 17978 plasmid 2). mRNA abundances were then quantified as transcripts per million using the kallisto (version 0.46.2)⁴⁵ “quant” function, and the parameter “-b 100” with paired-end reads. All downstream analyses were performed using R (version 4.1.0)⁴⁶. Expression differences between abaucin-treated samples and non-treated controls were quantified using the DESeq2 pipeline (version 1.34.0)⁴⁷. A cut-off of $\log_2(\text{FC}) \geq 1.5$ and $\text{padj} \leq 0.01$ was defined to identify differentially expressed genes, as highlighted in the volcano plots. For gene ontology (GO) enrichment, differentially expressed *A. baumannii* transcripts for which no gene name was identified in CP053098.1, CP053099.1, or CP053100.1 were subject to blastp analysis against *E. coli* MG1655 (<https://blast.ncbi.nlm.nih.gov/>). *A. baumannii* proteins with E-values $< 10^{-50}$, percent identify $> 40\%$, and query coverage $> 80\%$ to

annotated *E. coli* proteins were binned and GO-enriched with EcoCyc Pathway Tools^{48–50} using the Fisher exact test for statistical significance of GO term enrichment.

[00139] CRISPRi LolE knockdown. Three independent 20 base pair sgRNAs targeting *lolE* (sgRNA_{lolE}-1 – 5'-TAAACGTAAGCCAAGCGAAT-3' ; sgRNA_{lolE}-2 – 5'-CAAATTCTTCACAATGTCAT-3' ; sgRNA_{lolE}-3 – 5'-TTTAAGTGAGTCGAGGCTAC-3') were designed using predictive software⁵¹ to maximize on-target activity and minimize off-targeting binding in *A. baumannii* ATCC 17978. sgRNAs were then cloned into pFD152 (Addgene plasmid 125546; provided by David Bikard) using a single-step golden gate assembly reaction, as described previously⁵². sgRNA sequences were verified using Sanger sequencing and constructs were subsequently transformed into *A. baumannii* ATCC 17978 for downstream experiments. pFD152 was selected in *A. baumannii* using spectinomycin (250 µg/ml in liquid media; 500 µg/ml on solid media). To determine whether LolE knockdown enhanced the growth inhibitory potency of abaucin, *A. baumannii* ATCC 17978 harboring pFD152, pFD152-sgRNA_{lolE}-1, pFD152-sgRNA_{lolE}-2 or pFD152-sgRNA_{lolE}-3 were grown overnight in LB medium, diluted 1/10,000 into fresh LB, and grown mid-log phase (OD ~ 0.2) at 37°C. Anhydrotetracycline (aTc) was added to the subcultures at a final concentration of 1 µg/ml to induce knockdown of *lolE* expression and cultures were incubated for an additional 2.5 hr at 37°C with shaking. Following this induction period, cultures were back-diluted to the equivalent optical density of 1/10,000 of a dense culture and introduced to varying concentrations of abaucin and 0.5 µg/ml of aTc. MICs were then determined as described above.

[00140] Quantitative reverse transcription (qRT)-PCR. Wildtype *A. baumannii* ATCC 17978 and the resistant mutants were grown in 3 ml LB medium

overnight at 37°C with shaking. Cells were diluted 1/10,000 into 15 ml fresh LB and grown to mid-log at 37°C with shaking, at which time cells were pelleted by centrifugation at 4000 x g for 15 min at 4°C and frozen for ~2 hours. Cells were lysed in 100 µl of 1 mg/ml lysozyme and incubated at room temperature for 10 min. RNA was isolated using the RNEasy Mini Kit (Qiagen), with some modifications. Briefly, to each sample, 3.5 µl of 2-mercaptoethanol, 250 µl of RLT buffer, and 250 µl of 95% ethanol was added. 700 µl of each sample was then loaded onto a spin column and incubated at room temperature for 5 min. The sample was centrifuged at 8,000 x g for 1 min. The column was washed with 700 µl RW1 buffer and centrifuged at 8,000 x g for 1 min. The column was washed with 700 µl RPE buffer and centrifuged at 8,000 x g for 2 min. The column was washed again with 500 µl RPE buffer and centrifuged at 8,000 x g for 2 min. The column was transferred to a new collection tube and incubated at room temperature for 5 min. The empty column was centrifuged at 13,000 x g for 2 mins to remove any remaining liquid and incubated at room temperature for 5 min. The column was then placed into a fresh 1.5 ml microcentrifuge tube and 45 µl of RNase-free water was added to the centre of the column and incubated at room temperature for 5 min. To elute RNA, the column was centrifuged at 8,000 x g for 2 min. RNA was subject to DNase I treatment for 10 min at room temperature. The resulting RNA was quantified using a Nanodrop. Next, the RNA integrity was assessed through agarose gel electrophoresis. For cDNA synthesis, the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems ThermoFisher) was used as specified by the manufacturer's instructions. The cDNA was then amplified with iTaq Universal SYBR Green Supermix (Bio-Rad) via a Bio-Rad CFX96 (Bio-Rad) following the manufacturer's instructions. The target amplification efficiency was evaluated by

generating a standard curve with dilutions of cDNA (>95% amplification efficiency for each primer pair). Primers lolE195F + lolE195R were used to amplify *lolE*; rpoD182F + rpoD182R, glt188F + glt188R, and gyrB196F + gyrB196R were used to amplify the house keeping genes *rpoD*, *gltA*, and *gyrB*. Triple technical replicates were examined per biological sample, with controls omitting reverse-transcriptase to verify a lack of genomic DNA contamination, and cDNA to verify the absence of external nucleic acid contamination and primer dimer formation. Transcript level of *lolE* from each mutant was evaluated using the comparative $2^{-\Delta\Delta C_t}$ method to the wild-type strain, normalizing to the $2^{-\Delta\Delta C_t}$ value of housekeeping genes.

lolE195F – 5'-CAGCTGGAGATGGAGTCGCT-3'

lolE195R – 5'-TGGTGACGGGTATTGACCCT-3'

rpoD182F – 5'-ACATGGCCGTAATAGCCCTGA-3'

rpoD182R – 5'-CCATACGGCCACGACGTACT-3'

glt188F – 5'-GCGACAGCTTCATGCGAGTC-3'

glt188R – 5'-TGAGCACGAACCTTTCGCATCG-3'

gyrB196F – 5'-ATTAGTGCTGATGCGCCTGC-3'

gyrB196R – 5'-CTTGATCCGCCCATTGCTGG-3'

[00141] Structural protein prediction. The wildtype *A. baumannii* protein sequence of interest (the protein in which abaucin resistance mutations were observed) was first aligned against the UniProt/SwissProt database⁵³ using blastp (version 2.13.0+)^{54,55} through the NCBI blast platform (<https://blast.ncbi.nlm.nih.gov/>), and a multiple sequence alignment was obtained. This alignment identified the *E. coli* K12

LolE protein as a homologue with E-value = 6×10^{-88} . Next, the *E. coli* LolE protein, the *A. baumannii* wildtype LolE protein, and the *A. baumannii* abaucin -resistant A362T mutant LolE protein were aligned using locally installed Geneious Prime software (version 2021.2.2) (<https://www.geneious.com>); a “global alignment with free end gaps” was applied as the alignment type and “blosum62” was applied as the cost matrix, with parameters gap open penalty = 12; gap extension penalty = 3; and refinement iterations = 2. The structure of the wildtype *A. baumannii* LolE protein and A362T mutant protein sequences were then predicted with RoseTTAfold³⁰ through the Robetta platform (<https://rosetta.bakerlab.org/>). The protein structure of the wildtype *E. coli* LolCDE in complex with a lipoprotein was obtained from the study by Tang *et al*¹⁷, accession code PDB:7ARH. The structures of the wildtype *A. baumannii* LolE protein and the LolCDE complex were then aligned with Maestro (version 12.9.137) from Schrödinger and ChimeraX (version 1.3rc202112020528)^{56,57}. In Maestro, the default structural alignment parameters were used. With ChimeraX, the “Needleman-Wunsch” alignment algorithm with “blosum62” as the similarity matrix was used, with parameters “bb” as chain pairing; 0.3 as SS fraction; 1 as gap extend; 18/18/6 as gap open (HH/SS/other); and 2 as iteration cutoff. Final figures of structural alignments were generated with ChimeraX (version 1.3rc202112020528)^{56,57}.

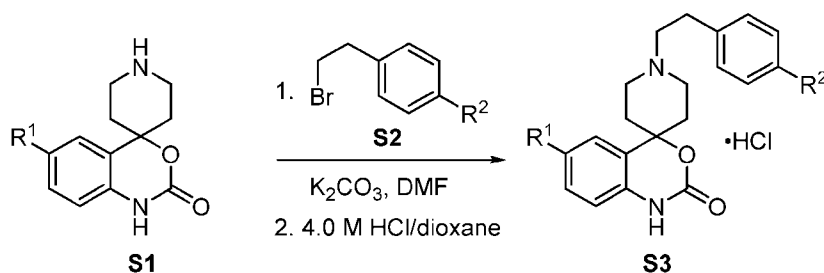
[00142] Quantitative microscopy. *A. baumannii* ATCC 17978 was grown overnight in 2 ml LB medium and diluted 1/10,000 into fresh LB. In 96-well flat-bottom plates, cells were then introduced to compound at the indicated concentrations, in final volumes of 100 μ l. Plates were grown to mid-exponential phase in a Biotek Epoch 2 plate reader with shaking at 37°C. Cell suspensions were transferred to a poly-lysine coated 0.17mm glass-bottom imaging plate (Brooks Scientific), and incubated with FM

4-64 (1 µg/ml final concentration) and DAPI (0.2 µg/ml final concentration) probes for 10 minutes in the dark. Samples were imaged using a Nikon Eclipse Ti inverted microscope at 100x magnification, and cell widths were assessed using Nikon Elements software. Cell widths for each untreated or treated sample were summarized in R using the density function to explore population shifts in cell swelling as a function of drug concentration.

[00143] Animal models. Mouse model experiments were conducted according to the guidelines set by the Canadian Council on Animal Care, using protocols approved by the Animal Review Ethics Board and McMaster University under Animal Use Protocol #20-12-41. Before infection, mice were relocated at random from housing cages to single-occupancy control or treatment cages. No animals were excluded from analysis, and blinding was considered unnecessary. Six- to eight-week-old female C57BL/6N mice were pretreated with 150 mg/kg (day T-4) and 100 mg/kg (day T-1) of cyclophosphamide to render mice neutropenic. On day T-0, mice were anesthetized using isoflurane and administered buprenorphine as an analgesic at 0.1 mg/kg intraperitoneally. A 2 cm² abrasion on the dorsal surface of the mouse was inflicted through tape-stripping to the basal layer of the epidermis using approximately 30-35 pieces of autoclave tape. Mice were immediately infected with $\sim 6.5 \times 10^6$ CFU *A. baumannii* ATCC 17978 directly pipetted onto the wound bed. The infection was left to establish for one hr prior to the first treatment with Glaxal Base supplemented with vehicle (1.65% DMSO) or abaucin (4% w/v). Mice (n=5 or 6) were treated 1 hr, 2 hr, 3 hr, 4 hr, 6 hr, 10 hr, 21 hr, and 24 hr post-infection with ~ 10 -20 µl Glaxal Base with abaucin (treatment) or DMSO (control). Mice were euthanized at experimental endpoint (25 hr post-infection) and wound tissue was collected, homogenized in

phosphate buffered saline, and plated on solid LB medium supplemented with chloramphenicol to quantify bacterial load. For chemical preparation, abaucin was weighed and solubilized in 1.65% DMSO and then added to a predetermined amount of Glaxal Base to a final concentration of 4% w/v. The solution was mixed thoroughly to ensure even distribution of compound in the carrier. For control groups, the same amount of vehicle (DMSO) was measured and mixed through Glaxal Base.

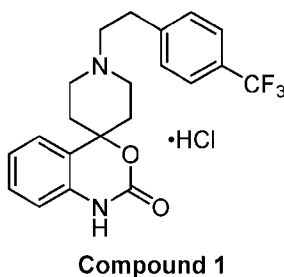
[00144] Chemical synthesis. Reagents, substrates, and solvents were purchased from commercial suppliers and used without purification unless otherwise specified. Reaction progress was monitored by analytical thin-layer chromatography, which involved precoated aluminum-backed plates (silica gel F254 SiliCycle Inc.), visualized under UV light. Purification of compounds were carried out either by an automated flash chromatography system (Teledyne CombiFlash Rf 200) on silica gel (40–60 μ M particle size) or by Preparative HPLC (Agilent 1290 Infinity II Preparative LCMSD) on a Prep-C18 column (5 μ m, 50 x 30.0 mm). NMR spectra were recorded at 25°C either using a Bruker NEO 400 (^1H at 400 MHz and DEPTQ ^{13}C at 101 MHz) or a Bruker AVIII 700 (^1H at 700 MHz, DEPTQ ^{13}C at 176 MHz, ^{19}F at 659 MHz). Chemical shifts in ^1H NMR, ^{13}C NMR and ^{19}F spectra are reported in parts per million (ppm) with reference to residual solvents or internal standards as follows: MeOD-*d*₄ (referenced to 4.87 ppm for ^1H and 49.00 ppm for ^{13}C), CDCl₃ (referenced to 7.26 ppm for ^1H and 77.16 ppm for ^{13}C). Coupling constants (J) are reported in hertz and peak multiplicities are reported using the following abbreviations: m = multiplet; s = singlet; d = doublet; t = triplet; q = quartet; dd = doublet of doublets, td = triplet of doublets.



[00145] General procedure - A solution of spirocycle **S1** (1 equiv.) in dry DMF (0.1 M) with phenethyl bromide **S2** (1.5 equiv.) and potassium carbonate (K_2CO_3 , 3 equiv.) was stirred at room temperature for 16 hr. The mixture was poured into water (25 mL) and extracted with EtOAc (2×25 mL). The organic layers were combined and washed with 10% aq. LiCl (2×50 mL), followed by a brine wash and then dried over MgSO_4 . The crude mixture was concentrated *in vacuo* and purified by Method A or Method B (described below), as specified.

[00146] Method A - The crude mixture was purified by column chromatography using a gradient of hexanes and ethyl acetate (EtOAc) as eluent. The purified compound was then dissolved in minimal EtOAc and stirred in 0.5 mL of 4.0 M HCl/dioxane for 30 minutes to yield the HCl salt. Compound **S3** was concentrated down and then freeze dried (via lyophilizer) from a mixture of water and acetonitrile (MeCN) (1:3) to produce a fine powder.

[00147] Method B - The crude mixture was purified by preparative HPLC using a gradient of 0.1% formic acid in water and acetonitrile as eluent. The purified compound was dissolved in minimal methanol and then stirred in 0.5 mL of 4.0 M HCl/dioxane for 30 minutes to yield the HCl salt. The compound was concentrated down and then freeze dried (via lyophilizer) from a mixture of H_2O /MeCN (1:3) to produce a fine powder.

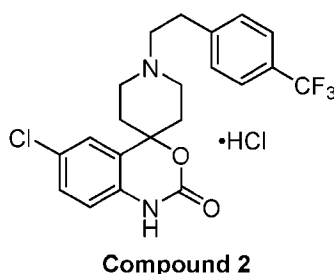


[00148] The general procedure was employed with spiro[benzo[d]1,3-oxazine-4,4'-piperidine]-2-one HCl (0.698 g, 2.74 mmol), 1-(2-bromoethyl)-4-(trifluoromethyl)benzene (1.04 g, 4.11 mmol), K₂CO₃ (1.16 g, 8.39 mmol) in DMF (25 mL). Reaction was worked up after 16 hr. The crude mixture was purified as per **Method A** to yield **Compound 1** (ABAUCIN) as a white powder.

[00149] **Yield** = 0.313 g, 0.733 mmol, 27%; **R_f** = 0.67 (10% MeOH in CH₂Cl₂);

[00150] **¹H NMR (400 MHz, MeOD) δ** 7.63 (d, *J* = 8.0 Hz, 2H), 7.49 (d, *J* = 8.0 Hz, 2H), 7.36 – 7.23 (m, 2H), 7.12 (td, *J* = 7.6, 1.2 Hz, 1H), 6.92 (dd, *J* = 7.9, 1.1 Hz, 1H), 3.20 (d, *J* = 11.9 Hz, 2H), 3.11 – 2.85 (m, 6H), 2.38 – 2.12 (m, 4H);

[00151] **¹³C NMR (176 MHz, MeOD) δ** 153.2, 144.9, 136.1, 130.5 (3C), 129.9 (q, *J* = 32.2 Hz), 126.5 (q, *J* = 3.6 Hz, 2C), 125.8, 125.8 (q, *J* = 271.0 Hz, CF₃), 124.8, 124.4, 115.7, 81.2, 60.0, 49.2 (2C), 35.3 (2C), 33.1.



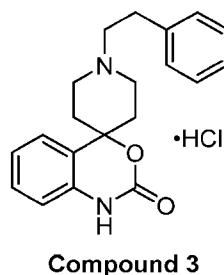
[00152] The general procedure was employed with 6-chlorospiro[1H-3,1-benzoxazine-4,4'-piperidine]-2-one (0.118 g, 0.467 mmol), 1-(2-bromoethyl)-4-

(trifluoromethyl)benzene (0.182 g, 0.719 mmol), K_2CO_3 (0.200 g, 1.45 mmol) in DMF (5 mL). Reaction was worked up after 16 hr. The crude mixture was purified as per **Method B** to yield **Compound 2** as a white powder.

[00153] **Yield** = 0.0456 g, 0.0988 mmol, 21%; R_f = 0.76 (10% MeOH in CH_2Cl_2);

[00154] ^1H NMR (700 MHz, MeOD) δ 7.69 (d, J = 7.7 Hz, 2H), 7.58 (d, J = 7.8 Hz, 2H), 7.44 – 7.30 (m, 2H), 6.96 (d, J = 8.4 Hz, 1H), 3.81 – 3.69 (m, 2H), 3.53 (d, J = 11.8 Hz, 4H), 3.33 – 3.25 (m, 2H), 2.48 (m, 4H);

[00155] ^{13}C NMR (176 MHz, MeOD) δ 151.7, 142.2, 135.0, 131.0, 130.7 (2C), 130.6 (q, J = 32.2 Hz), 129.9, 126.9 (q, J = 3.5 Hz, 2C), 125.9, 125.7 (q, J = 271.0 Hz), 124.9, 117.5, 79.0, 58.6, 49.3 (2C), 33.8 (2C), 31.1.

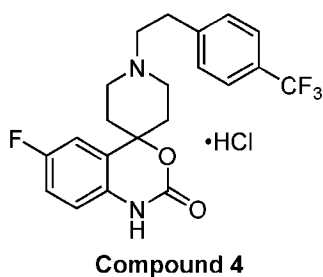


[00156] The general procedure was employed with spiro[benzo[d]1,3-oxazine-4,4'-piperidine]-2-one HCl (0.114 g, 0.524 mmol), 2-bromoethylbenzene (0.145 g, 0.785 mmol), K_2CO_3 (0.217 g, 1.57 mmol) in DMF (5 mL). Reaction was worked up after 16 hr. The crude mixture was purified as per **Method B** to yield **Compound 3** as a white powder.

[00157] **Yield** = 0.0475 g, 0.132 mmol, 25%; R_f = 0.64 (10% MeOH in CH_2Cl_2);

[00158] ^1H NMR (400 MHz, MeOD) δ 7.44 – 7.20 (m, 7H), 7.14 (td, J = 7.6, 1.2 Hz, 1H), 6.93 (dd, J = 8.0, 1.1 Hz, 1H), 3.46 (dt, J = 12.2, 2.6 Hz, 2H), 3.29 – 3.19 (m, 4H), 3.12 – 3.02 (m, 2H), 2.52 – 2.20 (m, 4H);

[00159] ^{13}C NMR (176 MHz, MeOD) δ 152.7, 138.8, 136.1, 130.8, 129.9 (2C), 129.8 (2C), 128.0, 125.0, 124.9, 124.4, 115.8, 80.3, 59.7, 49.1 (2C), 34.4 (2C), 32.3.



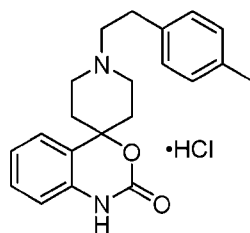
[00160] The general procedure was employed with 6-fluorospiro[1H-3,1-benzoxazine-4,4'-piperidine]-2-one (0.109 g, 0.461 mmol), 1-(2-bromoethyl)-4-(trifluoromethyl)benzene (0.175 g, 0.692 mmol), K_2CO_3 (0.181 g, 1.31 mmol) in DMF (5 mL). Reaction was worked up after 16 hr. The crude mixture was purified as per **Method B** to yield **Compound 4** as a white powder.

[00161] **Yield** = 0.0582 g, 0.131 mmol, 28%; R_f = 0.71 (10% MeOH in CH_2Cl_2);

[00162] ^1H NMR (400 MHz, MeOD) δ 7.69 (d, J = 7.9 Hz, 2H), 7.58 (d, J = 7.9 Hz, 2H), 7.21 – 7.09 (m, 2H), 6.98 (dd, J = 8.7, 4.6 Hz, 1H), 3.81 – 3.67 (m, 2H), 3.53 (d, J = 11.1 Hz, 4H), 3.37 – 3.21 (m, 3H), 2.55 (td, J = 14.2, 13.4, 4.3 Hz, 2H), 2.42 (d, J = 15.0 Hz, 2H);

[00163] ^{13}C NMR (176 MHz, MeOD) δ 160.5 (d, J = 241.4 Hz, Ar-F), 151.9, 142.2, 132.4, 130.7 (2C), 130.6 (q, J = 32.1 Hz), 126.9 (q, J = 3.5 Hz, CF_3), 125.9 (d, J

= 7.1 Hz), 125.7 (q, J = 271.1 Hz, 2C), 117.7 (d, J = 23.3 Hz), 117.5 (d, J = 8.0 Hz), 111.7 (d, J = 25.6 Hz), 79.0, 58.6, 49.3 (2C), 33.7 (2C), 31.1.



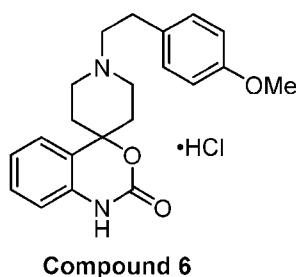
Compound 5

[00164] The general procedure was employed with substitution of the base to triethylamine. Spiro[benzo[d]1,3-oxazine-4,4'-piperidine]-2-one HCl (0.0598 g, 0.235 mmol), 4-methylphenethyl bromide (0.105 g, 0.527 mmol), triethylamine (0.0713 g, 0.705 mmol) in DMF (15 mL). Reaction was worked up after 16 hr. The crude mixture was purified as per **Method A** to yield **Compound 5** as an off-white powder.

[00165] **Yield:** 0.0205 g, 0.0550 mmol, 23%; **R_f** = 0.51 (10% MeOH in CH₂Cl₂);

[00166] **¹H NMR (400 MHz, CDCl₃)** δ 8.87 (s, 1H), 7.27 – 7.22 (m, 1H), 7.18 (dd, J = 7.8, 1.4 Hz, 1H), 7.11 (s, 4H), 7.07 (td, J = 7.6, 1.2 Hz, 1H), 6.87 (dd, J = 7.9, 1.1 Hz, 1H), 2.96 – 2.91 (m, 2H), 2.86 – 2.79 (m, 2H), 2.76 – 2.66 (m, 4H), 2.33 (s, 3H), 2.20 – 2.14 (m, 4H);

[00167] **¹³C NMR (101 MHz, CDCl₃)** δ 152.5, 137.2, 135.7, 134.5, 129.3 (2C), 129.2, 128.7 (2C), 125.4, 123.9, 123.4, 114.8, 81.8, 60.7, 48.3 (2C), 35.6 (2C), 33.5, 21.2.

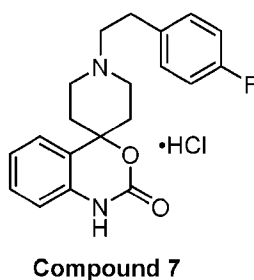


[00168] The general procedure was employed with substitution of the base to triethylamine. Spiro[benzo[d]1,3-oxazine-4,4'-piperidine]-2-one HCl (0.0533 g, 0.209 mmol), 4-methoxyphenethyl bromide (0.149 g, 0.693 mmol), triethylamine (0.0632 g, 0.625 mmol) in DMF (15 mL). Reaction was worked up after 16 hr. The crude mixture was purified as per **Method A** to yield **Compound 6** as an off-white powder.

[00169] **Yield:** 0.0292 g, 0.0750 mmol, 36%; **R_f** = 0.59 (10% MeOH in CH₂Cl₂);

[00170] **¹H NMR (400 MHz, CDCl₃)** δ 8.98 (s, 1H), 7.27 – 7.21 (m, 1H), 7.18 (dd, J = 7.8, 1.4 Hz, 1H), 7.16 – 7.11 (m, 2H), 7.07 (td, J = 7.6, 1.2 Hz, 1H), 6.88 (dd, J = 7.9, 1.1 Hz, 1H), 6.86 – 6.82 (m, 2H), 3.79 (s, 3H), 2.97 – 2.90 (m, 2H), 2.85 – 2.78 (m, 2H), 2.77 – 2.65 (m, 4H), 2.26 – 2.13 (m, 4H);

[00171] **¹³C NMR (101 MHz, CDCl₃)** δ 158.1, 152.6, 134.5, 132.3, 129.7 (2C), 129.2, 125.4, 123.9, 123.4, 114.8, 114.0 (2C), 81.7, 60.7, 55.4, 48.3 (2C), 35.6 (2C), 32.9.

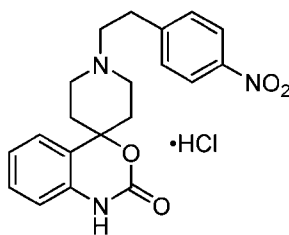


[00172] The general procedure was employed with substitution of the base to triethylamine. Spiro[benzo[d]1,3-oxazine-4,4'-piperidine]-2-one HCl (0.0526 g, 0.207 mmol), 4-fluorophenethyl bromide (0.137 g, 0.675 mmol), triethylamine (0.0632 g, 0.625 mmol) in DMF (15 mL). Reaction was worked up after 16 hr. The crude mixture was purified as per **Method A** to yield **Compound 7** as an off-white powder.

[00173] **Yield:** 0.0277 g, 0.0735 mmol, 36%; **R_f** = 0.49 (10% MeOH in CH₂Cl₂);

[00174] **¹H NMR (400 MHz, CDCl₃)** δ 9.00 (s, 1H), 7.28 – 7.22 (m, 1H), 7.17 (ddd, J = 8.7, 5.0, 2.2 Hz, 3H), 7.07 (td, J = 7.6, 1.2 Hz, 1H), 7.01 – 6.94 (m, 2H), 6.88 (dd, J = 7.9, 1.1 Hz, 1H), 3.01 – 2.89 (m, 2H), 2.86 – 2.78 (m, 2H), 2.78 – 2.63 (m, 4H), 2.19 – 2.16 (m, 4H);

[00175] **¹³C NMR (101 MHz, CDCl₃)** δ 161.6 (d, J = 243.8 Hz, Ar-F), 152.6, 135.9 (d, J = 3.3 Hz), 134.5, 130.2 (d, J = 7.8 Hz, 2C), 129.2, 125.3, 123.9, 123.4, 115.3 (d, J = 21.1 Hz, 2C), 114.8, 81.7, 60.4, 48.3 (2C), 35.5 (2C), 33.0.



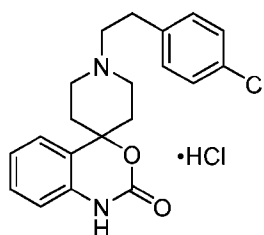
Compound 8

[00176] The general procedure was employed with substitution of the base to triethylamine. Spiro[benzo[d]1,3-oxazine-4,4'-piperidine]-2-one HCl (0.0531 g, 0.208 mmol), 4-nitrophenethyl bromide (0.101 g, 0.439 mmol), triethylamine (0.0632 g, 0.625 mmol) in DMF (15 mL). Reaction was worked up after 16 hr. The crude mixture was purified as per **Method A** to yield **Compound 8** as an off-white powder.

[00177] **Yield:** 0.0116 g, 0.0287 mmol, 14%; **R_f** = 0.54 (10% MeOH in CH₂Cl₂);

[00178] **¹H NMR (700 MHz, MeOD) δ** 8.33 – 8.15 (m, 2H), 7.71 – 7.57 (m, 2H), 7.35 (td, *J* = 7.7, 1.3 Hz, 1H), 7.29 (dd, *J* = 7.8, 1.3 Hz, 1H), 7.16 (td, *J* = 7.6, 1.1 Hz, 1H), 6.95 (dd, *J* = 8.0, 1.1 Hz, 1H), 3.74 – 3.71 (m, 2H), 3.58 – 3.53 (m, 4H), 3.33 – 3.30 (m, 2H), 2.51 – 2.46 (m, 2H), 2.44 – 2.40 (m, 2H);

[00179] **¹³C NMR (176 MHz, MeOD) δ** 152.2, 148.8, 145.3, 136.1, 131.2 (2C), 131.1, 125.0, 125.0 (2C), 124.3, 124.1, 116.0, 79.2, 58.3, 49.3 (2C), 34.0 (2C), 31.1.



Compound 9

[00180] The general procedure was employed with substitution of the base to triethylamine. Spiro[benzo[d]1,3-oxazine-4,4'-piperidine]-2-one HCl (0.0534 g, 0.210 mmol), 4-chlorophenethyl bromide (0.138 g, 0.629 mmol), triethylamine (0.0639 g, 0.631 mmol) in DMF (15 mL). Reaction was worked up after 16 hr. The crude mixture was purified as per **Method A** to yield **Compound 9** as an off-white powder.

[00181] **Yield:** 0.0202 g, 0.0514 mmol, 24%; **R_f** = 0.59 (10% MeOH in CH₂Cl₂);

[00182] **¹H NMR (400 MHz, CDCl₃) δ** 8.72 (s, 1H), 7.31 – 7.22 (m, 3H), 7.18 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.16 – 7.13 (m, 2H), 7.08 (td, *J* = 7.6, 1.2 Hz, 1H), 6.86 (dd, *J* = 7.9, 1.1 Hz, 1H), 2.93 – 2.91 (m, 2H), 2.86 – 2.79 (m, 2H), 2.77 – 2.65 (m, 4H), 2.18 – 2.16 (m, 4H);

[00183] ¹³C NMR (101 MHz, CDCl₃) δ 152.4, 138.8, 134.4, 132.0, 130.2 (2C), 129.2, 128.7 (2C), 125.3, 123.9, 123.4, 114.8, 81.6, 60.2, 48.3 (2C), 35.5 (2C), 33.2.

[00184] **Results**

[00185] **Machine learning-guided discovery of abaucin.** Recent work has highlighted the utility of machine learning in discovering novel antibacterial molecules using *E. coli* K12 as a model organism⁵. Building off this prior research, a message passing deep neural network¹⁸ was applied to discover new antibiotics against *A. baumannii*, a problematic nosocomial Gram-negative pathogen that commonly displays multidrug resistance and, increasingly, pan-drug resistance (FIG. 1A). A diverse collection of 7,684 small molecules was first screened at 50 μM for those that inhibited the growth of *A. baumannii* ATCC 17978 in LB medium (FIG. 1B, FIG. 5A Figure 2a). This chemical collection consisted of both off-patent drugs (2,341 molecules) and synthetic chemicals (5,343 molecules) curated from various high-throughput screening sub-libraries at the Broad Institute. Using a conventional hit cut-off of one standard deviation below the mean growth of the entire dataset, this resulted in 480 molecules being defined as “active” and 7,204 being defined as “inactive”.

[00186] Next, this dataset was used to train a binary classifier to predict whether structurally novel molecules may display activity against *A. baumannii*. Briefly, a directed message passing neural network architecture was leveraged, which translates the graph structure of a molecule into a continuous vector¹⁸ (FIG. 1A). This type of model operates by passing messages along bonds that encode information about neighboring atoms and bonds. By applying this message passing operation repeatedly, the model constructs higher-level bond messages that contain information about multi-atom substructures within the chemical. The highest-level bond messages are then

combined into a single continuous vector representing the entire molecule. This learned representation was augmented with molecular features computed by RDKit¹⁹, yielding a hybrid molecular representation with both learned features and computed molecular-level features. The algorithm's robustness was further increased by using an ensemble of 10 classifiers. The final model achieved an area under the precision-recall curve of 0.337 ± 0.088 and area under the receiver-operating characteristic curve of 0.792 ± 0.042 , providing confidence in leveraging the model for predictions in new chemical spaces.

[00187] After model training, the ensemble of 10 RDKit-augmented models was applied to identify antibacterial molecules with activity against *A. baumannii* from the updated Drug Repurposing Hub¹⁵, consisting of 6,680 molecules at various stages of preclinical and clinical investigation. This chemical library was selected as a proof-of-concept due to the structural diversity inherent to this collection, as well as the favorable cytotoxicity and drug-like properties that are observed for many Drug Repurposing Hub molecules. Herein, prediction scores for each compound were determined (FIG. 1C), molecules were ranked based on their probability of displaying growth inhibition against *A. baumannii*, and strongly predicted molecules with structures that were different from training dataset "actives" were prioritized for *in vitro* antibacterial validation. Importantly, this process of performing predictions and prioritizing molecules for validation was completed within a couple of hours. A message passing neural network model that was not augmented with RDKit features was also trained and applied as a baseline reference. This model did not perform as well as the RDKit-augmented primary model, achieving an area under the precision-recall curve of 0.266 ± 0.070 and area under the receiver-operating characteristic curve of 0.756 ± 0.050 . The

reduced performance of the model omitting RDKit features highlights the importance of these computable molecular features in maximizing predictive utility in the context of the training dataset.

[00188] Using a prediction score threshold of >0.2 (FIG. 1C) and subsequently filtering these predicted compounds based on a Tanimoto nearest neighbor similarity of <0.3 to molecules that were “active” in the training dataset, 240 priority molecules were identified that met these highly stringent criteria. These molecules were acquired and tested against *A. baumannii* at a concentration of 50 μM in LB medium – the same conditions in which the training data were acquired. Using another stringent cut-off of $>80\%$ growth inhibition, it was observed that nine of the tested molecules displayed antibacterial activity against *A. baumannii*, emphasizing the ability of this model to generalize to compounds that are highly divergent from the chemical space on which the model was trained. It is to be noted that $>80\%$ growth inhibition is a significantly more stringent cut-off than that used for model training, which was a conventional one standard deviation below the mean growth of the dataset, or $\sim 20\%$ growth inhibition. This stringent $>80\%$ growth inhibition cut-off was applied to efficiently prioritize the most potent predicted molecules on which to conduct further experimentation. For reference, if a hit cut-off of 20% growth inhibition was defined for prediction selection – similar to that used for model training – 41 molecules would be classified as active predictions. The 240 molecules with the lowest prediction scores were also tested and it was observed that none displayed antibacterial activity as defined by $>80\%$ growth inhibition, emphasizing the discriminatory utility of the model. Furthermore, testing the 240 molecules with the highest prediction scores, without considering Tanimoto nearest neighbor similarity to training set “actives”, resulted in 40 molecules that passed the

stringent >80% growth inhibition cut-off, indicating that the model has high predictive value over traditional chemical screening.

[00189] The nine priority molecules were subsequently assessed to remove (a) those with major structural features that are observed in known antibiotics; (b) those with reported antibacterial activity from the scientific or patent literature; and (c) those with possible non-specific membrane activity as assessed by the presence of acyclic aliphatic moieties. This structure-based filtering resulted in the retention of two molecules – abaucin and serdemetan. Abaucin is a well-studied CCR2-selective chemokine receptor antagonist^{20,21} that displayed a minimum inhibitory concentration (MIC) of ~2 µg/ml against *A. baumannii* ATCC 17978 (FIGS. 1D-1E); serdemetan^{22,23} is an antagonist of the transcription factor HDM2 that displayed an MIC of ~32 µg/ml. Given that abaucin was substantially more potent at inhibiting the growth of *A. baumannii*, subsequent investigations focused on this molecule.

[00190] Upon further experimentation with abaucin to assess *A. baumannii* viability after treatment, modest bactericidal activity against *A. baumannii* in LB medium was observed (FIG. 1F). Indeed, upon removal of abaucin from *A. baumannii* cultures *in vitro* after 6 hours of treatment, *A. baumannii* regrowth was observed, wherein the apparent lag period increased with increasing concentrations of abaucin (FIG. 5D). No discernable activity was observed in nutrient-deplete phosphate-buffered saline (PBS) (FIG. 1G). Collectively, these data suggest that abaucin displayed its antibacterial efficacy through inhibition of a biological process that was maximally active during growth and division²⁴, consistent with most known antibiotics²⁵. These data also suggest that abaucin is not membrane active via physical disruption of the phospholipid bilayer, a mechanism of action that was deliberately avoided during the

prediction filtering process. Indeed, membrane-active molecules generally retain bactericidal efficacy in nutrient-deplete conditions²⁴.

[00191] ABAUCIN has a narrow phylogenetic spectrum of antibacterial activity. After probing the activity of abaucin against *A. baumannii* ATCC 17978, growth inhibitory activity was next tested against clinical isolates of *A. baumannii*. Herein, 41 strains of *A. baumannii* were acquired from the Center for Disease Control and Prevention Antibiotic Resistance Isolate Bank (ARIsolate Bank) (Table 1), and tested abaucin at a range of concentrations below and above MIC. Remarkably, it was observed that abaucin could overcome all intrinsic and acquired resistance mechanisms within the *A. baumannii* isolates from this diverse clinical strain library (FIG. 3A, Table 1).

[00192] Next, the phylogenetic spectrum of activity displayed by abaucin was investigated by testing this compound against 24 carbapenem-resistant Enterobacteriaceae strains (Table 2), 24 *Pseudomonas aeruginosa* strains (Table 3), and 14 *Staphylococcus aureus* strains (Table 4), all from the ARIsolate Bank (antibiogram data can be found at <https://wwwn.cdc.gov/arisolatebank/>). In stark contrast to the observations with *A. baumannii*, abaucin did not display any growth inhibitory activity against these pathogenic species up to 20x the MIC in *A. baumannii* ATCC 17978 (FIGs. 2B=2D). In the context of clinical bacterial pathogens, these data provide strong evidence that abaucin has narrow-spectrum antibacterial activity, which is advantageous towards decreasing the inter-pathogen dissemination of resistance. A modest collection of structural analogs of abaucin displayed varying levels of activity against *A. baumannii* but avoided any discernable activity against lab strains of the bacterial species mentioned above (Table 5). This initial structure-activity relationship

investigation provides strong support that exploration of the chemical space around abaucin towards developing a medicinal chemistry optimized narrow-spectrum antibiotic against *A. baumannii* is feasible without being hampered by broad spectrum antibacterial activity.

[00193] Given these observations, it was hypothesized that abaucin would display minimal growth inhibitory activity against human commensal species. Indeed, currently employed antibiotics often induce dysbiosis during treatment²⁶, resulting in a wide array of complications, including secondary infections caused by opportunistic pathogens – *C. difficile* gut infections being a common example²⁷. To test this hypothesis, panels of 34 diverse human gut commensal isolates (Table 6) and 19 diverse human skin commensal isolates (Table 7) were curated, then the growth inhibitory properties of abaucin were tested at varying concentrations, as well as ampicillin and ciprofloxacin at their respective MIC concentrations in *A. baumannii* ATCC 17978. As expected, ampicillin and ciprofloxacin displayed antibacterial activity across a wide range of commensal isolates, whereas abaucin largely avoided growth inhibition of commensal species, even up to 20x MIC (FIG. 2E). Indeed, of the 53 isolates tested, abaucin only displayed *bona fide* growth inhibition against the gut isolates *Bifidobacterium breve* and *B. longum*, and this occurred above the MIC observed in *A. baumannii* ATCC 17978 (FIGs. 6B-6D). Given that *Bifidobacterium* is a Gram-positive genus that is phylogenetically divergent from Gram-negative *Acinetobacter*, it is likely that the lower potency activity of abaucin against *Bifidobacterium* is through a mechanism that is unrelated to that in *Acinetobacter*. This statement is elaborated below.

[00194] **ABAUCIN inhibits lipoprotein trafficking in *A. baumannii*.** Given the *A. baumannii* selectivity displayed by abaucin, the mechanism underlying its narrow-spectrum functionality was next elucidated. To this end, abaucin-resistant mutants were first selected for using wildtype *A. baumannii* growing on solid media supplemented with varying concentrations of abaucin. Using 4 µg/ml and 5 µg/ml, abaucin-resistant clones that did not display cross-resistance to functionally diverse antibiotics were isolated (FIG. 3A, FIGs. 7A-7H). Whole genome sequencing of four independent isolates revealed mutations in or upstream of the gene encoding LolE, an essential inner membrane protein involved in lipoprotein trafficking that has become a target of strong interest for novel Gram-negative antibiotic development^{16,17,28}. GenBank accession numbers for sequencing of abaucin resistant mutants are: BankIt2629921 – OP677864, OP677865, OP677866, OP677867. Two mutants (Y394F and an upstream G to A mutation) displayed 4-fold resistance to abaucin relative to wildtype *A. baumannii*, and two independent mutants contained an identical A362T mutation that resulted in 16-fold resistance. It is noted that the frequency of resistance to abaucin *in vitro* is 10^{-8} – 10^{-7} (Table 8), largely consistent with known antibiotics that target a single protein²⁹. Interestingly, *A. baumannii* LolE position A362 is homologous to *E. coli* LolE position I365, which resides near the acyl chains of the nascent lipoprotein during transport¹⁷. The structure of *A. baumannii* LolE was predicted using RoseTTAFold^{30,31} (FIGs. 7I-7J) and it was observed that *Ab*LolE position A362 is near *Ec*LolE position I365 in space, and both are adjacent to the accommodated acyl chains of the nascent lipoprotein (FIG. 3B).

[00195] These mutational data, combined with *in silico* structural insights, provided evidence suggesting that abaucin may disrupt lipoprotein accommodation and

transport facilitated by LolE. To further explore the hypothesis that abaucin was interfering with lipoprotein trafficking, wildtype *A. baumannii* was treated with 5x MIC of abaucin, or no compound, for varying durations and the cells were subjected to RNA sequencing. A transcriptomics approach would afford systems-level insight that could provide additional indirect evidence to support or negate the initial mechanistic hypothesis. GEO accession numbers for RNA sequencing datasets are GSE214305 – GSM6603484, GSM6603485, GSM6603486, GSM6603487, GSM6603488, GSM6603489, GSM6603490. After sequencing, differential expression analyses was performed between the no-drug control cultures and the abaucin-treated cultures to quantify up- and down-regulated transcripts. Differentially abundant transcripts were then clustered based on gene ontology (GO) term to identify the biological processes that most significantly changed due to abaucin exposure. Through this method, it was observed that abaucin caused a downregulation of genes involved in the aerobic electron transport chain and transmembrane ion transport (FIG. 3C and FIG. 7K). This transcriptional response is consistent with activation of the Cpx two-component envelope stress response that has been well-characterized in the model bacterium *E. coli*^{32,33}. Indeed, CpxAR plays a principal role in monitoring lipoprotein trafficking from the inner membrane to the outer membrane in Gram-negative bacteria³⁴. When lipoprotein trafficking is perturbed, CpxA autophosphorylates upon associated membrane stress, prior to phosphotransfer to the transcriptional regulator CpxR and subsequent transcriptional remodelling to restore envelope homeostasis³². These transcriptomics data further strengthen the hypothesis that abaucin displays antibacterial efficacy through perturbation of Lol complex-mediated lipoprotein trafficking. It is noted that the *A. baumannii* Lol system has not yet been thoroughly

explored, necessitating interpretation of these data in the context of the better-studied *E. coli* model.

[00196] With mutational, *in silico* structural, and transcriptional data providing support that abaucin perturbs lipoprotein trafficking, modulation of LolE expression based on changing abaucin potency was next investigated. First, it was hypothesized that the MIC of abaucin would decrease with decreased LolE expression³⁵. To test this, *A. baumannii* was engineered with an inducible CRISPRi system using three distinct guide RNAs targeting *AbLolE*. As expected, induction of the CRISPRi construct resulted in 4-8-fold decreased abaucin MIC relative to an empty vector control with no guide RNA (FIG. 3D). Uninduced cells with or without LolE-targeting guide RNAs all displayed identical abaucin sensitivities (FIG. 7L). Next, it was hypothesized that the abaucin-resistant mutant with the upstream intergenic mutation may be conferring resistance through increased expression of *lolE* – multicopy suppression-mediated resistance³⁶. This is the inverse experiment to that described above. To test this hypothesis, qPCR was performed on all four independent abaucin-resistant mutants, as well as the wildtype parent strain of *A. baumannii*, and observed that the mutant carrying the upstream G to A mutation displayed ~4-fold increased expression of *lolE* relative to all other strains tested (FIG. 3E, FIG. 7M). This 4-fold increase in *lolE* expression parallels the 4-fold increase in abaucin MIC observed relative to wildtype *A. baumannii*. Collectively, these data are consistent with the mechanistic model that abaucin targets LolE-mediated lipoprotein trafficking.

[00197] Prior work has shown that inhibition of lipoprotein transport in *E. coli* results in abnormal cell morphology characterized by significant bacterial cell swelling and loss of nucleoid condensation³⁷. It was hypothesized that abaucin treatment would

result in similar morphological features in *A. baumannii*. Therefore, wildtype *A. baumannii* was subjected to increasing concentrations of abaucin and imaged these cells using fluorescence microscopy. Here, *A. baumannii* cells were stained with DAPI and FM4-64, respectively, to visualize DNA and the cell envelope. Consistent with prior work in *E. coli*, abaucin-treated *A. baumannii* cells displayed increased swelling and a loss of intracellular nucleoid condensation as a function of concentration (FIG. 3F). These data provide compelling phenotypic support that abaucin disrupts lipoprotein trafficking.

[00198] ABAUCIN can suppress *A. baumannii* in a wound infection model.

A. baumannii is a problematic nosocomial pathogen that survives for prolonged periods on surfaces and has the ability to accumulate extracellular DNA¹, including antibiotic resistance genes³⁸. Indeed, *A. baumannii* is a major cause of multidrug-resistant infections in wounded military personnel³⁹. These data showing that abaucin inhibited the growth of a wide array of multidrug-resistant clinical isolates of *A. baumannii* – including pan-resistant strains – provided confidence that abaucin may be used to treat such problematic wound infections. To test the *in vivo* efficacy of abaucin, a wound infection was established on the dorsal surface of neutropenic C57BL/6 mice using *A. baumannii* ATCC 17978 ($\sim 6.5 \times 10^6$ CFU inoculum) and allowed the bacterial population to expand for one hour. Mice were subsequently treated with Glaxal Base Moisturizing Cream supplemented with vehicle (1.65% DMSO) or abaucin (4% w/v). Application of vehicle or abaucin occurred at 2 hr, 3 hr, 4 hr, 6 hr, 10 hr, 21 hr, and 24 hr post-infection. Mice were sacrificed at 25 hr post-infection, and tissue was aseptically dissected then plated to quantify *A. baumannii* viability. In vehicle-treated mice, $\sim 6.9 \times 10^8$ CFU/g were retrieved at the experimental endpoint and it was observed

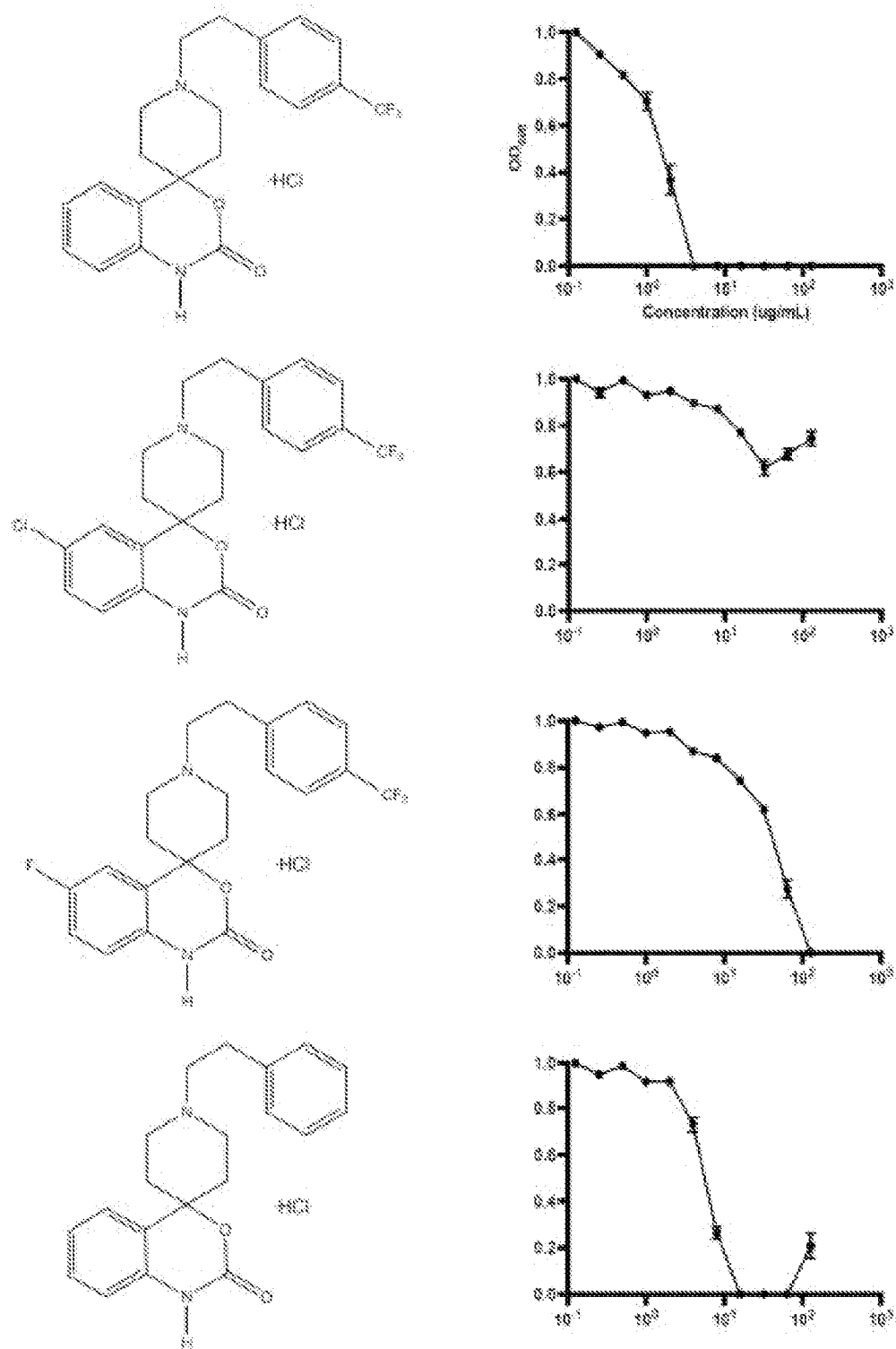
that the wounded tissues displayed significant inflammation. Contrarily, ABAUCIN-treated mice carried $\sim 4.0 \times 10^7$ CFU/g – nearly identical to the pre-treated infection control mice – and abaucin-treated tissues displayed markedly less inflammation (FIGs. 4A and 4B). These data show that abaucin can effectively suppress an *A. baumannii* wound infection, consistent with its effect on *A. baumannii* viability *in vitro* (FIG. 1F).

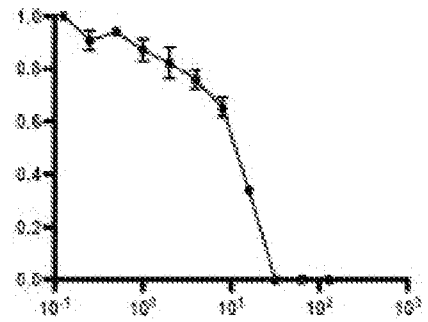
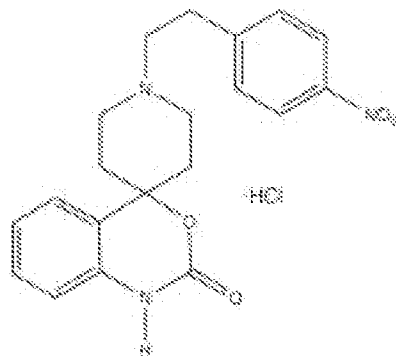
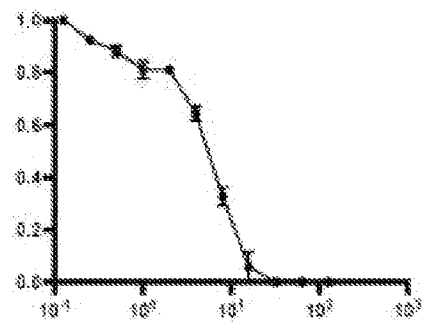
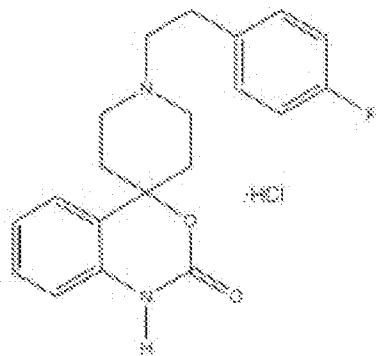
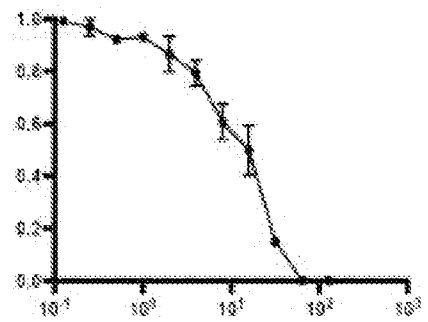
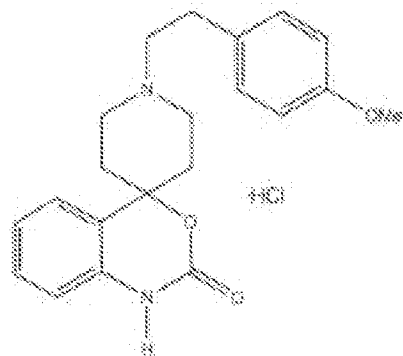
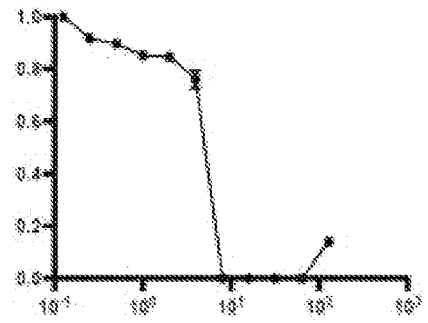
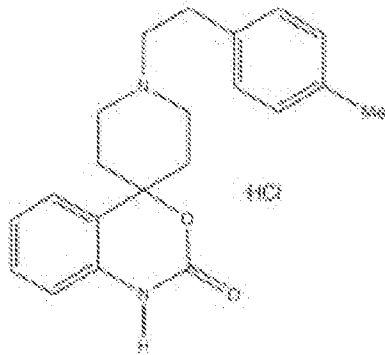
[00199] Discussion

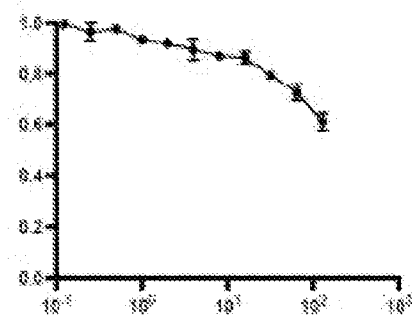
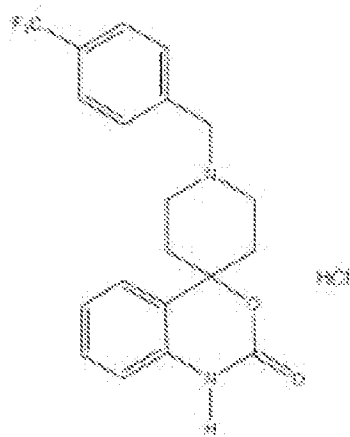
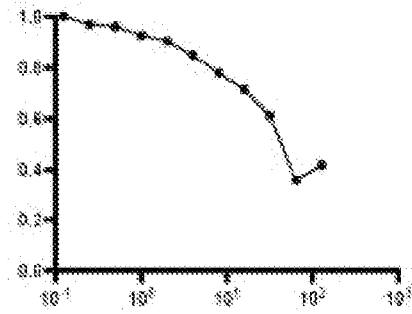
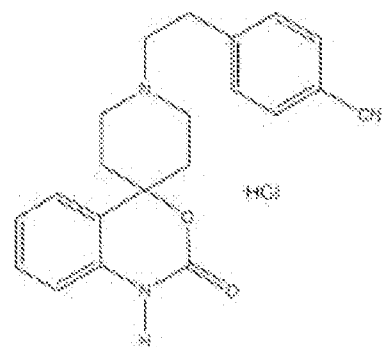
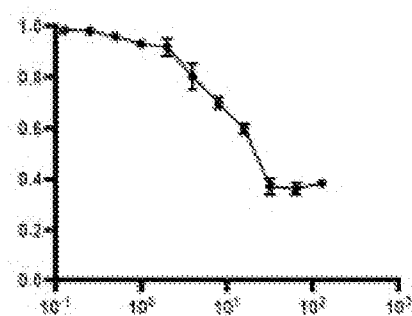
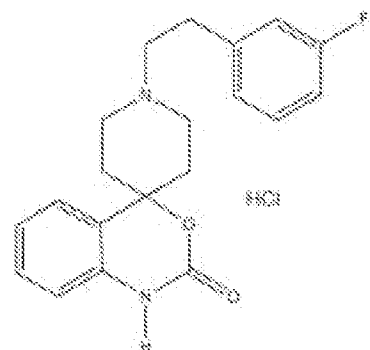
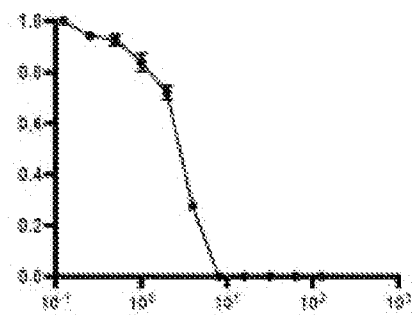
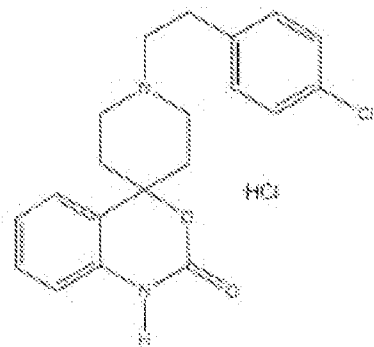
[00200] Structurally and functionally novel antibiotics are urgently needed for *A. baumannii*, which is notoriously difficult to eradicate due to its ability to uptake and retain antibiotic resistance determinants¹. Moreover, species-selective antibiotics hold promise to limit the horizontal dissemination of resistance determinants and decrease the likelihood of dysbiosis during treatment⁸. The machine learning-guided discovery of abaucin highlights the utility of algorithmic approaches to discover novel antibacterial molecules against *A. baumannii* and provides the field with a promising new narrow-spectrum molecular scaffold to address one of the world's most challenging Gram-negative pathogens. Lipoprotein trafficking is a highly sought-after antibiotic target that has yet to be perturbed by clinically used antibacterial drugs, which is advantageous towards increasing the duration of clinical utility of molecules that disrupt this process. Importantly, the observations herein – that abaucin displays narrow-spectrum activity through perturbation of LolE-mediated lipoprotein trafficking – can be explained, at least in part, due to the divergence of the *A. baumannii* Lol system relative to most other Gram-negative species¹⁶. Specifically, in most Gram-negative organisms, the inner membrane-associated lipoprotein transport machinery consists of LolC, LolD, and LolE in an asymmetric multi-protein complex. *A. baumannii*, on the

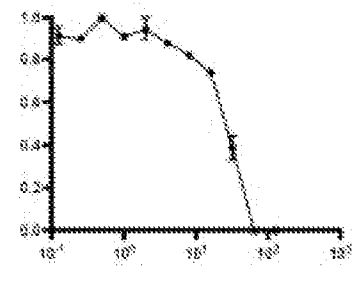
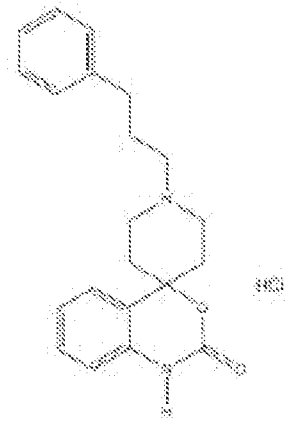
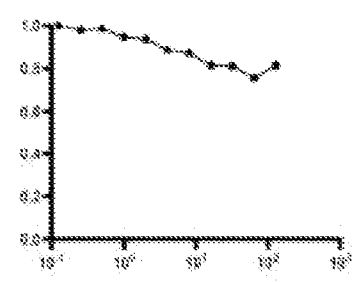
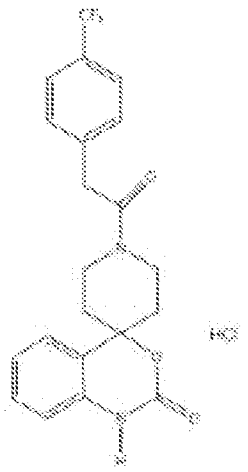
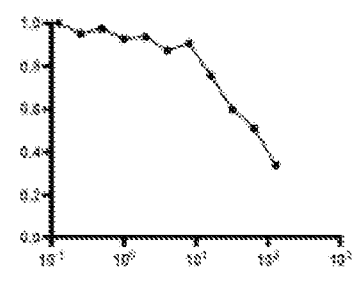
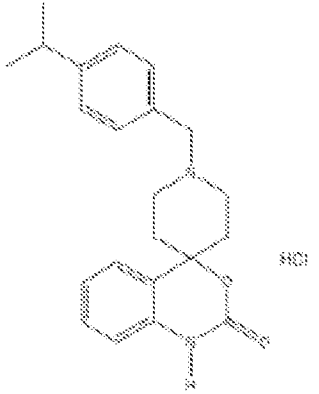
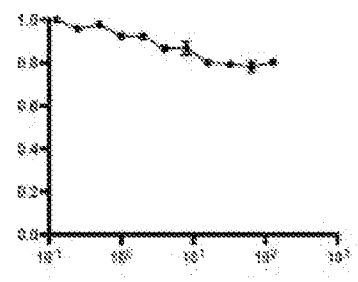
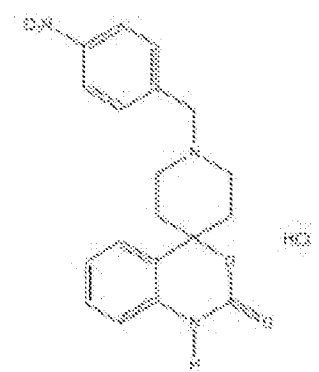
other hand, encodes a symmetric inner membrane complex containing LolD and two copies of LolE (also termed LolF), without LolC.

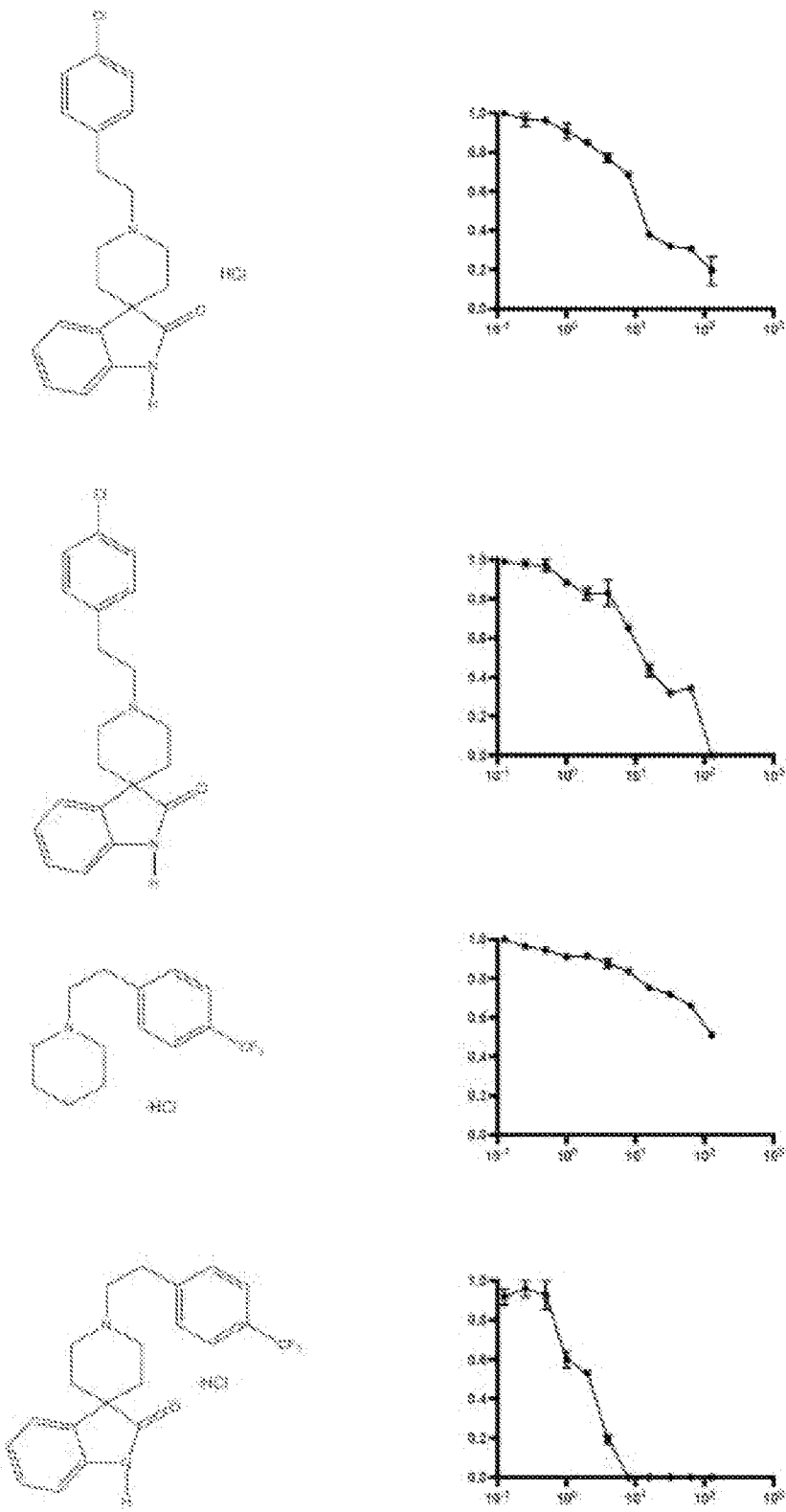
[00201] Provided below are compound of the present disclosure together with their respective inhibition curve relative to *A. Baumannii* with the X-axis representing concentration in ug/ml and the Y-axis representing spectrophotometer measurement at optical density (OD) 600 nm (OD600).











[00202] TABLES**[00203] Table 1.** *A. baumannii* strains tested against abaucin.

AR Bank #	Species	Resistance Mechanisms	Abaucin MIC (ug/ml)
AR-273	<i>Acinetobacter baumannii</i>	aac(3)-IIa, ADC-25, aph(3')-Ic, aph(3')-VIa, OXA-23, OXA-66, strA, strB, sul2	16
AR-274	<i>Acinetobacter baumannii</i>	aac(3)-Ia, ADC-25, aph(3')-Ic, OXA-66, OXA-72, strA, strB, sul1, sul2, TEM-1D	16
AR-275	<i>Acinetobacter baumannii</i>	ADC-25, aph(3')-Ic, armA, mph(E), msr(E), OXA-23, OXA-66, strA, strB, sul2, TEM-1D	8
AR-276	<i>Acinetobacter baumannii complex</i>		16
AR-277	<i>Acinetobacter baumannii</i>	aac(3)-IIa, OXA-24, OXA-65, strA, strB, sul2, TEM-1B	16
AR-278	<i>Acinetobacter baumannii</i>	ADC-25, aph(3')-Ic, aph(3')-VIa, armA, catB8, mph(E), msr(E), OXA-23, OXA-66, strA, strB, sul1, sul2, TEM- 1D	16
AR-279	<i>Acinetobacter baumannii</i>	ADC-25, aph(3')-Ic, aph(3')-VIa, armA, catB8, mph(E), msr(E), OXA-23, OXA-66, strA, strB, sul1, sul2, TEM- 1D	16
AR-280	<i>Acinetobacter baumannii</i>	aac(3)-Ia, ADC-25, aph(3')-Ic, OXA-66, strA, strB, sul1, TEM-1D	16
AR-281	<i>Acinetobacter baumannii</i>	aac(3)-Ia, ADC-25, aph(3')-Ic, OXA-82, strA, strB, sul1, sul2, TEM-1D	8

AR-282	<i>Acinetobacter baumannii</i>	ADC-25, aph(3')-Ic, armA, mph(E), msr(E), OXA-23, OXA-66, strA, strB, sul2, TEM-1D	16
AR-283	<i>Acinetobacter baumannii</i>	ADC-25, aph(3')-Ic, aph(3')-VIa, armA, catB8, mph(E), msr(E), OXA-23, OXA-66, strA, strB, sul1, TEM-1D	16
AR-284	<i>Acinetobacter baumannii</i>	aac(3)-IIa, OXA-24, OXA-65, strA, strB, sul2, TEM-1B	16
AR-285	<i>Acinetobacter baumannii</i>	aac(3)-IIa, OXA-24, OXA-65, strA, strB, sul2, TEM-1B	2
AR-286	<i>Acinetobacter baumannii</i>	ADC-25, aph(3')-Ic, armA, catB8, mph(E), msr(E), OXA-24, OXA-66, strA, strB, sul1	8
AR-287	<i>Acinetobacter baumannii</i>	ADC-25, OXA-66, OXA-72, strA, strB, sul2	16
AR-288	<i>Acinetobacter baumannii</i>	ADC-25, armA, catB8, mph(E), msr(E), OXA-23, OXA-66, strA, strB, sul1	16
AR-289	<i>Acinetobacter baumannii</i>	aac(3)-Ia, ADC-25, OXA-66, OXA-72, strA, strB, sul2	16
AR-290	<i>Acinetobacter baumannii</i>	ADC-25, aph(3')-Ic, aph(3')-VIa, armA, catB8, mph(E), msr(E), OXA-23, OXA-66, strA, strB, sul1, TEM-1D	16
AR-291	<i>Acinetobacter baumannii</i>	ADC-25, aph(3')-Ic, aph(3')-VIa, armA, catB8, mph(E), msr(E), OXA-23, OXA-66, strA, strB, sul1, TEM-1D	16
AR-292	<i>Acinetobacter baumannii</i>	aac(3)-Ia, OXA-66, OXA-72, strA, strB	4
AR-293	<i>Acinetobacter baumannii</i>	aac(3)-Ia, OXA-66, OXA-72, strA, strB	16
AR-294	<i>Acinetobacter baumannii</i>	aac(3)-IIa, aph(3')-VIa, OXA-23, OXA-65, strA, strB, sul2, TEM-1B	16
AR-295	<i>Acinetobacter baumannii</i>	ADC-25, OXA-23, OXA-66, strA, strB, sul2, TEM-1D	32

AR-296	<i>Acinetobacter baumannii</i>	aadA2, aadB, ADC-25, OXA-223, OXA-23, sul1	16
AR-297	<i>Acinetobacter baumannii</i>	ADC-25, mph(E), msr(E), OXA-23, OXA-66, strA, strB, sul2	32
AR-298	<i>Acinetobacter baumannii</i>	ADC-25, aph(3')-Ic, armA, catB8, mph(E), msr(E), OXA-237, OXA-66, strA, strB, sul1, sul2, TEM-1D	8
AR-299	<i>Acinetobacter baumannii</i>	aph(3')-VIa, armA, ARR-3, cmIA1, mph(E), msr(E), OXA-203, OXA-23, PER-7, strA, strB, sul1, sul2, tet(B)	32
AR-300	<i>Acinetobacter baumannii</i>	ADC-25, aph(3')-Ic, armA, catB8, mph(E), msr(E), OXA-66, strA, strB, sul1, sul2	16
AR-301	<i>Acinetobacter baumannii</i>	ADC-25, mph(E), msr(E), OXA-66, OXA-72, strA, strB, sul2	8
AR-302	<i>Acinetobacter baumannii</i>	aac(3)-Ia, armA, mph(E), msr(E), OXA-23, OXA-82, strA, strB, sul1	16
AR-303	<i>Acinetobacter baumannii</i>	ADC-25, aph(3')-Ic, aph(3')-VIa, armA, catB8, mph(E), msr(E), OXA-23, OXA-66, strA, strB, sul1, sul2	64
AR-304	<i>Acinetobacter baumannii</i>	aac(3)-Ia, ADC-25, OXA-66, OXA-72, strA, strB, sul2	4
AR-305	<i>Acinetobacter baumannii</i>	aac(3)-IIa, OXA-24, OXA-65, strA, strB, sul2, TEM-1B	16
AR-306	<i>Acinetobacter baumannii</i>	aac(3)-IIa, OXA-24, OXA-65, strA, strB, sul2, TEM-1B	16
AR-307	<i>Acinetobacter baumannii</i>	ADC-25, aph(3')-Ic, armA, catB8, mph(E), msr(E), OXA-237, OXA-66, strA, strB, sul1, sul2	16
AR-308	<i>Acinetobacter baumannii</i>	ADC-25, armA, catB8, mph(E), msr(E), OXA-71, strA, strB, sul1, TEM-1D	16
AR-309	<i>Acinetobacter baumannii</i>	aac(3)-Ia, armA, mph(E), msr(E), OXA-23, OXA-82, strA, strB, sul1	16

AR-310	<i>Acinetobacter baumannii</i>	aac(3)-Ia, armA, mph(E), msr(E), OXA-23, OXA-82, strA, strB, sul1	16
AR-311	<i>Acinetobacter baumannii</i>	ADC-25, aph(3')-Ic, armA, catB8, mph(E), msr(E), OXA-23, OXA-82, strA, strB, sul1	8
AR-312	<i>Acinetobacter baumannii</i>	aph(3')-Ic, catA1, OXA-69, sul2, tet(B)	16
AR-313	<i>Acinetobacter baumannii</i>	aac(3)-Ia, aph(3')-Ic, catA1, OXA-23, OXA-69, TEM-1D, tet(A)	16

[00204] Table 2. Carbapenem-resistant Enterobacteriaceae (CRE) strains tested against abaucin.

AR Bank #	Species	Resistance Mechanisms
AR-112	<i>Klebsiella pneumoniae</i>	aac(6'), aph(3'), aph(4), catA1, cmlA1, dfrA12, KPC-3 , mph(A), oqxA, oqxB, sul1, sul3
AR-113	<i>Klebsiella pneumoniae</i>	aac(6')-Ib, aph(3')-Ia, aph(4)-Ia, catA1, cmlA1, dfrA12, KPC-3 , mph(A), OmpK35, OmpK36, oqxA, oqxB, SHV-11, sul1, sul3
AR-114	<i>Escherichia coli</i>	aadB, cmlA1, dfrA5, KPC-3 , strA, strB, sul1, sul2, TEM-1B
AR-115	<i>Klebsiella pneumoniae</i>	aph(3')-Ia, aph(4)-Ia, catA1, cmlA1, dfrA12, KPC-3 , mph(A), OmpK35, oqxA, oqxB, sul1, sul3, TEM-1A

AR-116	<i>Citrobacter freundii</i>	aac(6')-If, aadA1, aadB, CMY-76, CMY-79, dfrA1, KPC-2 , OmpF, sul1, sul2
AR-117	<i>Klebsiella pneumoniae</i>	aadB, catB3, dfrA14, dfrA18, fosA, KPC-3 , oqxA, OXA-9, sul1, TEM-1A
AR-118	<i>Escherichia coli</i>	aac(3)-IIa, catA1, CMY-6, dfrA29, NDM-1 , OmpF, OXA-2, rmtC, strA, strB, sul1, TEM-1A
AR-119	<i>Escherichia coli</i>	aadA1, catA1, CMY-6, CTX-M-15, dfrA29, NDM-1 , OmpF, OXA-1, OXA-2, OXA-9, rmtC, strA, strB, sul1, TEM-1B
AR-121	<i>Serratia marcescens</i>	SME-3
AR-122	<i>Serratia marcescens</i>	SME-3
AR-123	<i>Serratia marcescens</i>	SME-3
AR-124	<i>Serratia marcescens</i>	SME-3
AR-125	<i>Klebsiella pneumoniae</i>	aph(3')-Ia, aph(4)-Ia, catA1, cmlA1, dfrA12, KPC-3 , mph(A), OmpK35, oqxA, oqxB, OXA-9, sul1, sul3, TEM-1B
AR-126	<i>Klebsiella pneumoniae</i>	, catB3, dfrA1, fosA, KPC-2 , OmpK36, oqxA, OXA-1, sul1, TEM-1B
AR-127	<i>Salmonella</i> Senftenberg	aac(3)-IId, armA, CMY-4, mph(E), msr(E), NDM-1 , strA, strB, sul1, TEM-1B

AR-128	<i>Escherichia coli</i>	aac(3)-IIa, aadA5, catA1, CMY-6, CTX-M-15, dfrA17, dfrA29, mph(A), NDM-1 , OmpF, OXA-2, rmtC, strA, strB, sul1, tet(B)
AR-129	<i>Klebsiella pneumoniae</i>	aac(6')-Ib, aadA2, aph(3')-Ia, catA1, dfrA12, KPC-3 , mph(A), OmpK35, oqxA, oqxB, sul1, TEM-1A
AR-130	<i>Serratia marcescens</i>	SME-3
AR-131	<i>Serratia marcescens</i>	SME-3
AR-132	<i>Enterobacter cloacae</i> group	fosA, NMC-A
AR-133	<i>Morganella morganii</i>	aadA1, dfrA1, dfrA14, KPC-2 , tet(D)
AR-134	<i>Raoultella ornithinolytica</i>	aac(6')-Ib, aadA1, dfrA5, ere(A), KPC-3 , OXA-9, strA, strB, sul1, sul2, TEM-1A
AR-135	<i>Klebsiella pneumoniae</i>	aac(3)-IIa, aac(6')-Ib, aph(3')-XV, catB2, dfrA14, OmpK35, oqxA, OXA-9, SHV-12, sul1, TEM-1A, tet(D), VIM-1
AR-136	<i>Enterobacter cloacae</i>	aac(6')-Ib, aadA1, dfrA14, KPC-3 , OmpF2, OXA-9, SHV-12, strA, strB, sul2, TEM-1A

[00205] **Table 3.** *P. aeruginosa* strains tested against abaucin.

AR Bank #	Species	Resistance Mechanisms
AR-229	<i>Pseudomonas aeruginosa</i>	OXA-50, PAO
AR-230	<i>Pseudomonas aeruginosa</i>	aac(3)-Id, aadA2, cmlA1, dfrB5, OXA-4, OXA-50, PAO, tet(G), VIM-2
AR-231	<i>Pseudomonas aeruginosa</i>	aac(6')-IIc, KPC-5 , OXA-2, OXA-50, PAO
AR-232	<i>Pseudomonas aeruginosa</i>	aadA6, OXA-50, PAO, strA, strB, sul1, tet(C)
AR-233	<i>Pseudomonas aeruginosa</i>	catB7, OXA-50, PAO
AR-234	<i>Pseudomonas aeruginosa</i>	aadA6, OXA-50, PAO, strA, strB, tet(C)
AR-235	<i>Pseudomonas aeruginosa</i>	aadB, OXA-10, OXA-50, PAO, sul1, VEB-1
AR-236	<i>Pseudomonas aeruginosa</i>	aadB, aph(3')-IIb, OXA-50, PAO
AR-237	<i>Pseudomonas aeruginosa</i>	catB7, OXA-50, PAO
AR-238	<i>Pseudomonas aeruginosa</i>	catB7, OXA-50, PAO
AR-239	<i>Pseudomonas aeruginosa</i>	aac(6')-IIa, aadB, aph(3')-Ic, cmlA1, dfrB5, GES-1, OXA-10, OXA-50, strA, strB, tet(G), VIM-11

AR-240	<i>Pseudomonas aeruginosa</i>	aadA6, OXA-50, PAO, sul1, VIM-2
AR-241	<i>Pseudomonas aeruginosa</i>	aac(6')-IIc, aadA7, catB7, IMP-1 , OXA-101, OXA-50, OXA-9, PAO, sul1
AR-242	<i>Pseudomonas aeruginosa</i>	aac(3)-Id, aadA2, cmlA1, dfrB5, OXA-4, OXA-50, PAO, VIM-2
AR-243	<i>Pseudomonas aeruginosa</i>	aac(3)-Id, aadA2, cmlA1, dfrB5, OXA-4, OXA-50, PAO, tet(G), VIM-2
AR-244	<i>Pseudomonas aeruginosa</i>	OXA-50
AR-245	<i>Pseudomonas aeruginosa</i>	aph(3'), OXA, PAO, sul1, VIM-2
AR-246	<i>Pseudomonas aeruginosa</i>	aadB, NDM-1 , OXA-10, OXA-50, PAO, rmtD2, tet(G), VEB-1
AR-247	<i>Pseudomonas aeruginosa</i>	OXA-50, PAO
AR-248	<i>Pseudomonas aeruginosa</i>	aac(3)-Id, aadA2, cmlA1, dfrB5, OXA-4, OXA-50, PAO, tet(G), VIM-2
AR-249	<i>Pseudomonas aeruginosa</i>	aac(3)-Id, aadA2, cmlA1, dfrB5, OXA-4, OXA-50, PAO, tet(G), VIM-2
AR-250	<i>Pseudomonas aeruginosa</i>	aadB, NDM-1 , OXA-10, OXA-50, PAO, rmtD2, tet(G), VEB-1
AR-251	<i>Pseudomonas aeruginosa</i>	catB7, OXA-50, PAO

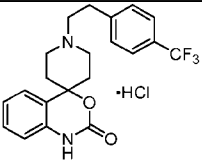
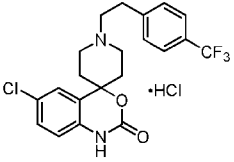
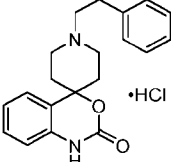
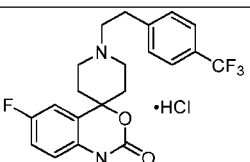
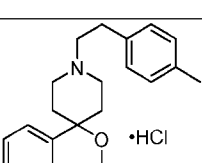
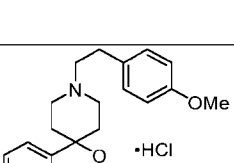
AR-252	<i>Pseudomonas aeruginosa</i>	aadA1, aadA6, OXA-2, OXA-50, PAO, sul1
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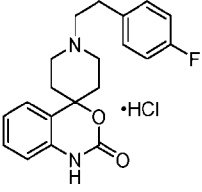
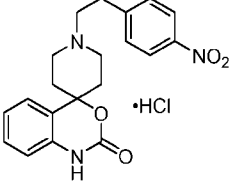
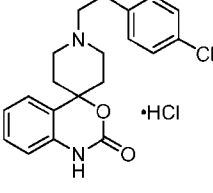
[00206] **Table 4.** *S. aureus* strains tested against abaucin.

AR Bank #	Species	Resistance Mechanisms
AR-215	<i>Staphylococcus aureus</i>	aadD, blaZ, erm(A), mecA, spc
AR-216	<i>Staphylococcus aureus</i>	aph(3')-III, mecA, mph(C), msr(A)
AR-217	<i>Staphylococcus aureus</i>	blaZ, dfrG, mecA
AR-218	<i>Staphylococcus aureus</i>	aph(3')-III, erm(A), mecA, spc, tet(K)
AR-219	<i>Staphylococcus aureus</i>	aac(6')-aph(2''), aadD, erm(A), mecA, spc, tet(M)
AR-220	<i>Staphylococcus aureus</i>	aadD, blaZ, erm(A), mecA, spc
AR-221	<i>Staphylococcus aureus</i>	aac(6')-aph(2''), mecA, tet(M)
AR-222	<i>Staphylococcus aureus</i>	blaZ
AR-223	<i>Staphylococcus aureus</i>	mecA
AR-224	<i>Staphylococcus aureus</i>	aph(3')-III, erm(A), mecA, spc, tet(K)
AR-225	<i>Staphylococcus aureus</i>	aph(3')-III, blaZ, mecA, mph(C), msr(A)
AR-226	<i>Staphylococcus aureus</i>	aph(3')-III, blaZ, mecA, mph(C), msr(A)
AR-227	<i>Staphylococcus aureus</i>	aadD, blaZ, erm(A), mecA, spc
AR-228	<i>Staphylococcus aureus</i>	aadD, blaZ, erm(A), mecA, spc

[00207] **Table 5. Minimum inhibitory concentration (MIC) of ABAUCIN analogs against a panel of bacterial species.** Growth inhibition of bacterial species by ABAUCIN and analogs thereof. Cells were grown in LB in the presence of varying concentrations of each molecule at 37°C and the MIC of each was determined. The strains shown are *E. coli* BW25113, *P. aeruginosa* PA01, *S. aureus* RN4220, and *A.*

baumannii ATCC 17978. Experiments were conducted in biological duplicate and resulted in identical MIC values.

	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>A. baumannii</i>
	>128 µg/ml	>128 µg/ml	>128 µg/ml	2 µg/ml
	>128 µg/ml	>128 µg/ml	>128 µg/ml	>128 µg/ml
	>128 µg/ml	>128 µg/ml	>128 µg/ml	8 µg/ml
	>128 µg/ml	>128 µg/ml	>128 µg/ml	64 µg/ml
	>128 µg/ml	>128 µg/ml	>128 µg/ml	32 µg/ml
	>128 µg/ml	>128 µg/ml	>128 µg/ml	64 µg/ml

	>128 µg/ml	>128 µg/ml	>128 µg/ml	16 µg/ml
	>128 µg/ml	>128 µg/ml	>128 µg/ml	32 µg/ml
	>128 µg/ml	>128 µg/ml	>128 µg/ml	8 µg/ml

[00208] **Table 6.** Human gut commensal strain panel.

Figure List	Surette Strain ID	NCBI Taxonomy	GTDB Phyla
1	GC684	<i>Eggerthella lenta</i>	Actinobacteriota
2	GC133	<i>Bifidobacterium longum</i>	Actinobacteriota
3	GC96	<i>Bifidobacterium breve</i>	Actinobacteriota
4	GC641	<i>Bifidobacterium adolescentis</i>	Actinobacteriota
5	GC267	<i>Parabacteroides distasonis</i>	Bacteroidota
6	GC187	<i>Bacteroides xylanisolvens</i>	Bacteroidota
7	GC186	<i>Bacteroides vulgatus</i>	Bacteroidota
8	GC239	<i>Bacteroides uniformis</i>	Bacteroidota
9	GC465	<i>Bacteroides thetaiotaomicron</i>	Bacteroidota
10	GC101	<i>Bacteroides stercoris</i>	Bacteroidota
11	GC137	<i>Bacteroides ovatus</i>	Bacteroidota

12	GC432	<i>Bacteroides intestinalis</i>	Bacteroidota
13	GC73	<i>Bacteroides fragilis</i>	Bacteroidota
14	GC201	<i>Bacteroides dorei</i>	Bacteroidota
15	GC74	<i>Bacteroides cellulosilyticus</i>	Bacteroidota
16	GC203	<i>Bacteroides caccae</i>	Bacteroidota
17	GC81	<i>Streptococcus salivarius</i>	Firmicutes A
18	GC104	<i>Streptococcus parasanguinis</i>	Firmicutes A
19	GC82	<i>Streptococcus anginosus</i>	Firmicutes A
20	GC4	<i>Lactobacillus plantarum</i>	Firmicutes A
21	GC8	<i>Lactobacillus paracasei</i>	Firmicutes A
22	GC23	<i>Enterococcus faecium</i>	Firmicutes A
23	GC27	<i>Enterococcus durans</i>	Firmicutes A
24	GC273	<i>Coprobacillus cateniformis</i>	Firmicutes A
25	GC458	<i>Faecalicatena orotica</i>	Firmicutes B
26	GC280	<i>Dorea formicigenerans</i>	Firmicutes B
27	GC218	<i>Coprococcus comes</i>	Firmicutes B
29	GC1500	<i>Clostridium sporogenes</i>	Firmicutes B
29	GC1584	<i>Clostridium perfringens</i>	Firmicutes B
30	GC501	<i>Blautia wexlerae</i>	Firmicutes B
31	GC279	<i>Blautia obeum</i>	Firmicutes B
32	GC253	<i>Escherichia fergusonii</i>	Proteobacteria
33	GC61	<i>Enterobacter himalayensis</i>	Proteobacteria
34	GC7	<i>Akkermansia muciniphila</i>	Verrucomicrobiota

[00209] **Table 7.** Human skin commensal strain panel.

Figure List	Surette Strain ID	NCBI Taxonomy	GTDB Phyla
35	GC1013	<i>Micrococcus luteus</i>	Actinobacteriota
36	GC1117	<i>Cutibacterium acnes</i> S2	Actinobacteriota
37	GC867	<i>Corynebacterium simulans</i>	Actinobacteriota
38	GC65	<i>Corynebacterium pseudodiphtheriticum</i>	Actinobacteriota
39	GC71	<i>Corynebacterium durum</i>	Actinobacteriota
40	GC45	<i>Corynebacterium coyleae</i>	Actinobacteriota
41	GC1149	<i>Corynebacterium aurimucosum</i>	Actinobacteriota
42	GC43	<i>Corynebacterium amycolatum</i>	Actinobacteriota
43	GC77	<i>Corynebacterium afermentans</i>	Actinobacteriota
44	GC256	<i>Streptococcus sanguinis</i>	Firmicutes
45	GC1490	<i>Streptococcus oralis</i>	Firmicutes
46	GC106	<i>Streptococcus mutans</i>	Firmicutes
47	C78	<i>Streptococcus mitis</i>	Firmicutes
48	GC1229	<i>Staphylococcus warneri</i>	Firmicutes
49	GC889	<i>Staphylococcus hominis</i>	Firmicutes
50	GC572	<i>Staphylococcus haemolyticus</i>	Firmicutes
51	GC1235	<i>Staphylococcus epidermidis</i> S2	Firmicutes
52	GC1217	<i>Staphylococcus epidermidis</i> S1	Firmicutes
53	GC1000	<i>Staphylococcus capitis</i>	Firmicutes

[00210] **Table 8. Frequency of resistance to ABAUCIN.** Wildtype *A. baumannii* was streaked onto solid media supplemented with varying concentrations of ABAUCIN for the noted durations, and emergent colonies were counted to quantify frequency of resistance as noted in the table. Dashed cells show conditions where lawns of *A. baumannii* was observed on the solid media plate.

Concentration	24 hours	48 hours	72 hours
0× MIC	--	--	--
0.5× MIC	--	--	--
1× MIC	--	--	--
2× MIC	1.58×10^{-5}	--	--
4× MIC	8.18×10^{-8}	1.45×10^{-7}	2.25×10^{-7}
8× MIC	0	0	0

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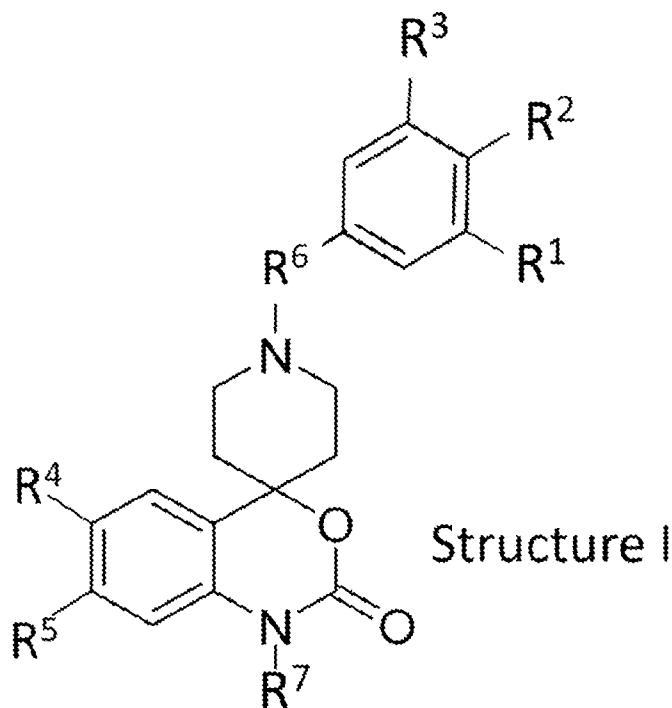
[00268] 57. Pettersen, E. F. *et al.* UCSF ChimeraX: Structure visualization for researchers, educators, and developers. *Protein Sci.* **30**, 70–82 (2021).

[00269] While the present disclosure has been described with reference to examples, it is to be understood that the scope of the claims should not be limited by the embodiments set forth in the examples but should be given the broadest interpretation consistent with the description as a whole.

[00270] All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety. Where a term in the present disclosure is found to be defined differently in a document incorporated herein by reference, the definition provided herein is to serve as the definition for the term.

WHAT IS CLAIMED IS:

1. A compound for inhibiting growth of an *Acinetobacter baumannii* bacterium, the compound having Structure I as follows:



or a pharmaceutically acceptable salt, solvate and/or prodrug thereof;

wherein:

$R^1 - R^3$ are independently selected from H, OH, CH₃, C₂H₅, NO₂, CN, halogen, alkoxy (e.g., methoxy), or trifluoromethyl, with at least one and more typically two, and quite possibly all three of $R^1 - R^3$ being H;

R^4 and R^5 are independently selected from H, OH, halogen or amine, with none or more typically one or both of R^4 and R^5 being H;

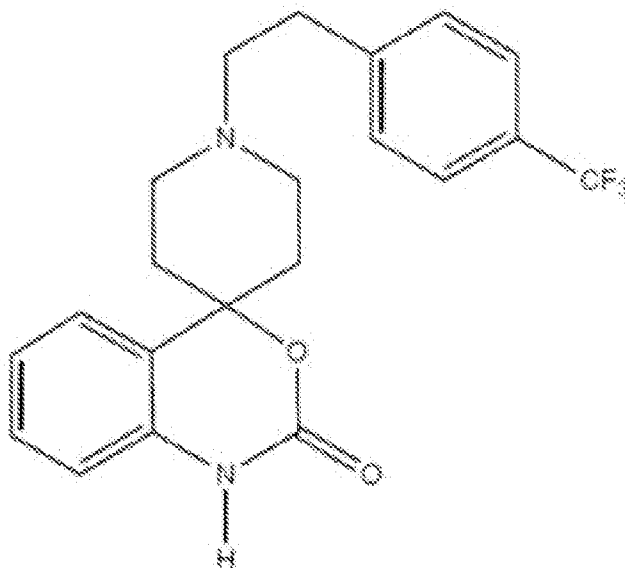
R^6 is C₁-C₃ alkyl or alkylene; and

R^7 is H or C₁-C₃ alkyl or alkylene.

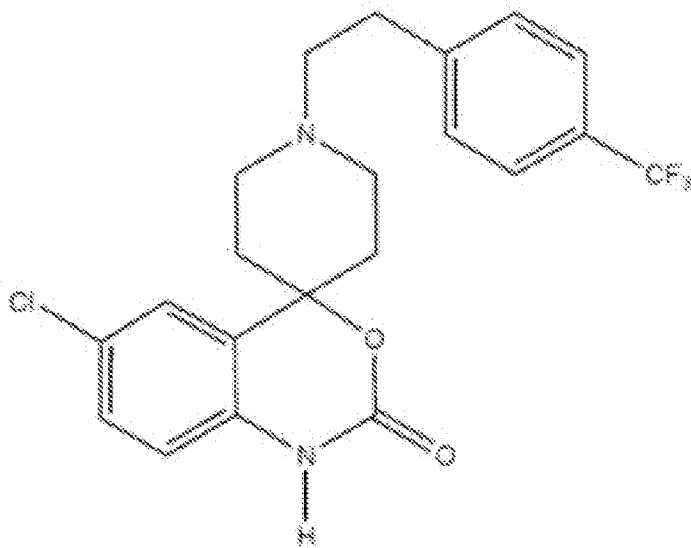
2. A compound as in claim 1, wherein R^1 and R^3 are H.
3. A compound as in claim 1 or 2, wherein R^2 is selected from H, OH, CH_3 , C_2H_5 , NO_2 , halogen, alkoxy or trifluoromethyl.
4. A compound as in claim 1, 2 or 3, wherein R^2 is selected from H, methoxy, halogen or trifluoromethyl.
5. A compound as in any one of claims 1 through 4, wherein R^4 is H.
6. A compound as in any one of claims 1 through 5, wherein R^5 is selected from H, OH, halogen or primary amine, or from H or halogen, with one or both of R^4 and R^5 being H.
7. A compound as in any one of claims 1 through 6, wherein R^6 is C_1 - C_2 alkyl.
8. A compound as in any one of claims 1 through 7, wherein R^6 is C_2 alkyl.
9. A compound as in any one of claims 1 through 8, wherein R^7 is H or C_1 - C_2 alkyl.
10. A compound as in any one of claims 1 through 9, wherein R^7 is H.
11. A compound as in any one of claims 1 through 10, wherein at least one of one of $R^1 - R^5$ is a halogen selected from F, Br, or Cl.
12. A compound as in any one of claims 1 through 11, wherein at least one of one of $R^1 - R^5$ is a halogen selected from F or Cl.

13. A compound as in any one of claim 1 through 12, wherein the compound exhibits a minimum inhibitory concentration (MIC) against *A. Baumannii*, particularly *A. baumannii* ATCC 17978 of no greater than 64 µg/ml, more typically no greater than 32 µg/ml, even more typically no greater than 16 µg/ml or even possibly no greater than 8 µg/ml or 2 µg/ml.

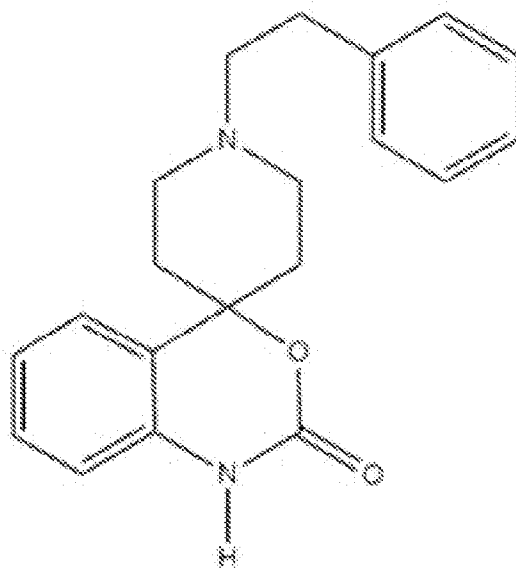
14. A compound as recited in claim 1 or, where appropriate, any one of claims 2 through 13, wherein the compound is selected from:



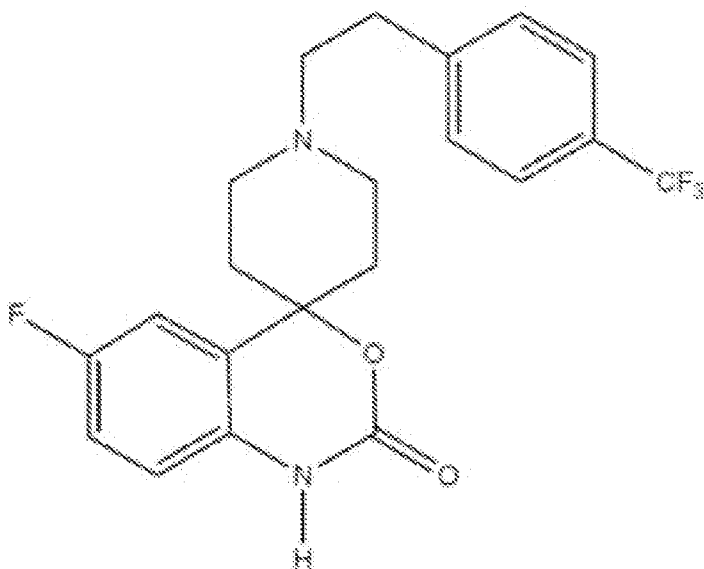
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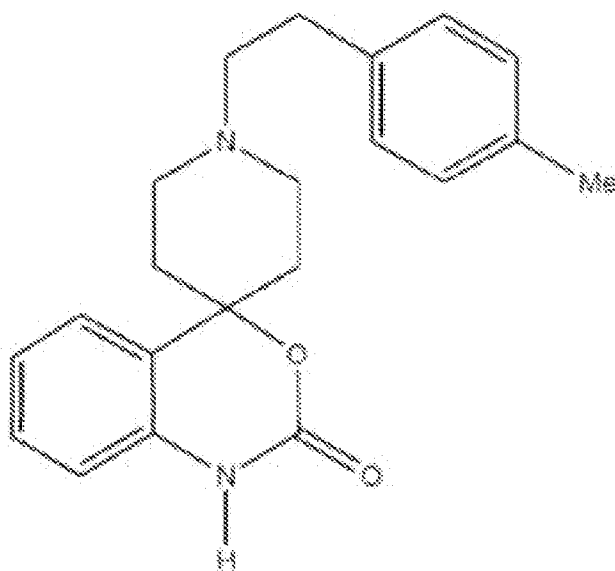
6-chloro-1'-(4-(trifluoromethyl)phenethyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



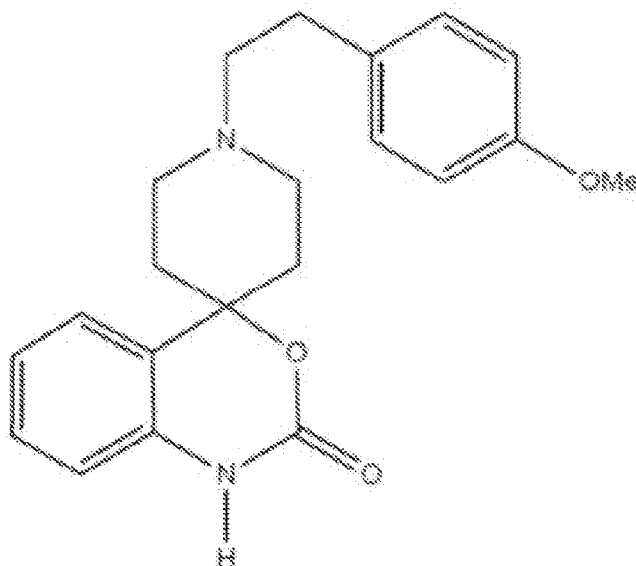
1'-phenethylspiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



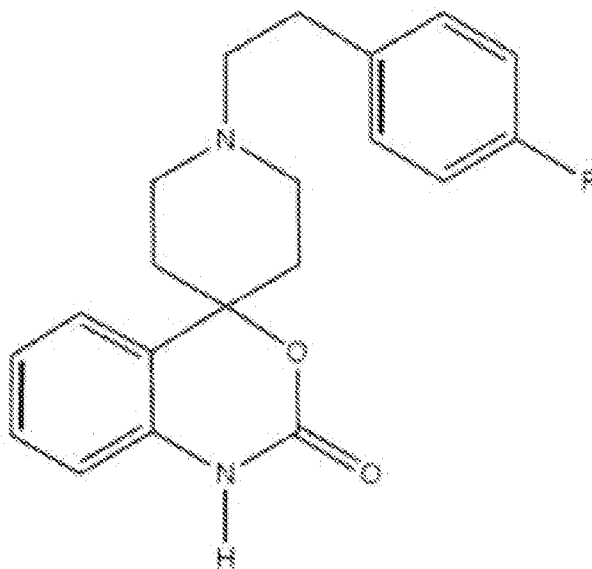
6-fluoro-1'-(4-(trifluoromethyl)phenethyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one.



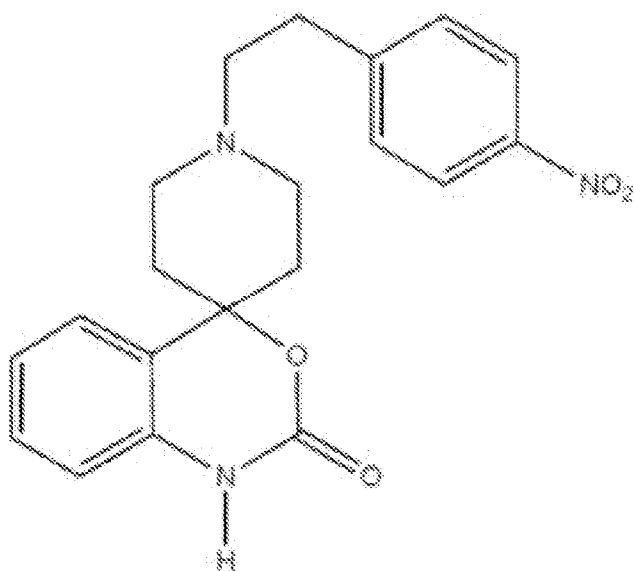
1'-(4-methylphenethyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



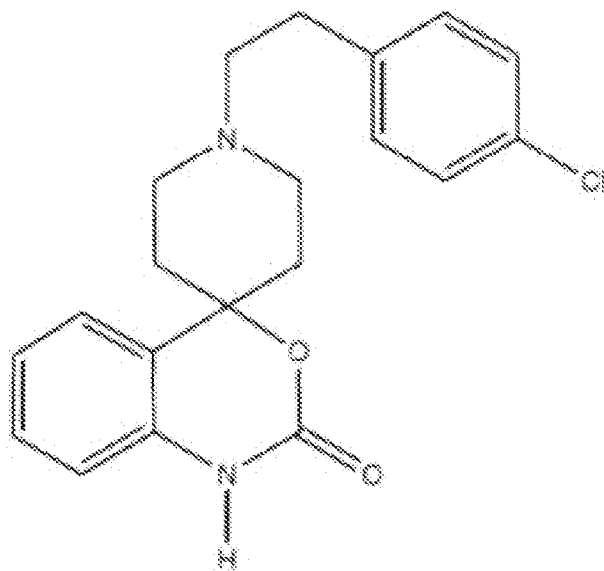
1'-(4-methoxyphenethyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



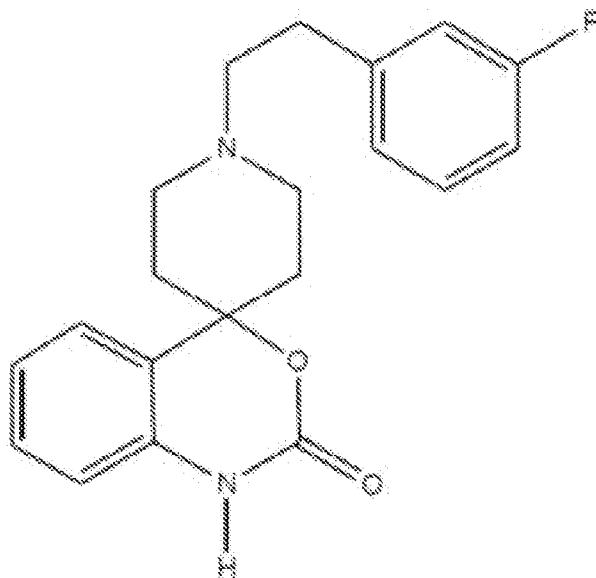
1'-(4-methoxyphenethyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



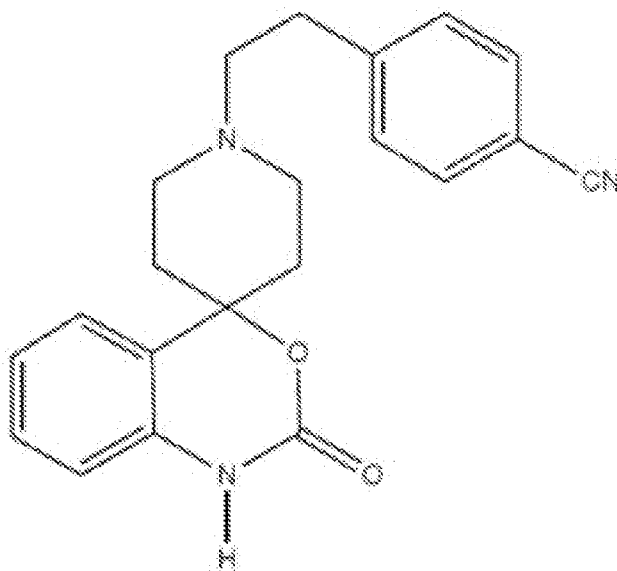
1'-(4-nitrophenethyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



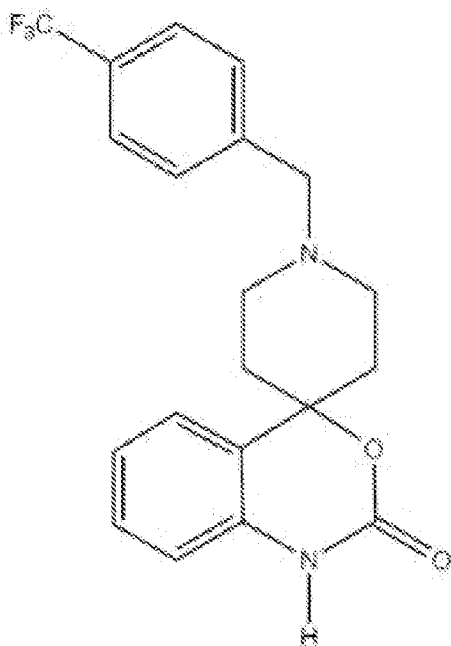
1'-(4-chlorophenethyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



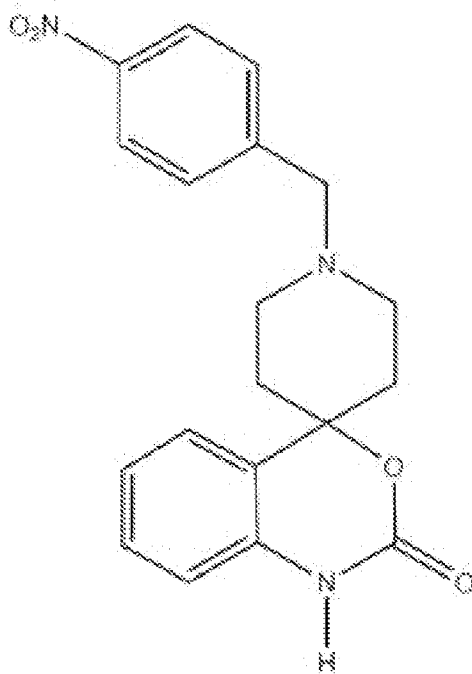
1'-(3-fluorophenethyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



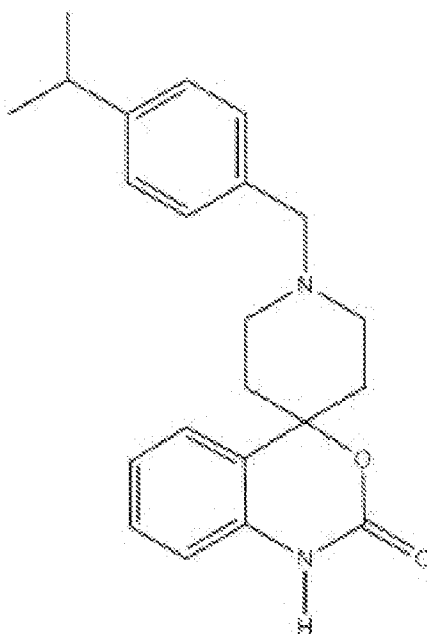
4-(2-(2-oxo-1,2-dihydrospiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-1'-yl)ethyl)benzonitrile,



1'-(4-(trifluoromethyl)benzyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,

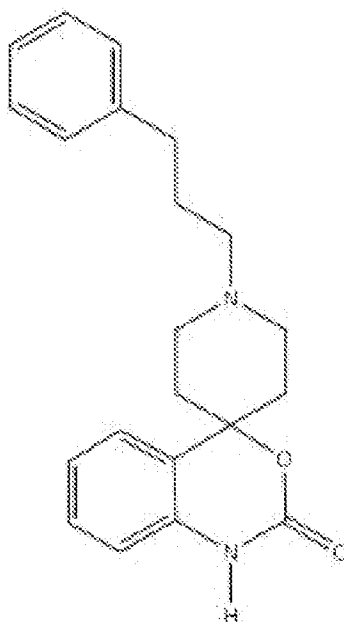


1'-(4-nitrobenzyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one,



1'-(4-isopropylbenzyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one

, or



1'-(3-phenylpropyl)spiro[benzo[*d*][1,3]oxazine-4,4'-piperidin]-2(1*H*)-one;

or a pharmaceutically acceptable salt, solvate and/or prodrug thereof.

15. A compound as in any one of claims 1 through 14, wherein the compound is provided as a HCl salt.

16. A composition comprising a compound of any one of claims 1 through 15, or a pharmaceutically acceptable salt, solvate and/or prodrug thereof, and a pharmaceutically acceptable carrier or vehicle.

17. A method for inhibiting growth of an *Acinetobacter baumannii* bacterium, comprising contacting the bacterium with an effective amount of a compound of any one of claims 1 through 16, or a pharmaceutically acceptable salt, solvate and/or prodrug thereof.

18. A method of treating an *Acinetobacter baumannii* bacterial infection comprising administering an effective amount of a compound of any one of claims 1 through 14, or a pharmaceutically acceptable salt, solvate and/or prodrug thereof, to a subject in need thereof.

19. Use of a compound of any one of claims 1 through 14, or a pharmaceutically acceptable salt pharmaceutically acceptable salt, solvate and/or prodrug thereof, to inhibit growth of an *Acinetobacter baumannii* bacterium.

20. Use of a compound of any one of claims 1 through 14, or a pharmaceutically acceptable salt pharmaceutically acceptable salt, solvate and/or prodrug thereof, to treat or prevent an *Acinetobacter baumannii* bacterial infection.

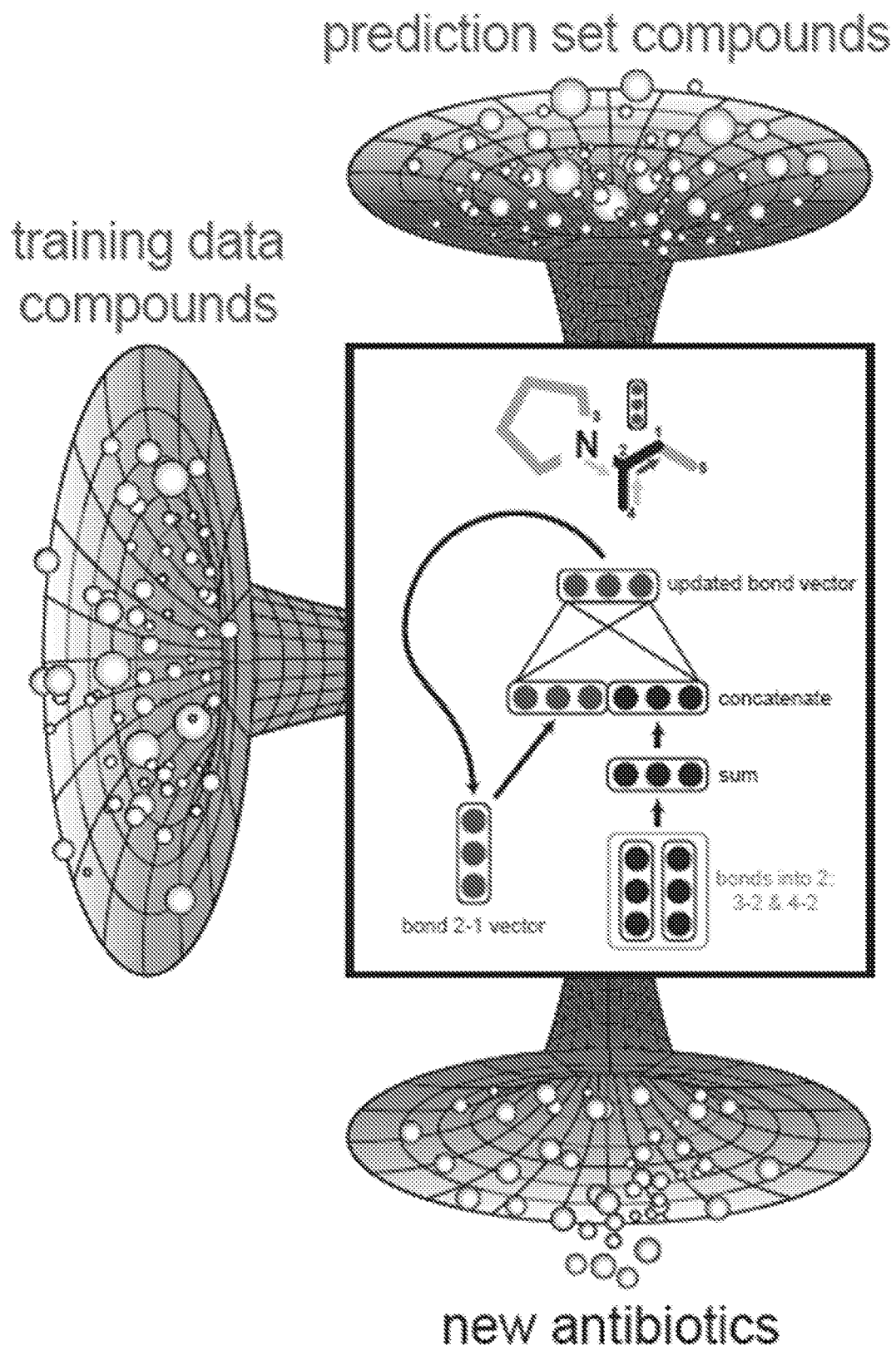


Fig. 1A

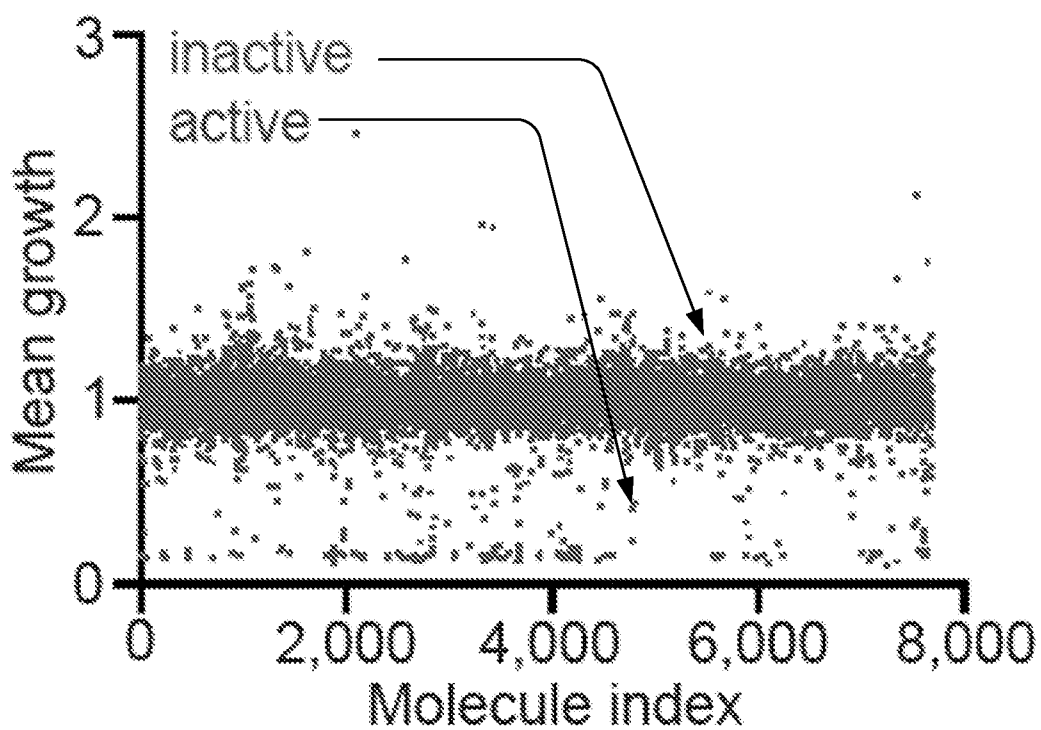


Fig. 1B

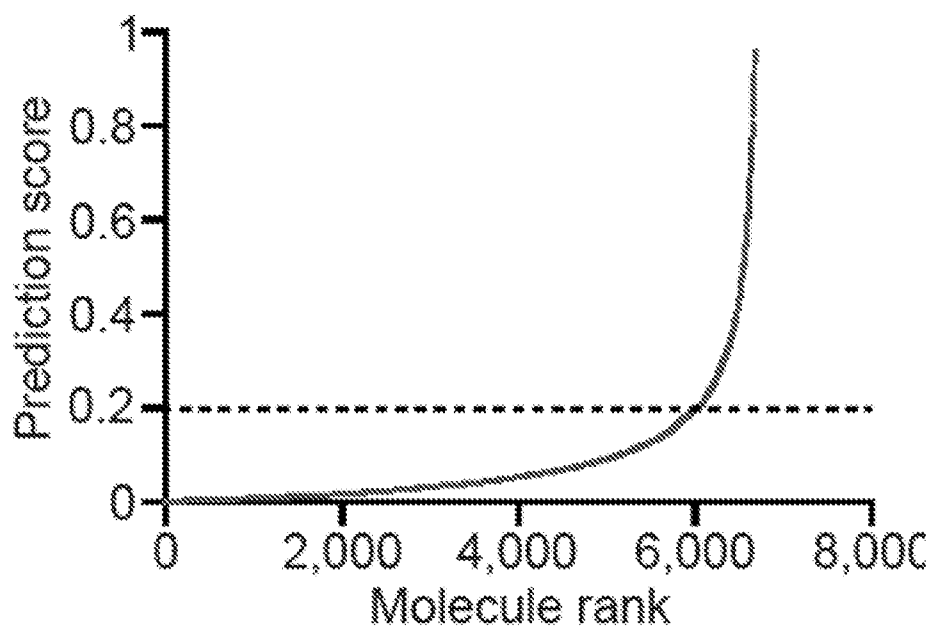


Fig. 1C

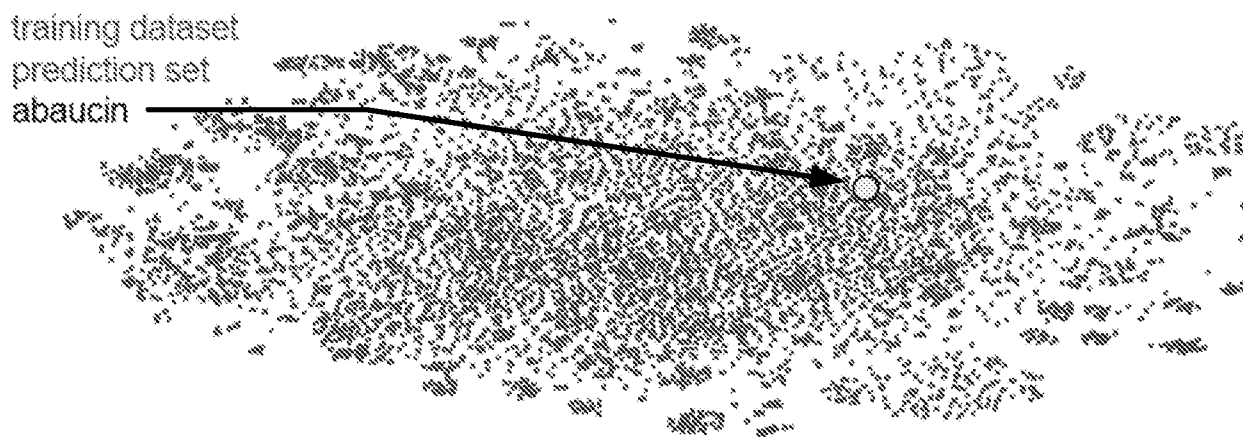


Fig. 1D

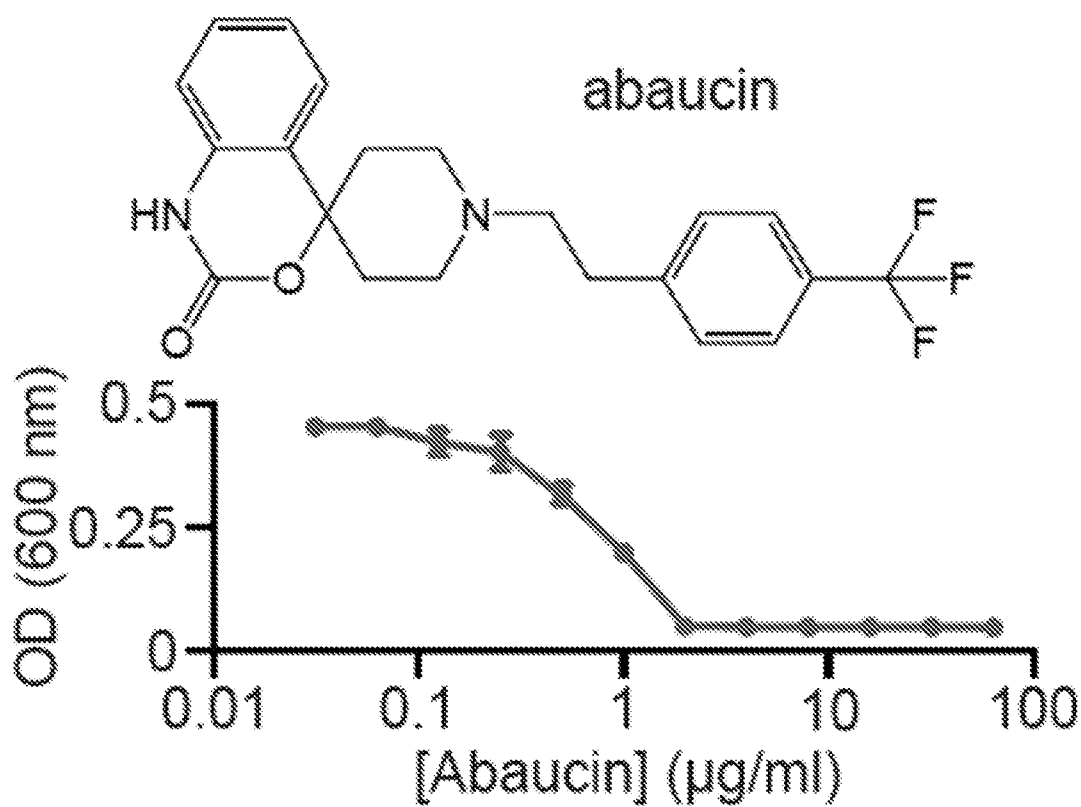


Fig. 1E

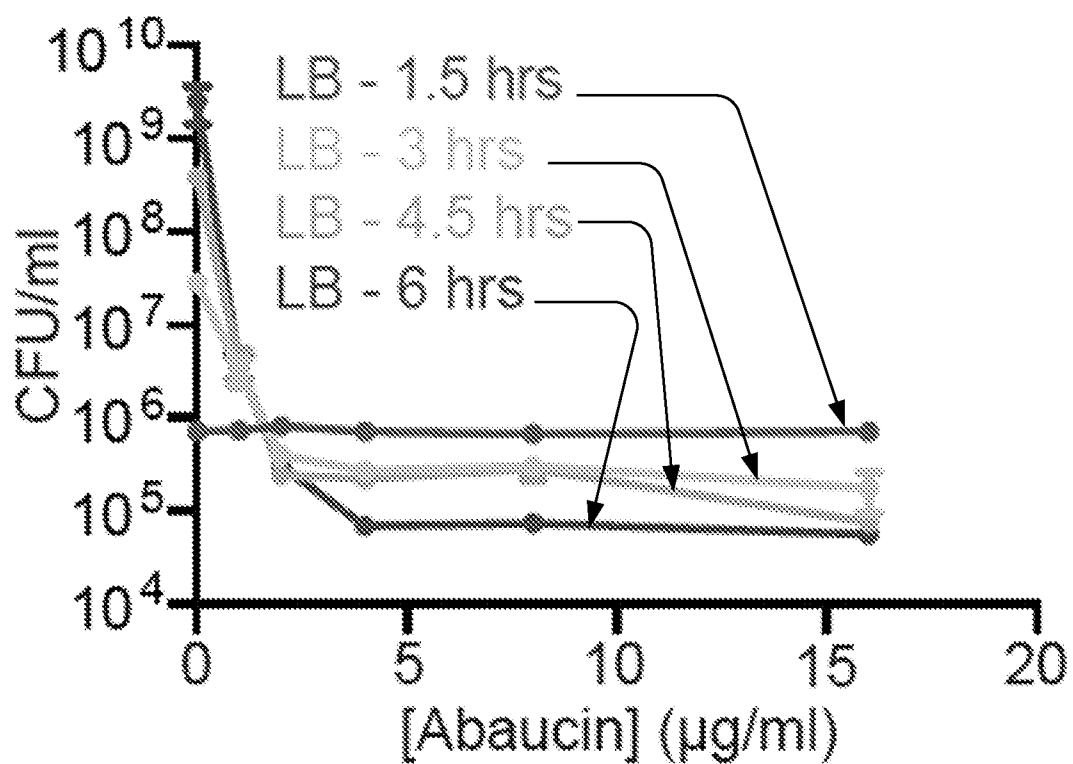


Fig. 1F

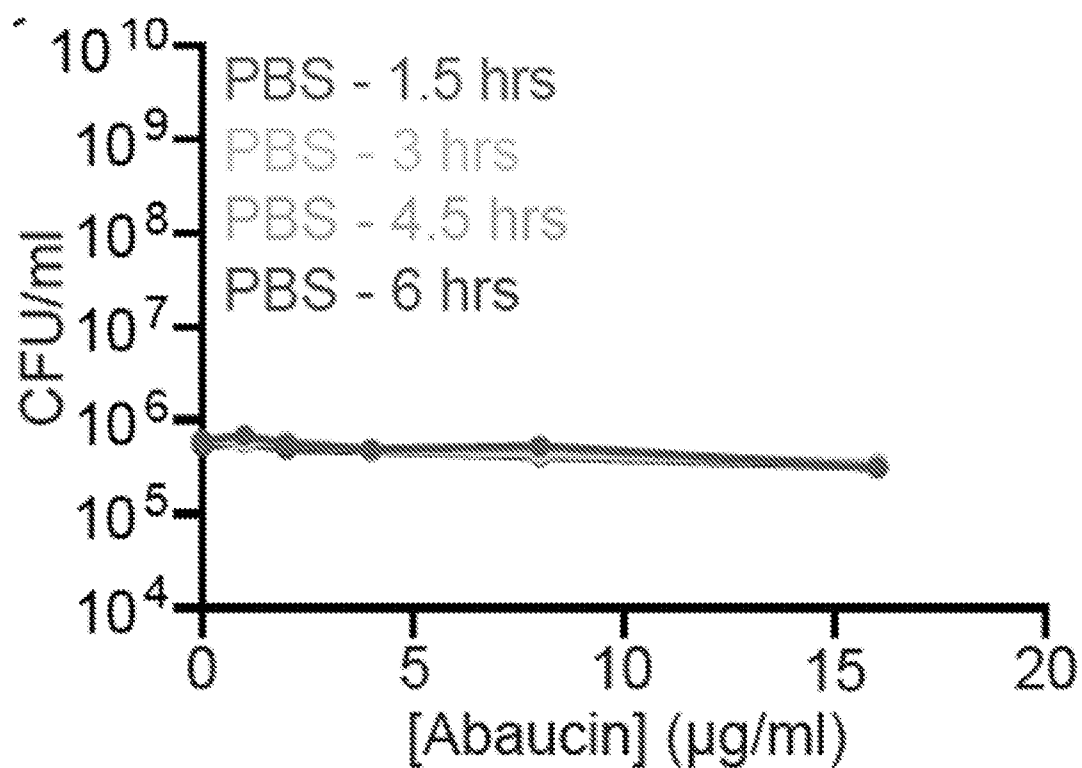


Fig. 1G

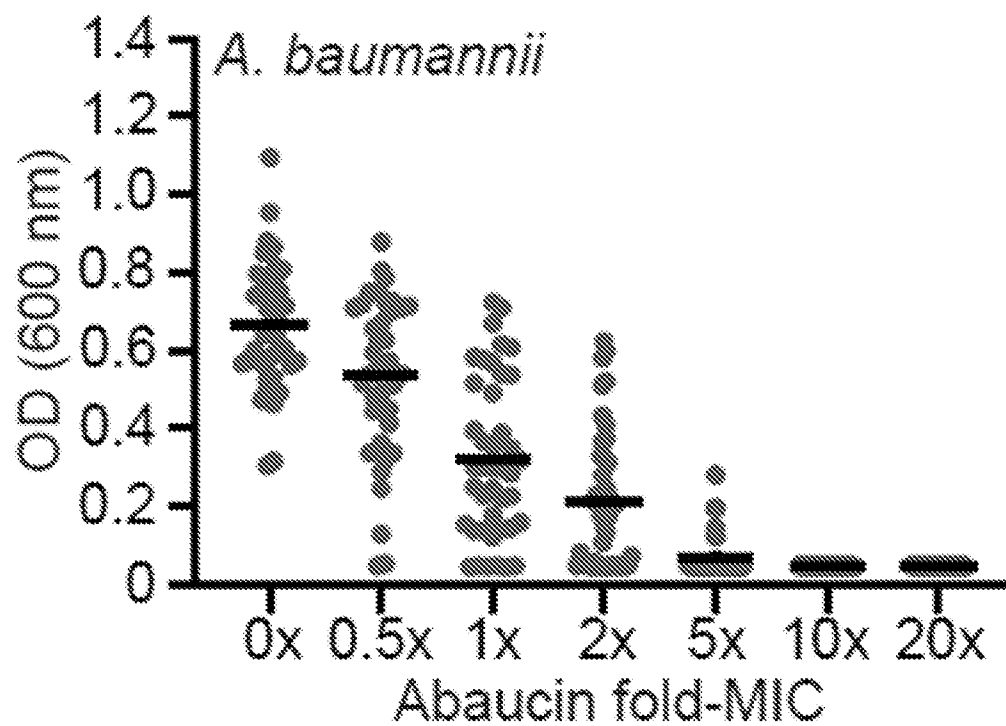


Fig. 2A

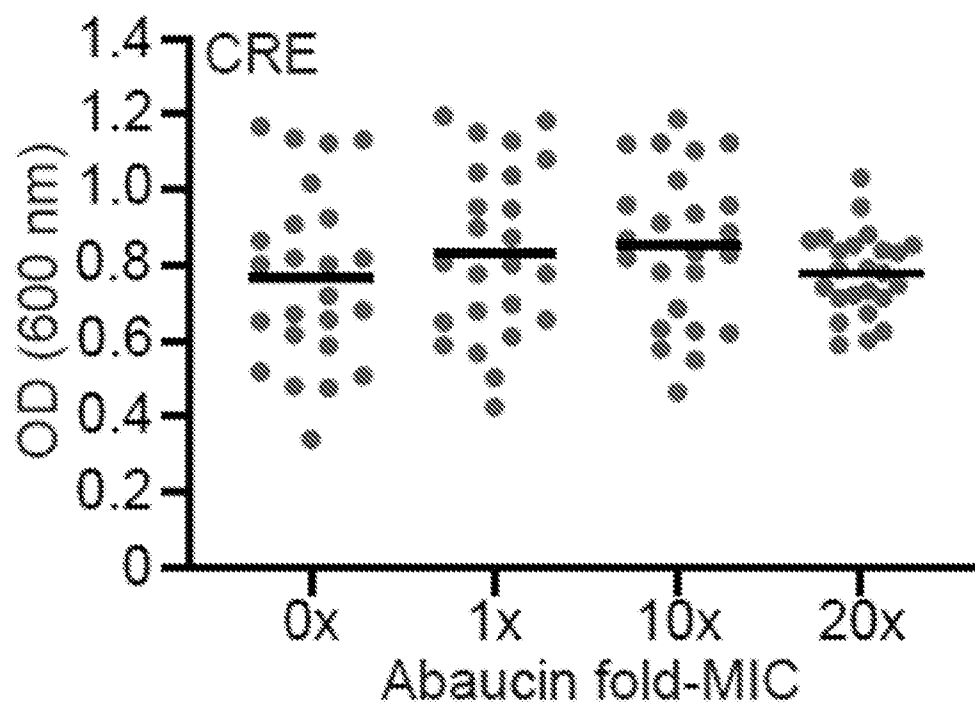


Fig. 2B

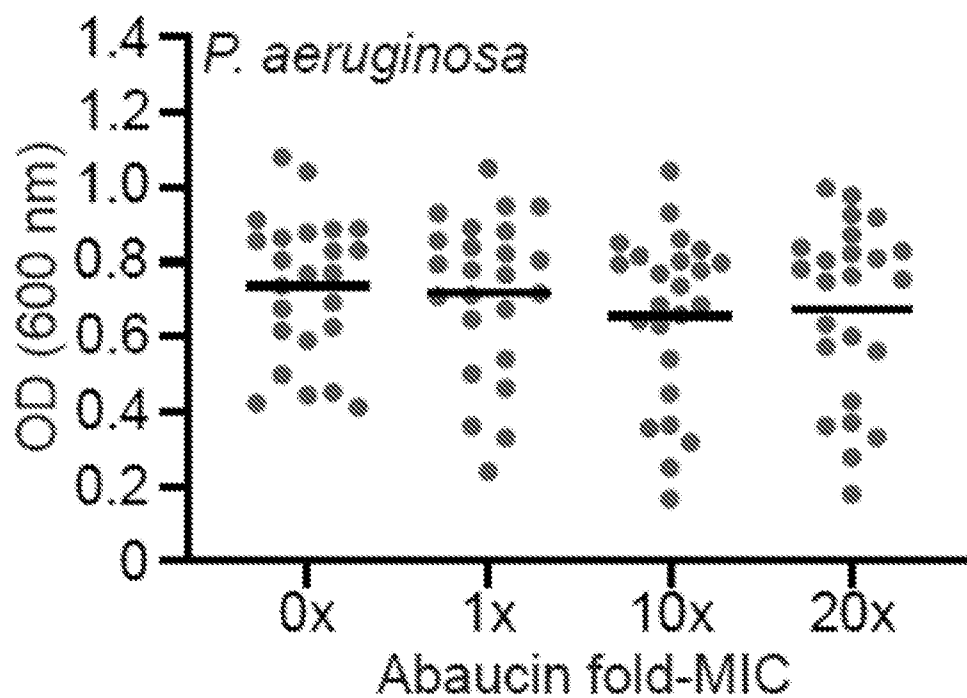


Fig. 2C

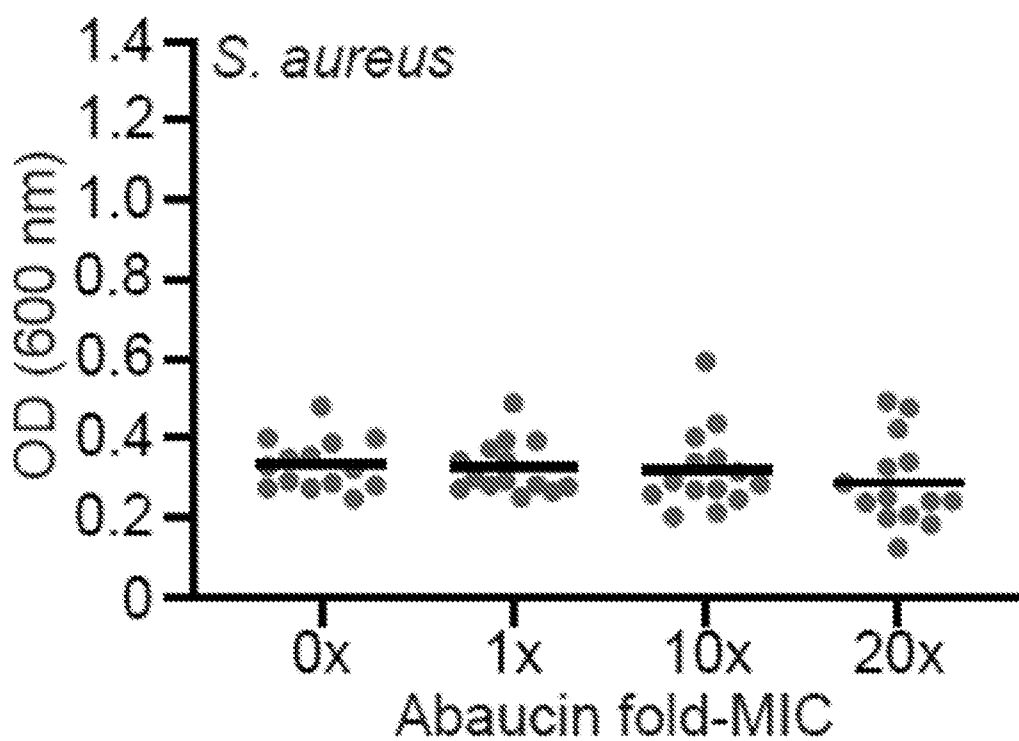


Fig. 2D

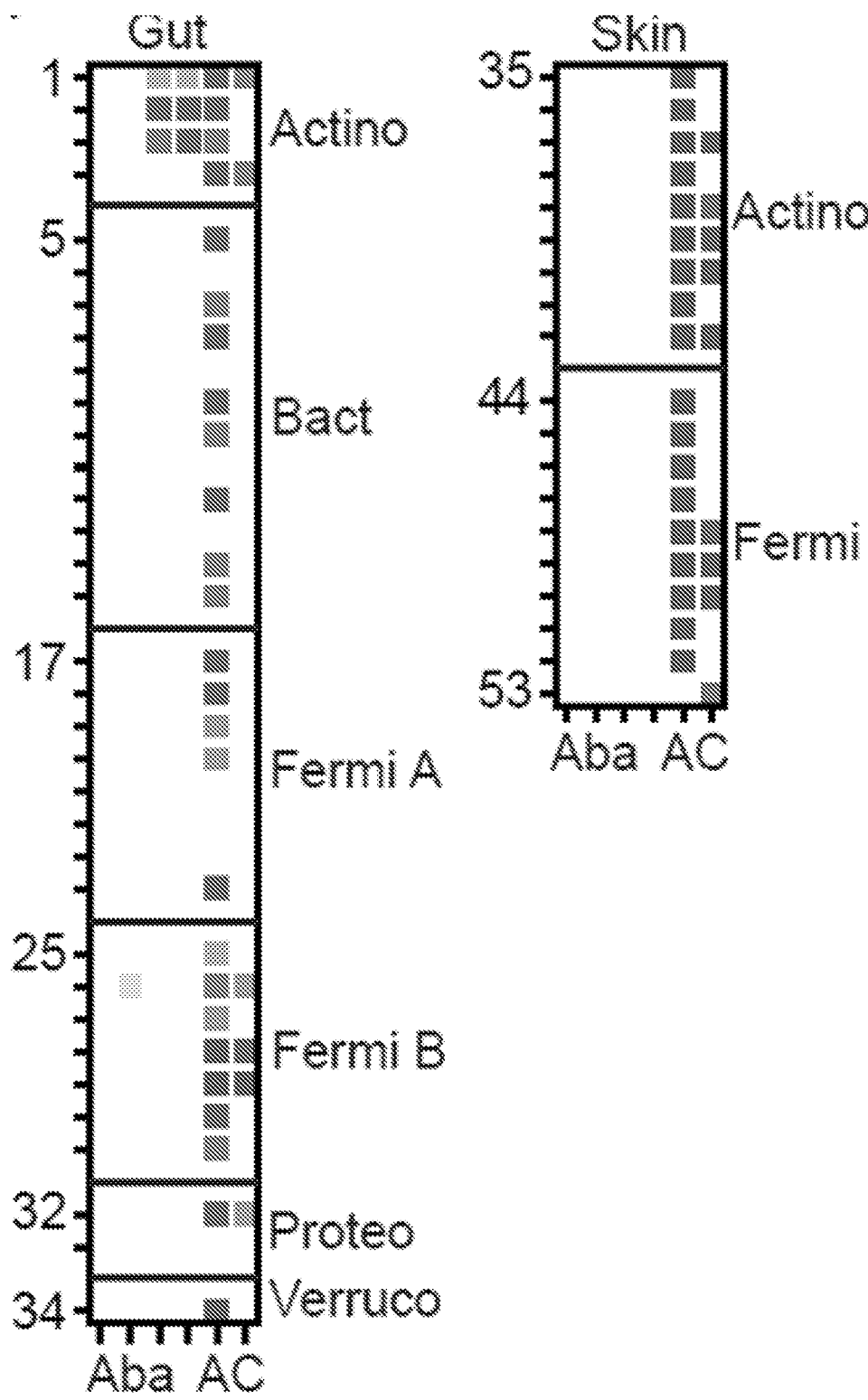


Fig. 2E

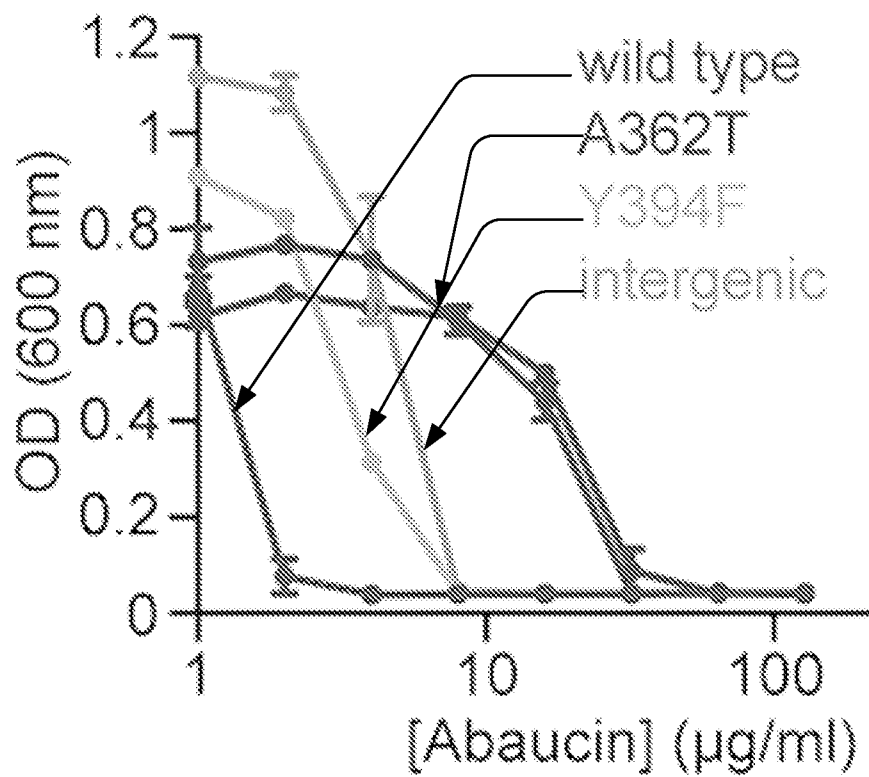


Fig. 3A

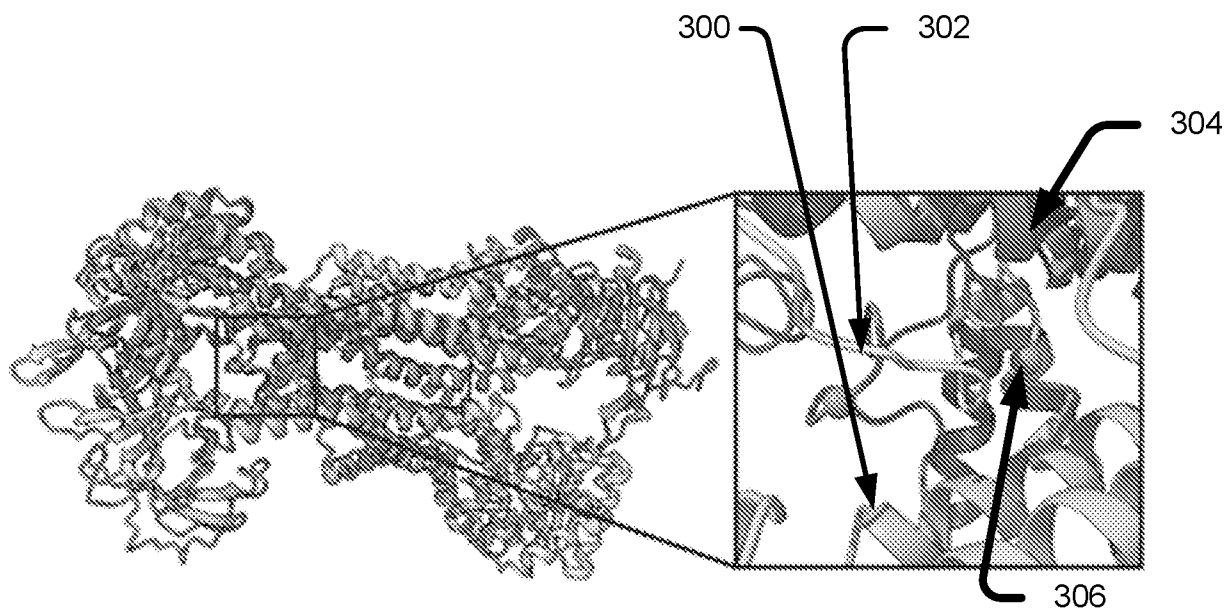


Fig. 3B

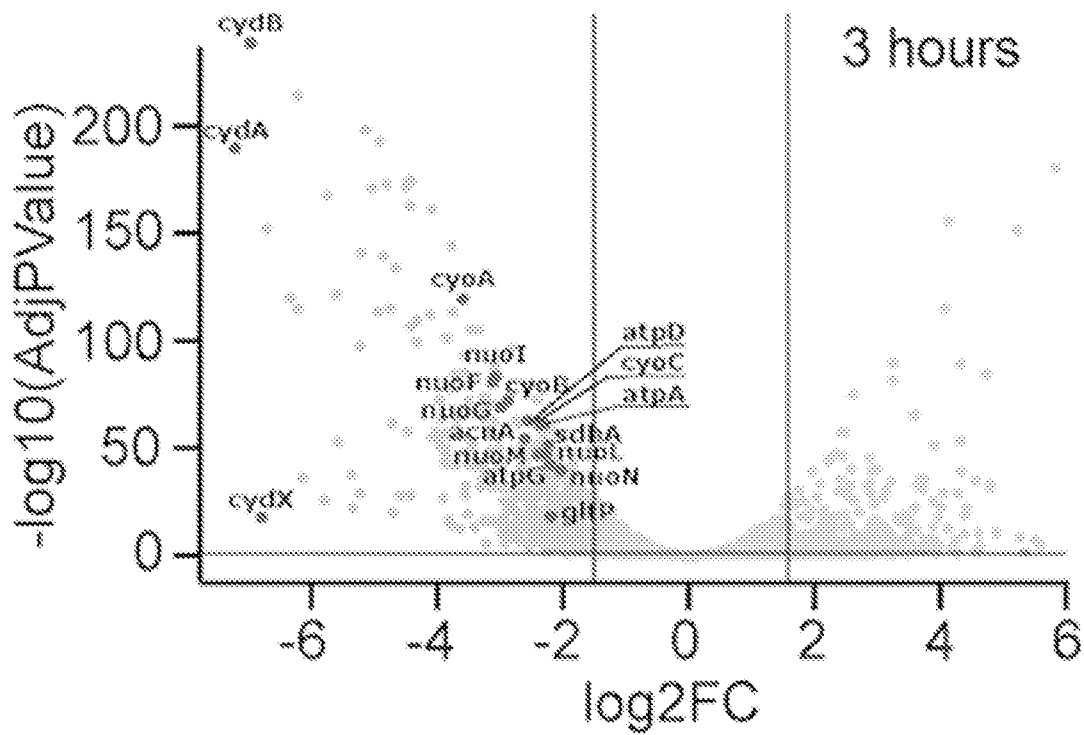


Fig. 3C

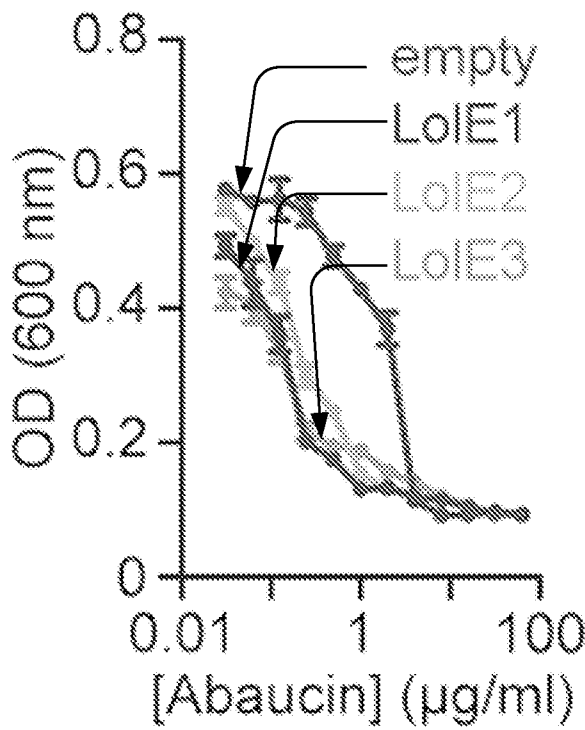


Fig. 3D

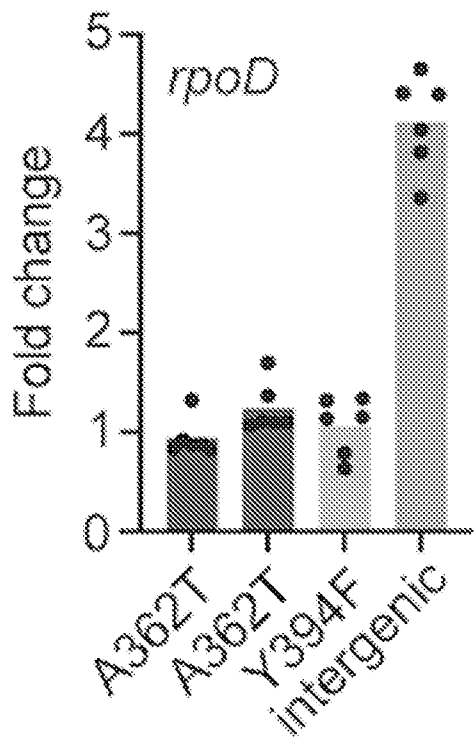


Fig 3E

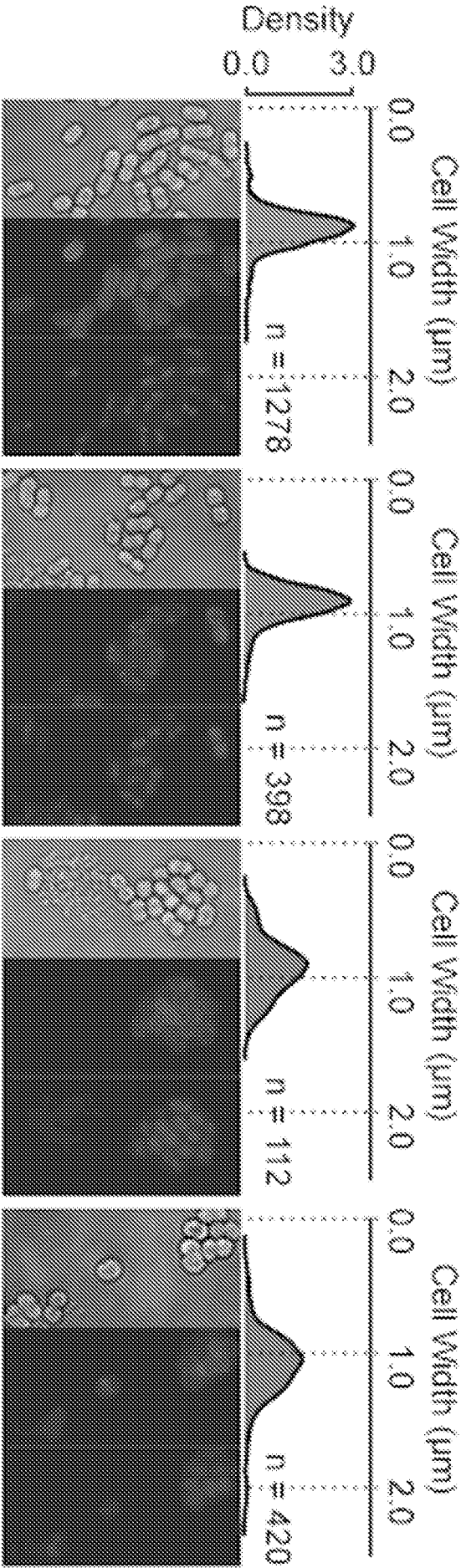


Fig. 3F

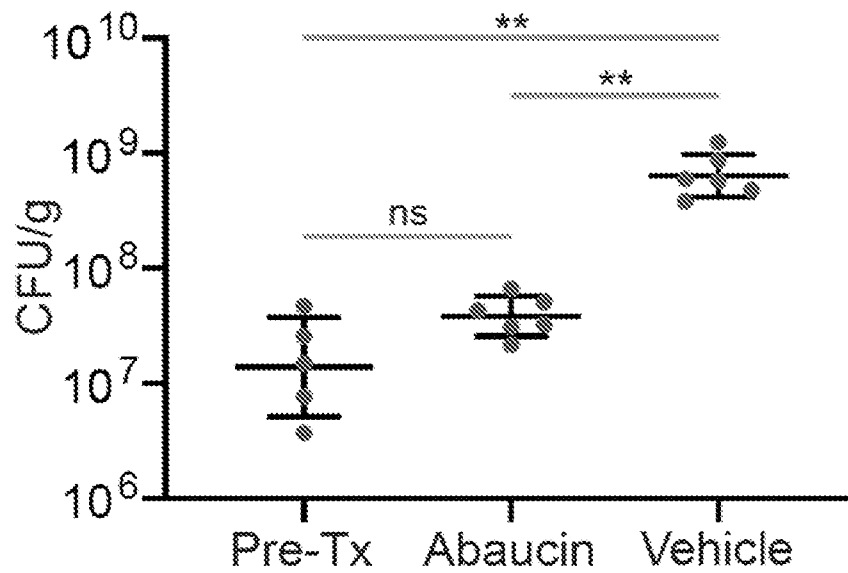
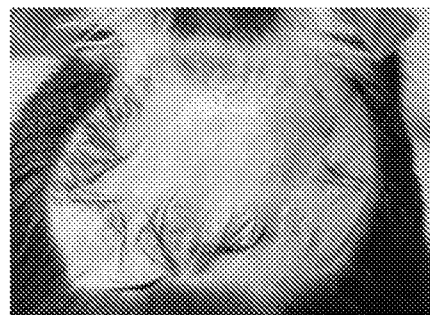
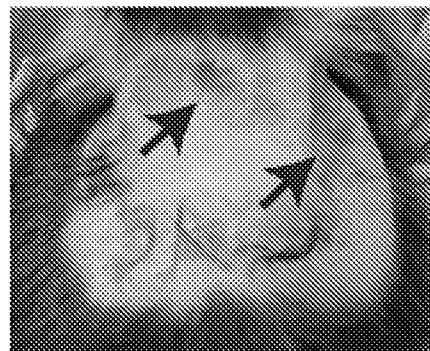


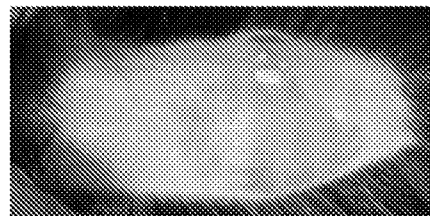
Fig. 4A



t=0



t=24 hrs
vehicle



t=24 hrs
abaucin

Fig. 4B

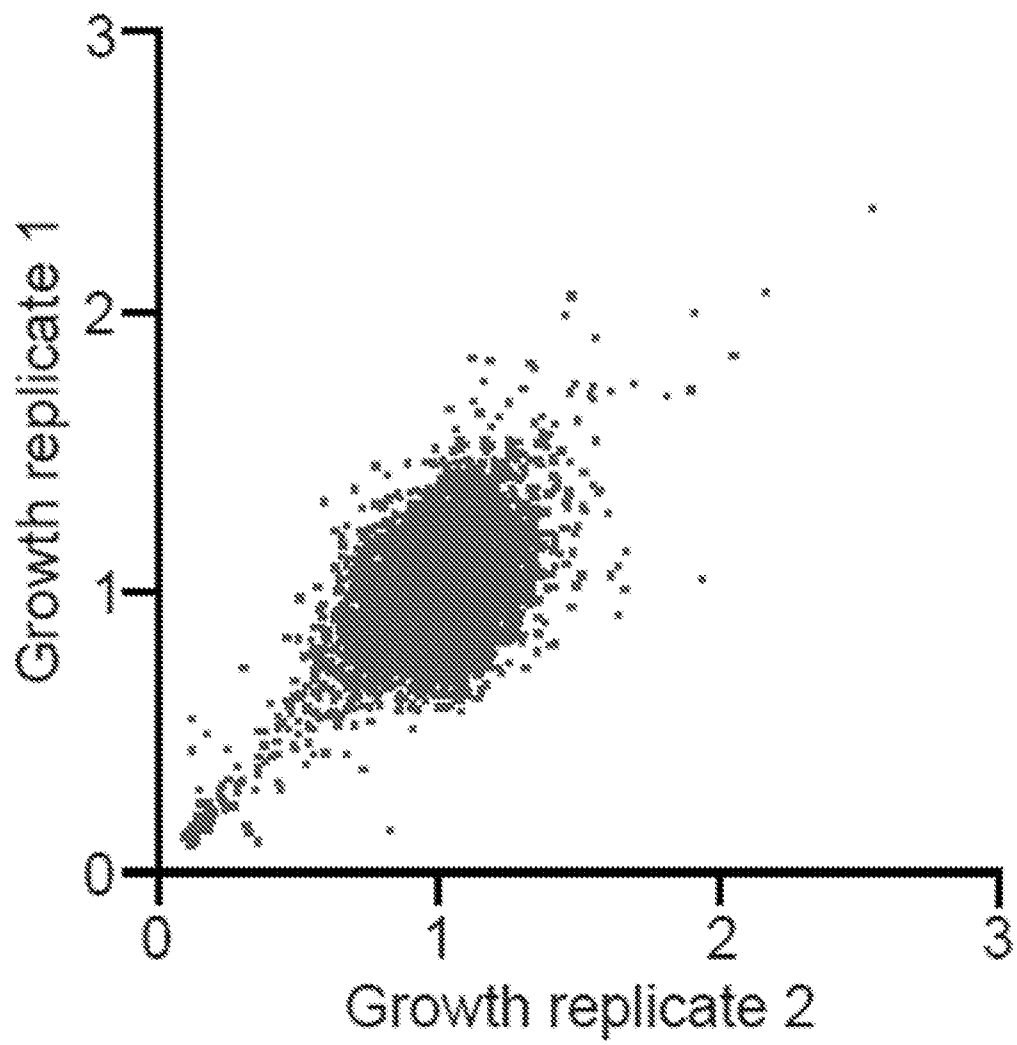
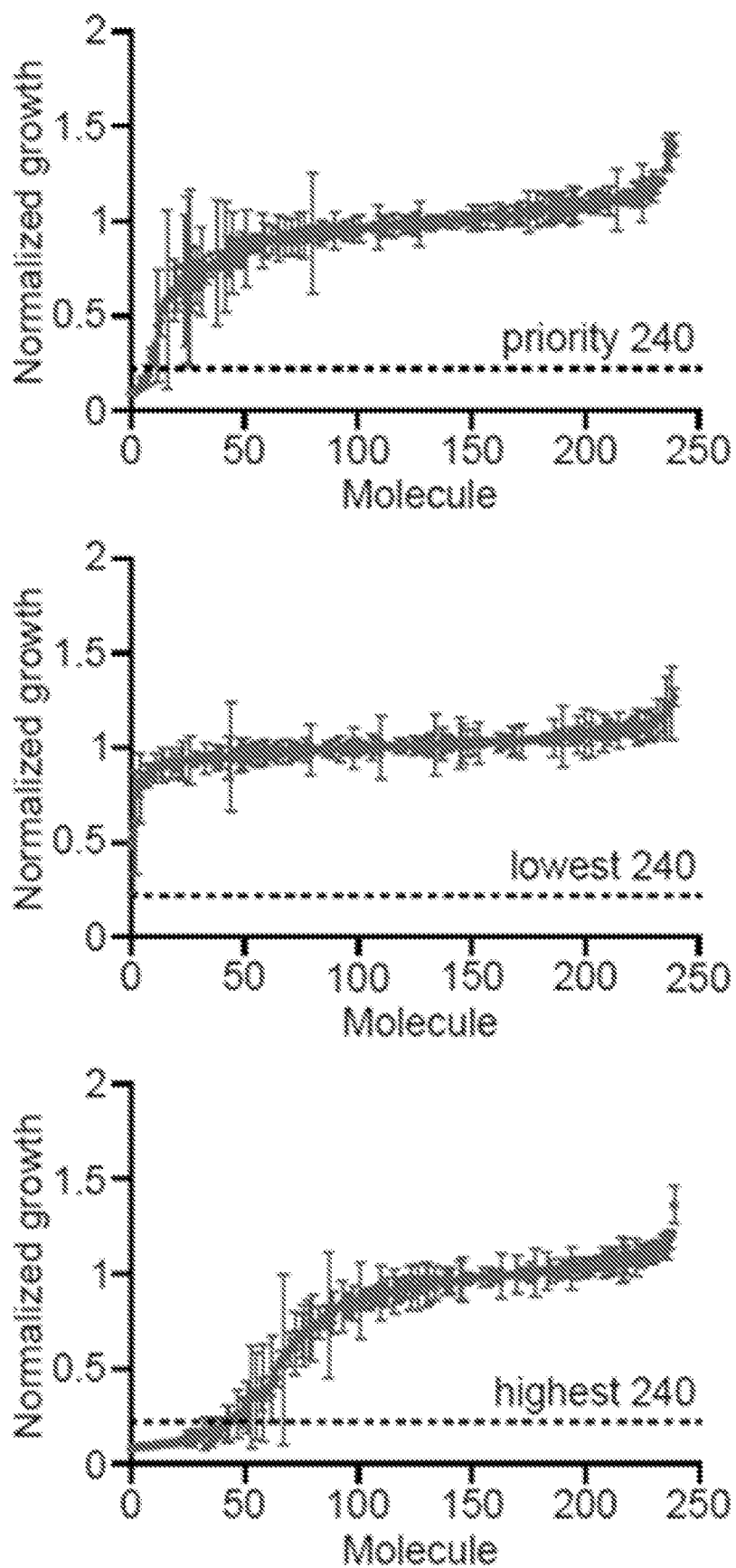


Fig. 5A

**Fig. 5B**

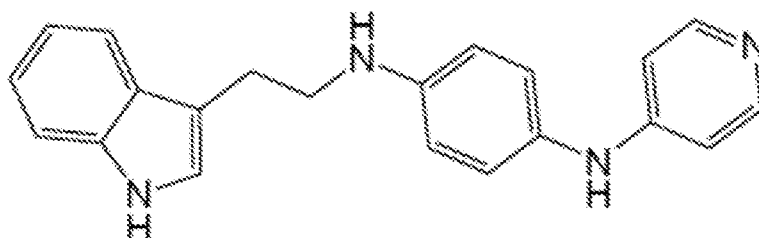
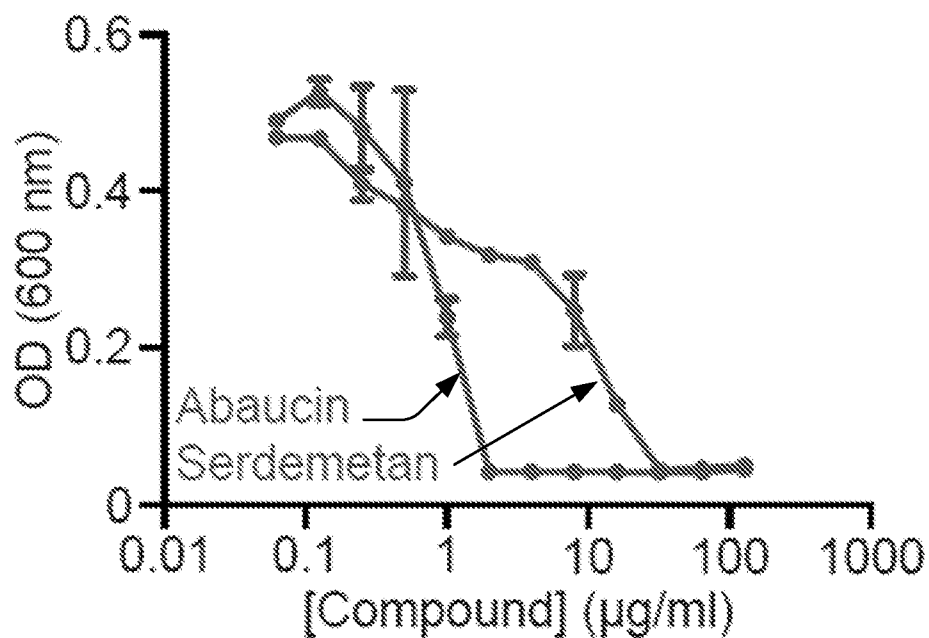


Fig. 5C

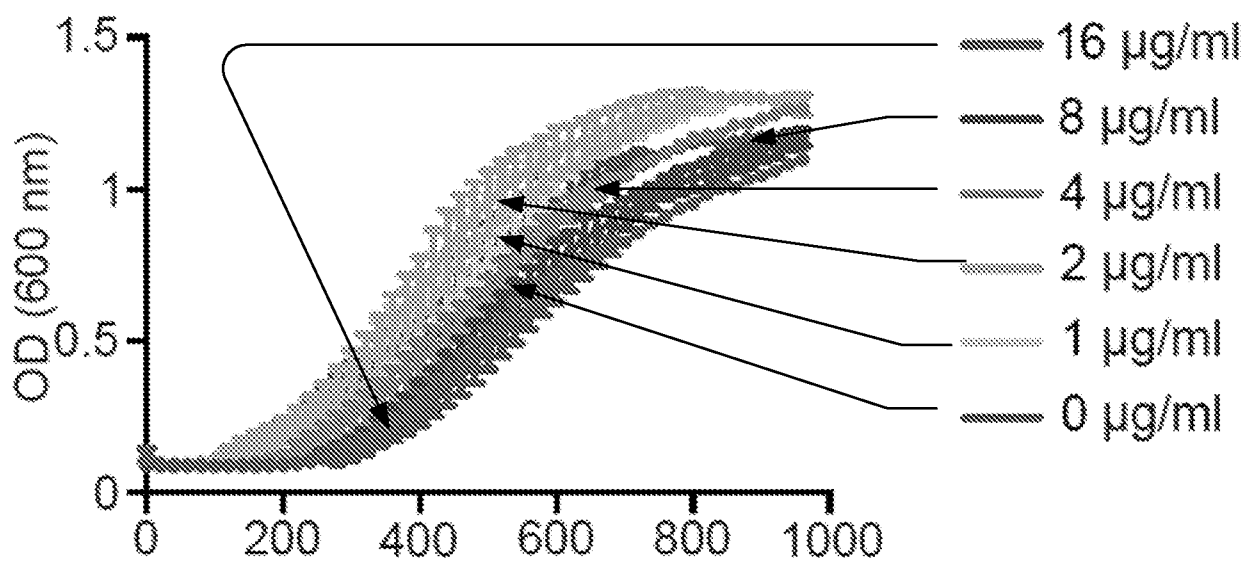


Fig. 5D

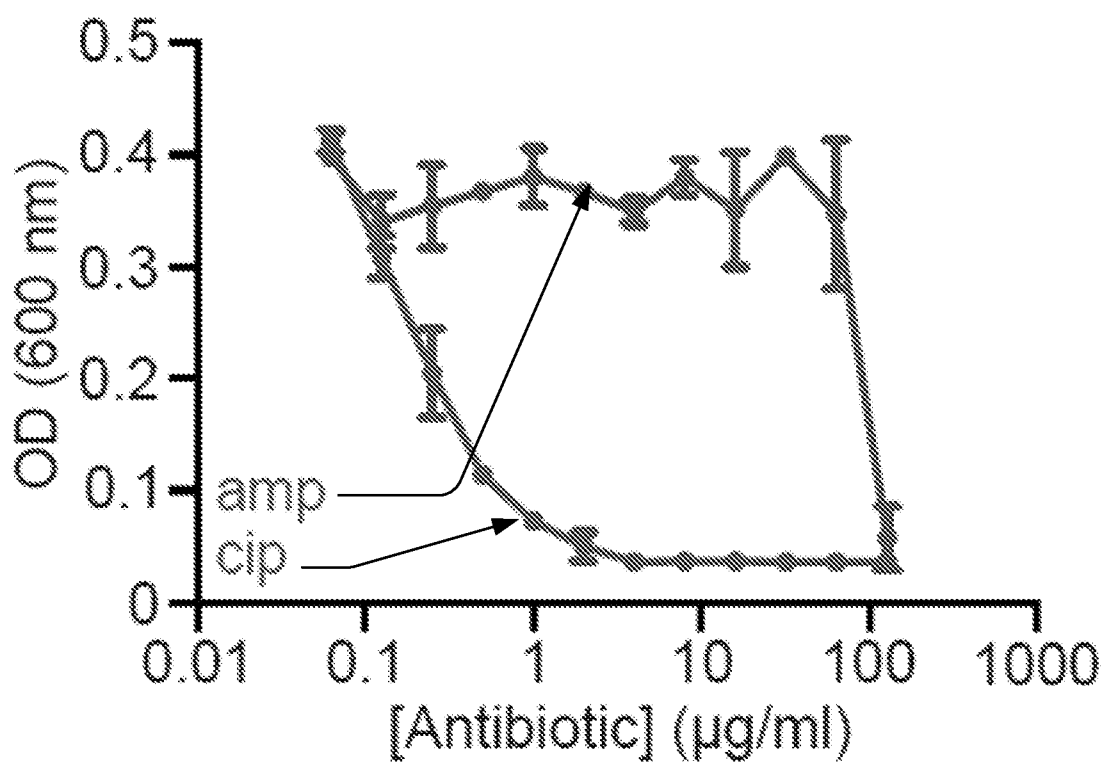


Fig. 6A

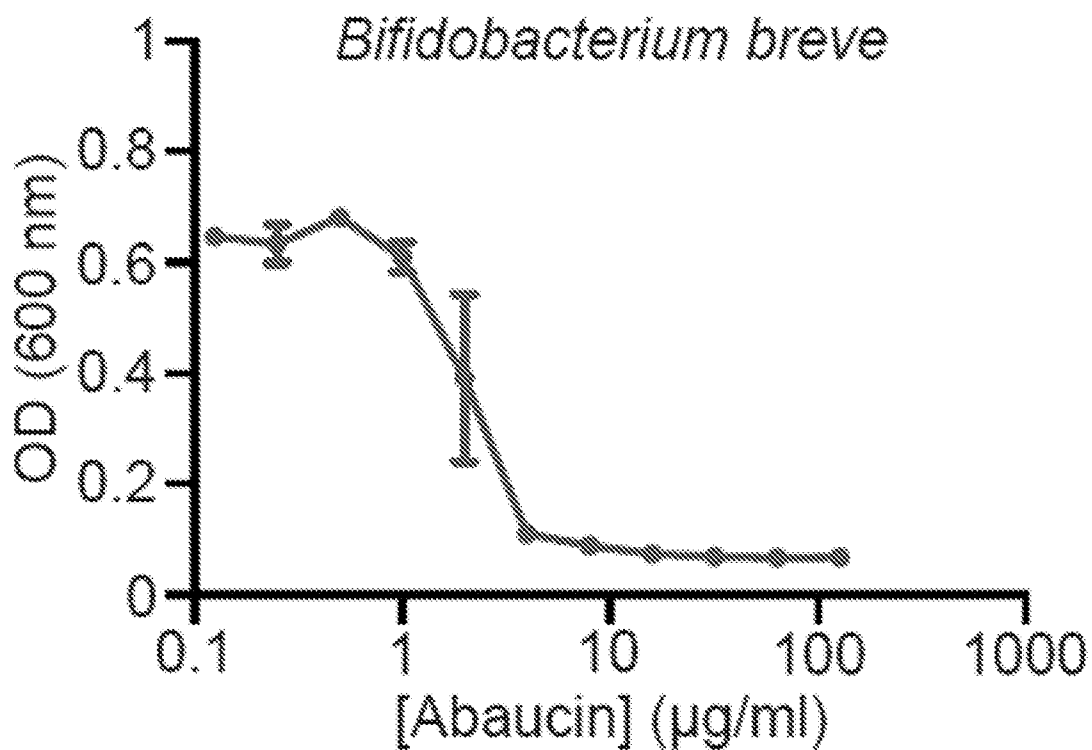


Fig. 6B

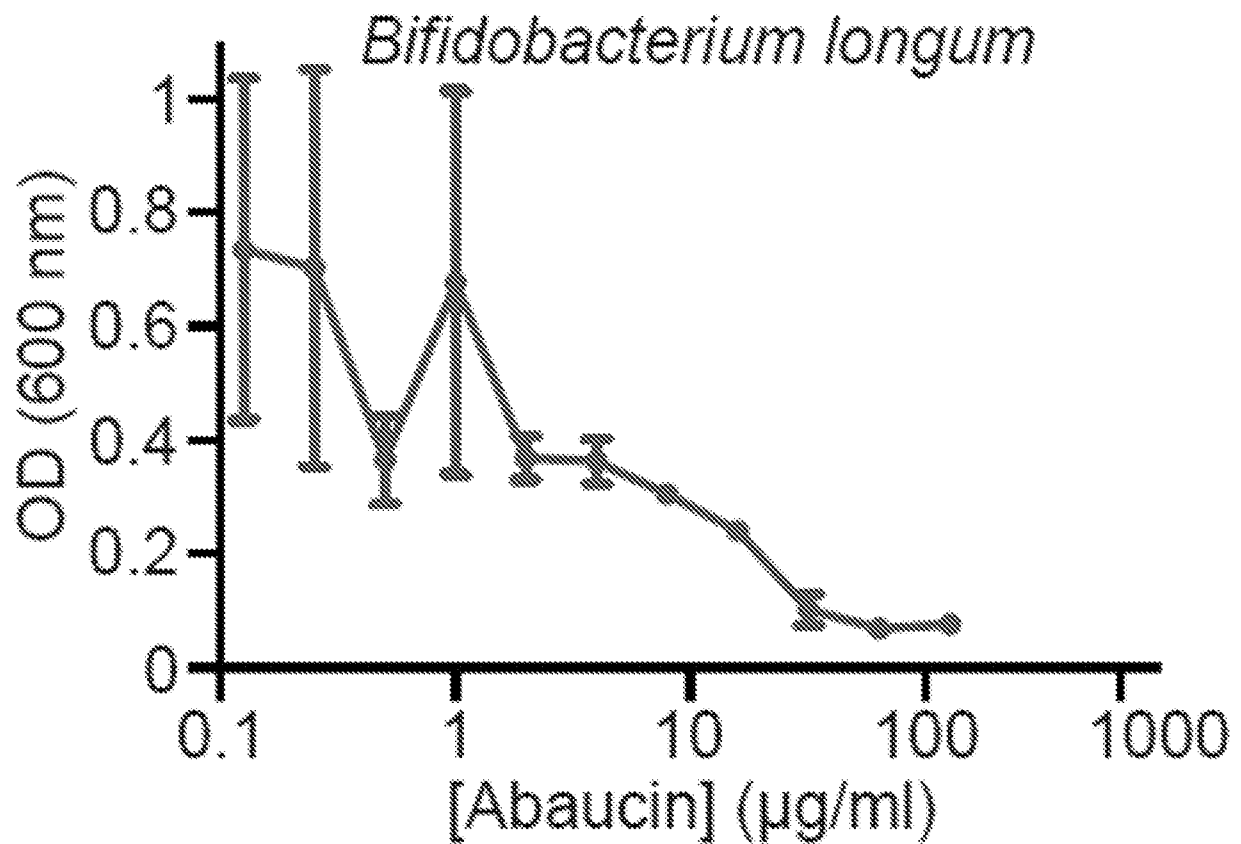


Fig. 6C

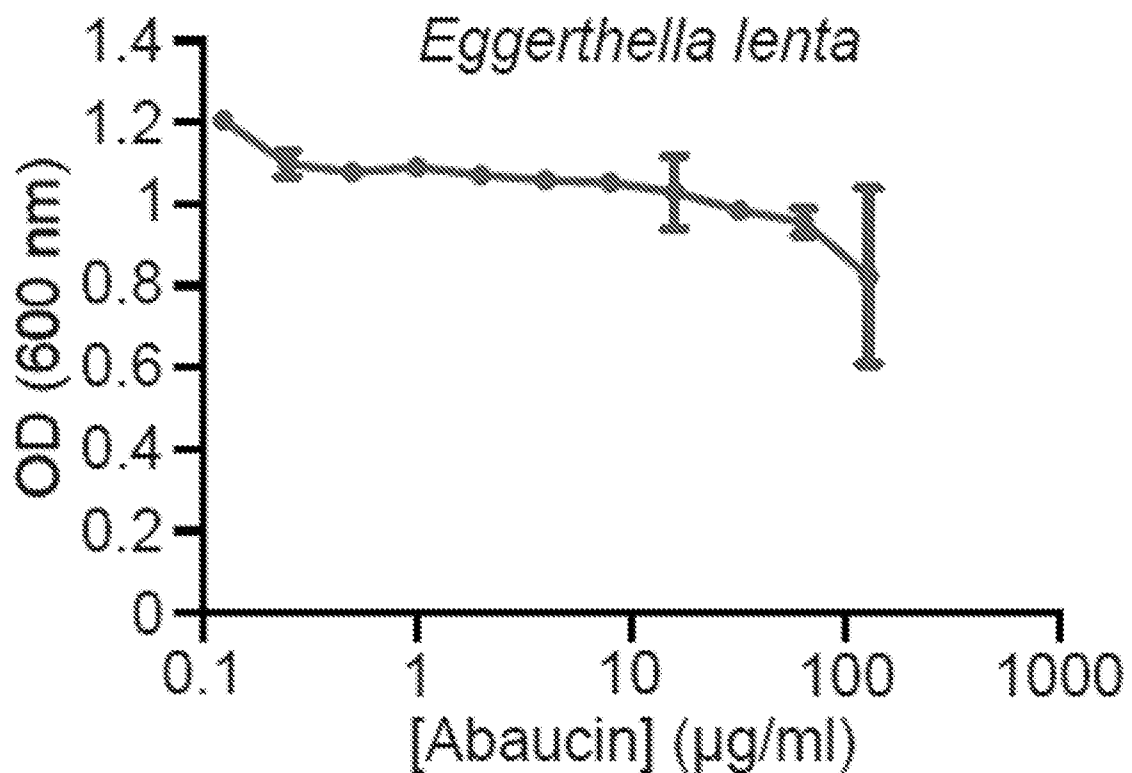


Fig. 6D

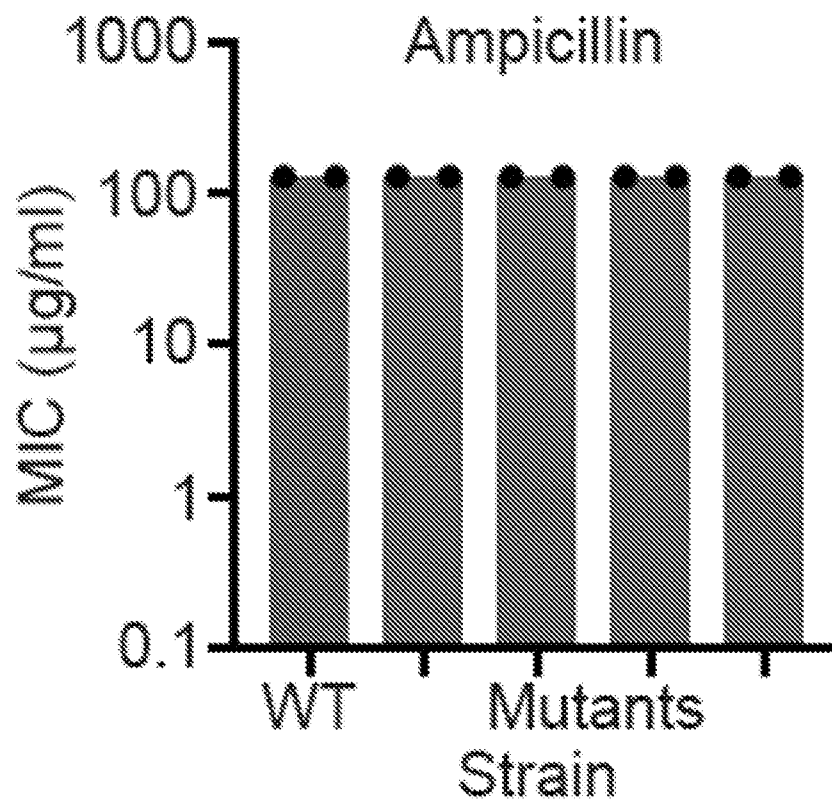


Fig. 7A

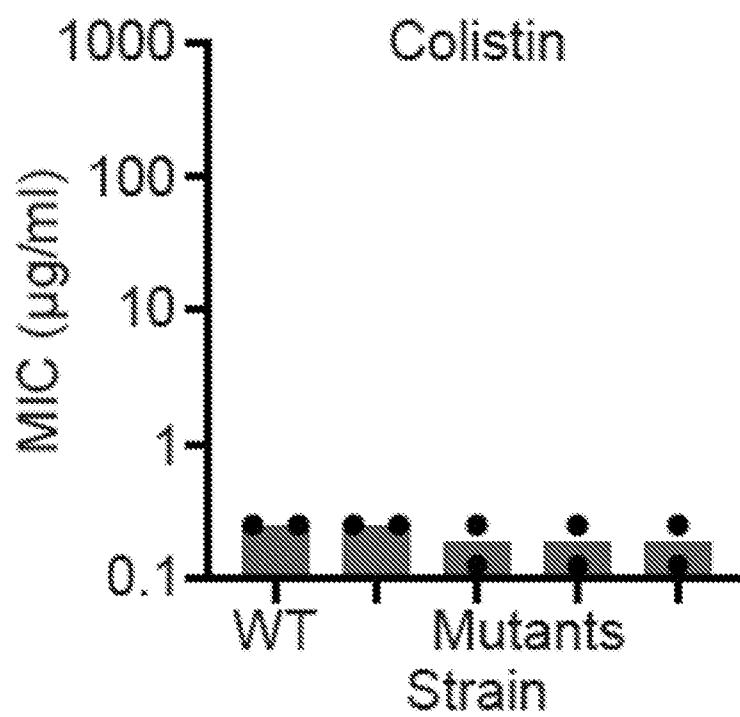


Fig. 7B

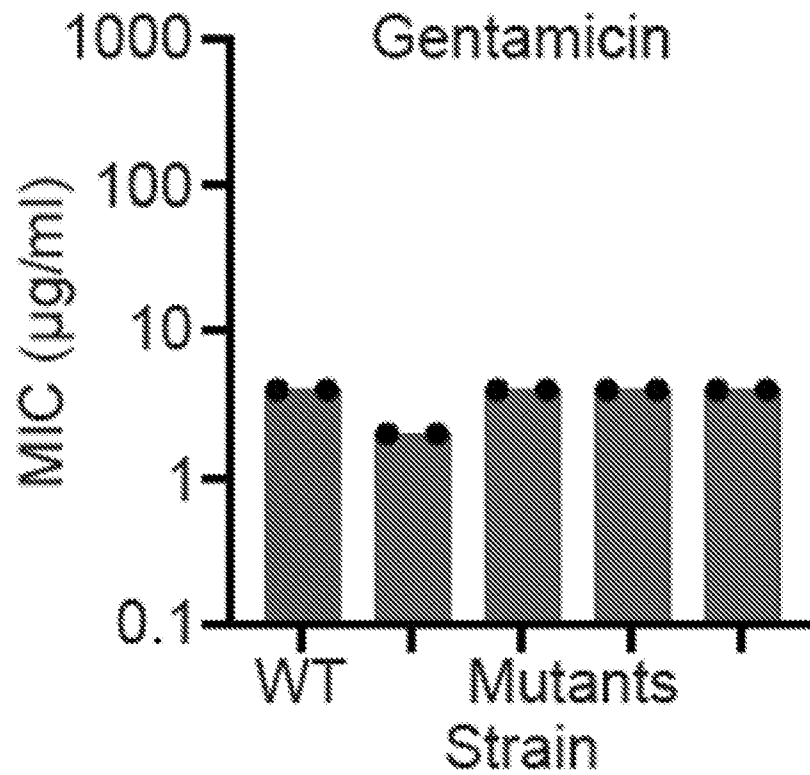


Fig. 7C

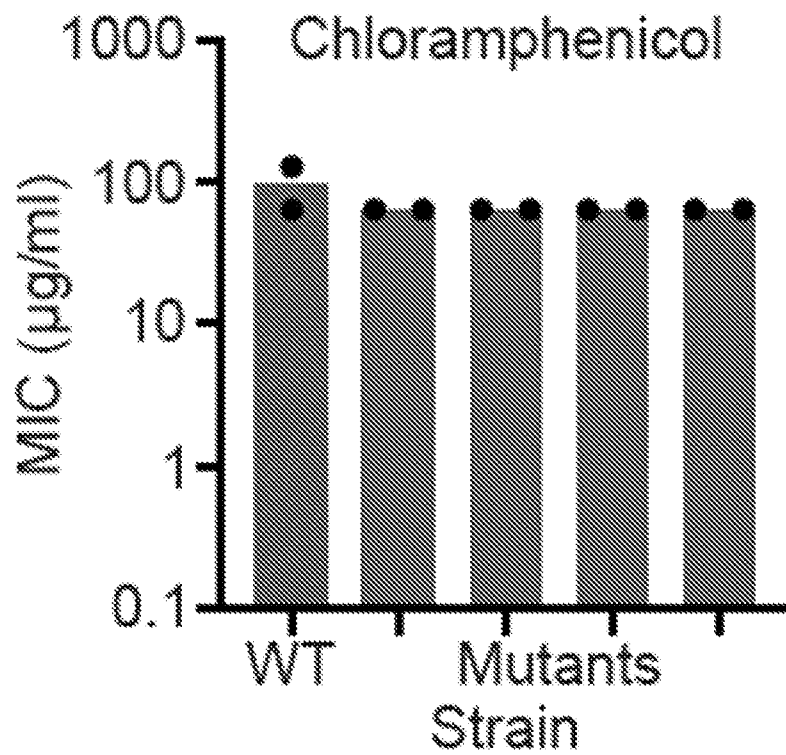


Fig. 7D

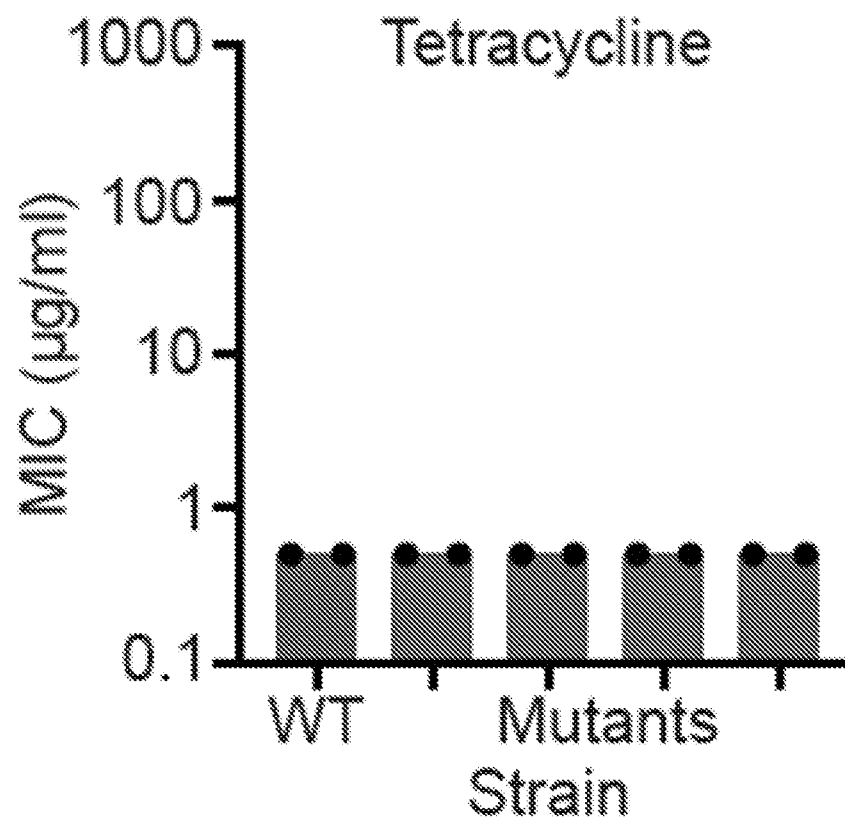


Fig. 7E

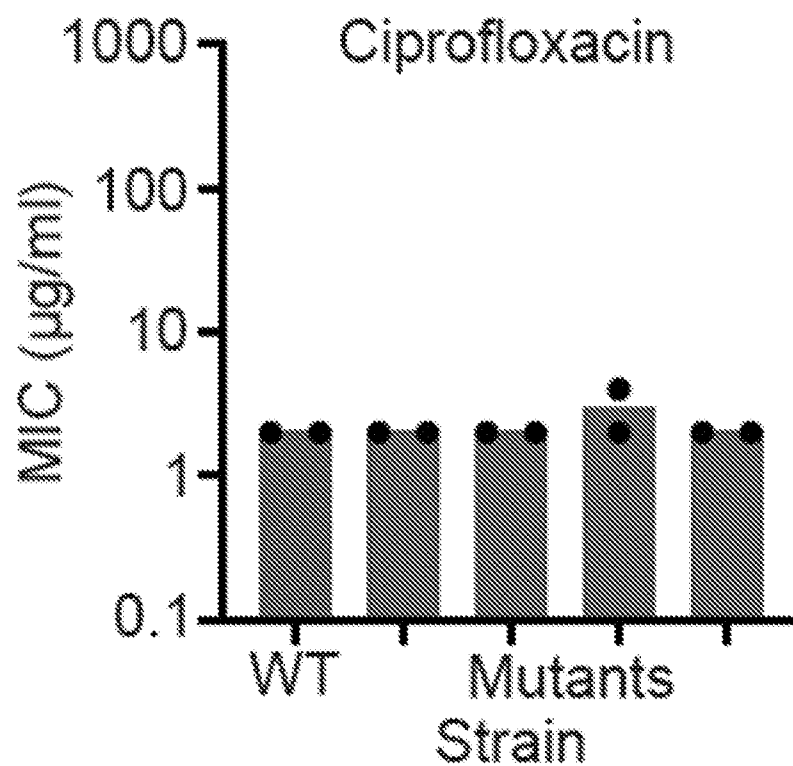


Fig. 7F

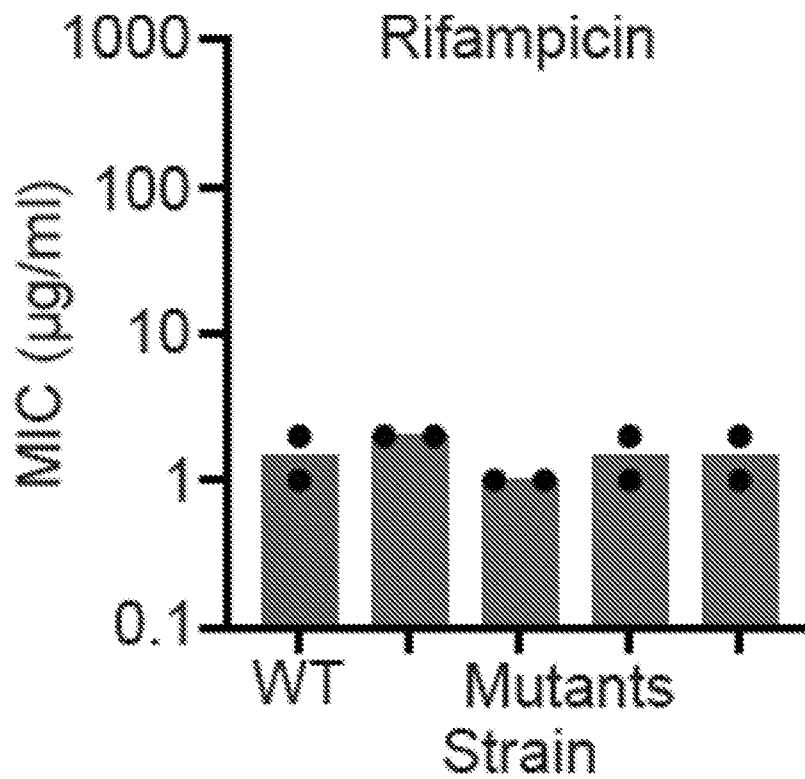


Fig. 7G

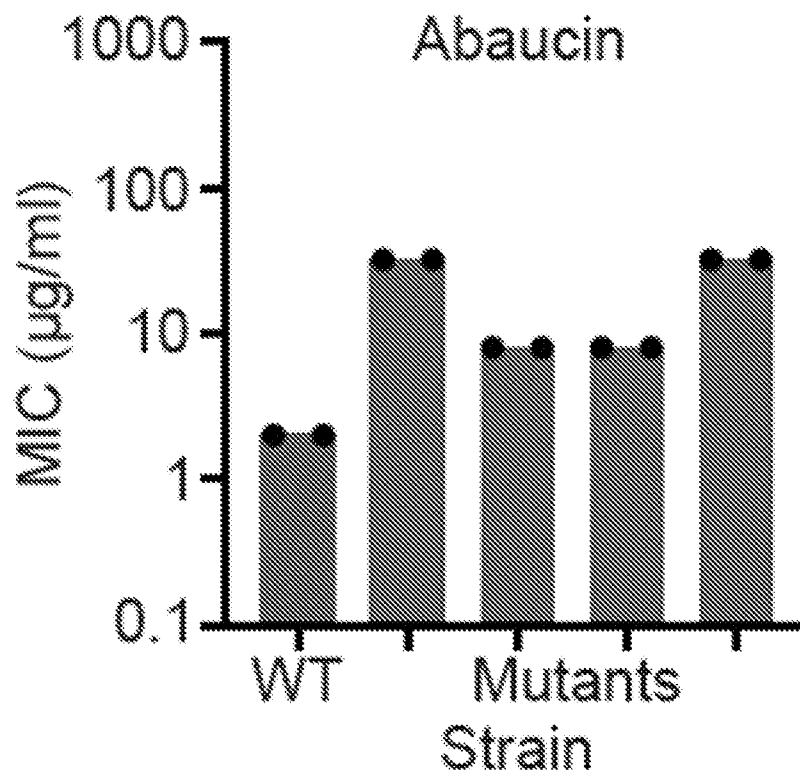


Fig. 7H

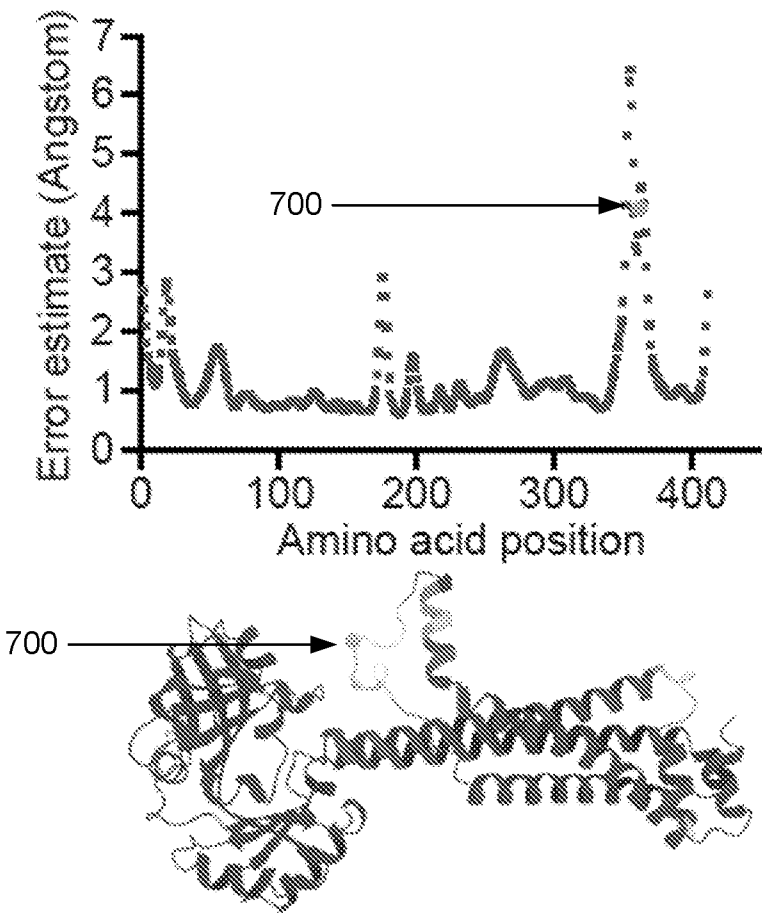


Fig. 7I

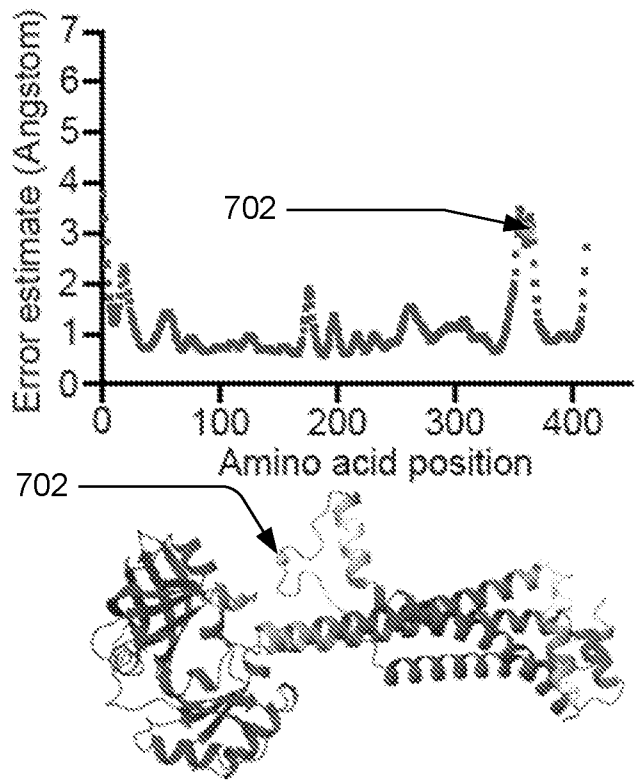


Fig. 7J

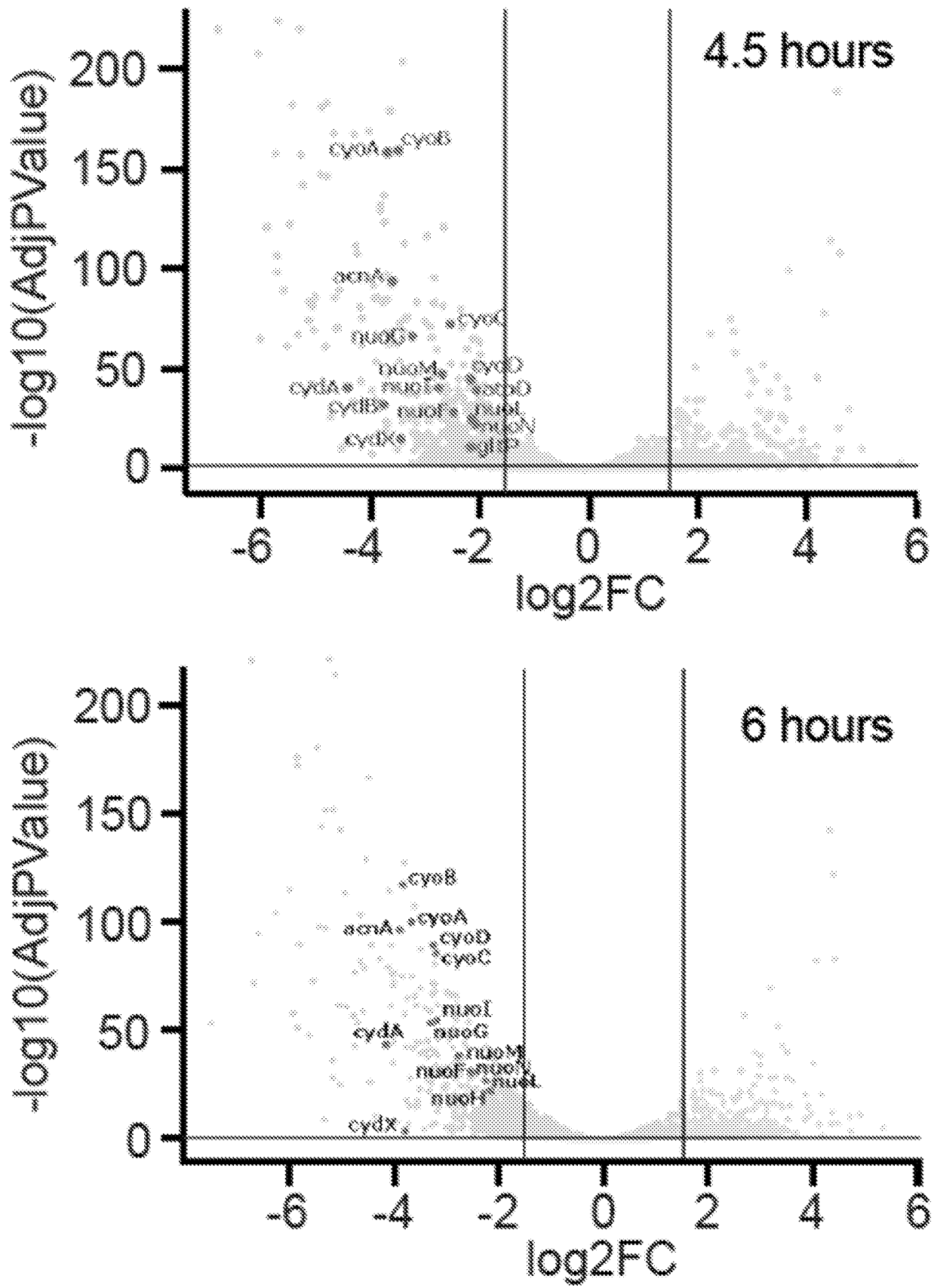


Fig. 7K

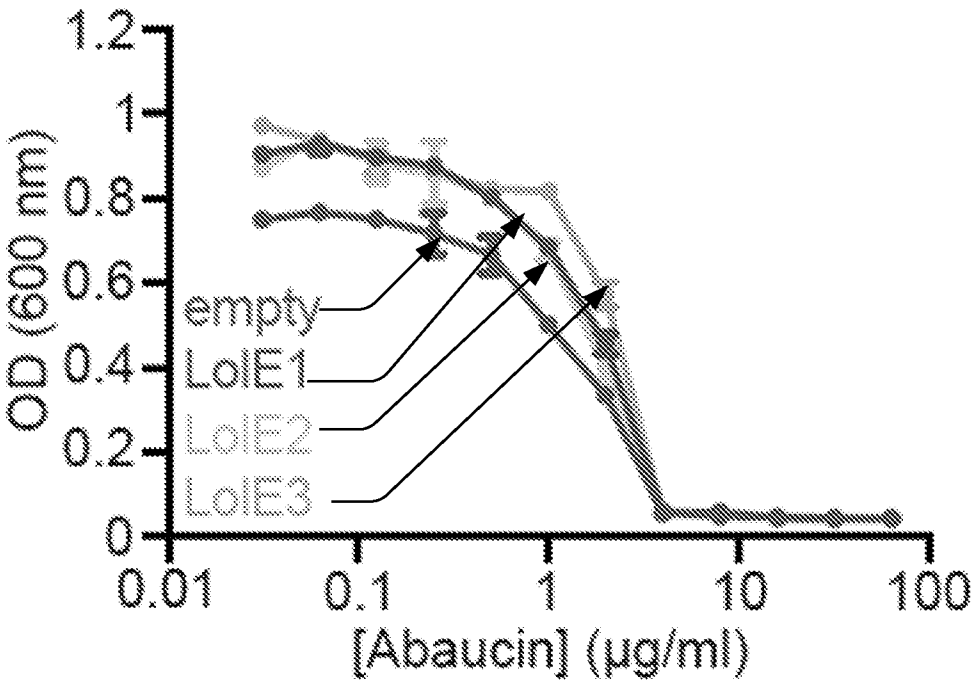


Fig. 7L

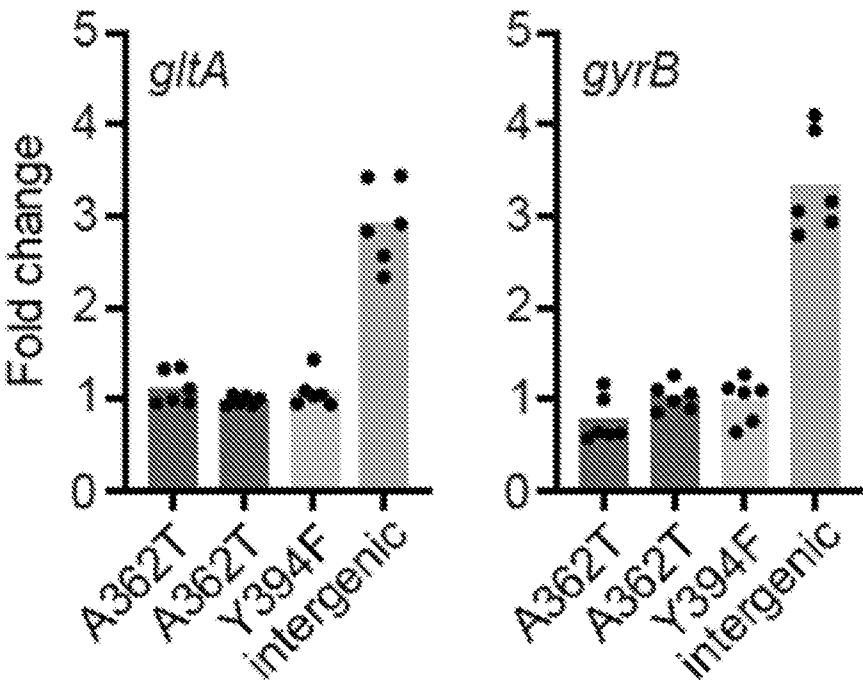


Fig. 7M

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2024/027486

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/536 A61K31/537 A61P31/04
ADD.

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)

A61K C07D A61P

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)

EPO-Internal, BIOSIS, EMBASE, WPI Data**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/021375 A2 (VERTEX PHARMA [US]; MAKINGS LEWIS R [US] ET AL.) 21 February 2008 (2008-02-21) page 70; compound 55 claim 1	1 - 16
A	BR 1020 1603 0039 A2 (UNIAO BRASILEIRA DE EDUCACAO E ASSISTENCIA MANTENEDORA DA PUCRS [BR]) 17 July 2018 (2018-07-17) the whole document	1 - 20



Further documents are listed in the continuation of Box C.



See patent family annex.

* 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

15 August 2024

Date of mailing of the international search report

19/09/2024

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2

NL - 2280 HV Rijswijk

Tel. (+31-70) 340-2040,

Fax: (+31-70) 340-3016

Authorized officer

Collins, Sally

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2024/027486

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	LIU GARY ET AL: "Deep learning-guided discovery of an antibiotic targeting <i>Acinetobacter baumannii</i>", NATURE CHEMICAL BIOLOGY, vol. 19, no. 11, 25 May 2023 (2023-05-25), pages 1342-1350, XP093194757, New York ISSN: 1552-4450, DOI: 10.1038/s41589-023-01349-8 Retrieved from the Internet: URL:https://www.nature.com/articles/s41589-023-01349-8> the whole document -----	1-20

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2024/027486

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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