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(54) **Title:** METHOD FOR TREATING MICROORGANISM INFECTIONS

(57) **Abstract:** The invention described herein provides methods for treating microorganism infection based on the relationship among a given set of parameters pertinent to microorganism infection.



METHOD FOR TREATING MICROORGANISM INFECTIONS

BACKGROUND OF THE INVENTION

Antibiotic tolerance and resistance has become a grave threat to the successful treatment of many common bacterial infections. Indeed, according to the Infectious Disease Society of America, methicillin resistant *Staphylococcus aureus* (MRSA) kills more Americans every year than emphysema, HIV/AIDS, Parkinson's disease and homicide combined. Not only is multi-drug resistance in common infectious gram positive and negative pathogens such as *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis* and *Enterobacter species* on the rise, but evidence of resistance is being seen in *Salmonella* and *Clostridium difficile*, and increasingly *Neisseria gonorrhoea* (Gerard D. Wright, "Antibiotics: A New Hope," 19 (2012) 3-10). Due to this increase in resistance, not only is the development of new antibiotics an important medical need, but determining efficacious and safe methods of administration is also vital so that antibiotic resistance does not increase for known antibiotics or arise with new classes of antibiotics.

SUMMARY OF THE INVENTION

One aspect of the invention provides a method of treating a microorganism infection in a subject in need of treatment, the method comprises:

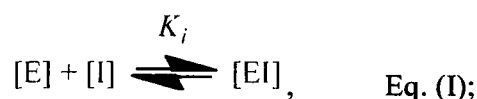
- (1) providing a compound that binds to and inhibits an enzyme of the microorganism, thereby inhibiting proliferation of the microorganism;
- (2) providing a dosing regimen for administering the compound to the subject;
- (3) administering the compound to the subject according to the dosing regimen, and initiating a time course of treatment;
- (4) dividing the time course of treatment into a series of successive intervals, wherein within each said interval, compound concentration at a site of microorganism infection remains substantially constant (*e.g.*, less than 5% deviation from the

average concentration);

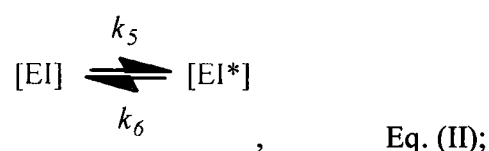
- (5) beginning from the first of said series of successive intervals, and for each said series of successive intervals, constantly determining, according to Equation (III), microorganism titer N at the end of each said series of successive intervals, using microorganism titer N_0 at the beginning of each said series of successive intervals, wherein N for one interval is N_0 for the immediate following interval; and,
- (6) terminating treatment according to the dosing regimen when microorganism titer N at a given interval falls below a pre-determined / target microorganism titer, thereby treating the microorganism infection;

wherein:

- (a) the compound binds to the enzyme *in vivo* to form a compound-enzyme complex ("EI"), with a binding constant K_i , according to Equation (I):



- (b) the compound-enzyme complex EI is in equilibrium with a tight compound-enzyme complex ("EI*"), with an on-rate constant k_5 and an off-rate constant k_6 , according to Equation (II):



- (c) the dosing regimen produces a pharmacokinetic (PK) profile of the compound in the subject (at the site of the microorganism infection);
- (d) Equation (III) is:

$$[N] = [N_0] \exp([\lambda + \varepsilon(\sigma - 1)]t + \varepsilon \rho \left(\frac{k_6}{2kb} - \frac{1}{2} \right) t^2 + \varepsilon \frac{\gamma}{k} (1 - e^{-kt}))$$

wherein:

N_0 is microorganism titer at the beginning of each said series of successive intervals;

N is microorganism titer at the end of each said series of successive intervals;

t is the duration of time for a given interval associated with N_0 ;

λ is intrinsic logarithmic growth rate of the microorganism;

ε is maximum kill rate of the microorganism by the compound;

ρ is replenishment rate of the enzyme;

$[I]$ is concentration of the compound at the site of enzyme function (e.g., inside the microorganism);

K_i is the binding constant defined in Eq. (I);

k_5 and k_6 are on- and off-rates defined in Eq. (II);

$\beta = 1 + K_M / [S] + (K_M / [S]) \times ([I] / K_i)$;

$k = k_6 + (k_5 / \beta) \times (K_M / [S]) \times ([I] / K_i)$;

$\sigma = (k \times (k_6 + \rho) / \beta - k_6 \times \rho / \beta) / k^2$;

K_M is the Michaelis-Menten constant of the enzyme;

$[S]$ is concentration of the substrate of the enzyme at the site of enzyme function (e.g., inside the microorganism);

for the first of said series of successive intervals, γ is a constant determined by Equation (IV):

$$\gamma = 1 / \beta + (k \times (k_6 + \rho) / \beta - k_6 \times \rho / \beta) / k^2, \quad \text{Eq. (IV);}$$

for each subsequent said series of successive intervals, γ is a constant determined by Equation (V):

$$\gamma = \frac{\beta_{old}[ES]_{\text{end-of-last-interval}}}{\beta_{new}} - \sigma_{new}, \quad \text{Eq. (V);}$$

wherein in Eq. (V):

β_{old} is β defined above for the immediate preceding interval;

β_{new} and σ_{new} are β and σ defined above for the current interval;

$[ES]_{\text{end}}$ is concentration of enzyme-substrate complex at the end of the immediate preceding interval, and is calculated according to Equation (VI),

wherein $b = (k_6 + \rho) / \beta$; $h = k_6 \times \rho / \beta$; and γ is as defined above for the immediate preceding interval:

$$[ES] = \frac{bk-h}{k^2} + \frac{h}{k} t + \gamma e^{-kt}, \quad \text{Eq. (VI).}$$

In certain embodiments, the microorganism is a bacterium.

In certain embodiments, the bacterium is *P. aeruginosa*, *E. coli*, *K. pneumonia*, *A. baumannii*, *M. tuberculosis*, *S. aureus*, *S. pneumonia*, or *H. influenzae*.

In certain embodiments, the infection is systemic. In other embodiments, the infection is local.

In certain embodiments, the subject is a human, or a non-human mammal (*e.g.*, a livestock animal, a pet, a lab animal *etc.*).

In certain embodiments, the compound is an antibiotic, such as Ampicillin, Cloxacillin, Methicillin, Cefotaxime, Ceftriaxone, Moxalactam, Imipenem, or Meropenem; vancomycin, erythromycin, Gentamicin, Amikacin, Tobramycin, Rifampin, Trimethoprim, or Ciprofloxacin.

In certain embodiments, the antibiotic exhibits a post-antibiotic effect (PAE) in treating infection by the microorganism.

In certain embodiments, k_6 is less than about 1 hr^{-1} .

In certain embodiments, the series of successive intervals are of identical duration.

In certain embodiments, at least two of the series of successive intervals are of different duration.

In certain embodiments, [I] is calculated / determined based on the PK profile of the compound.

Another aspect of the invention provides a method of treating a microorganism infection in a subject in need of treatment, the method comprising first determining a specific dosing regimen that would produce a desired treatment result based on calculations according to Equation (III), and then administering the drug to the host or patient according to the selected dosing regimen.

Yet another aspect of the invention provides a method for screening inhibitor compounds that may be used to achieve desired treatment time course and efficacy, while preferably avoiding or minimizing associated side effects or toxicological effects.

Still another aspect of the invention provides a method for determining or predicting efficacy of a compound administered according to a set dosing regimen, in the context of treating a specific microorganism infection in a specific host.

A further aspect of the invention provides a method for determining or predicting

efficacy of a pre-determined compound used according to a pre-determined dosing regimen, for treating a specific host with a given initial microorganism load.

It is contemplated that, where applicable, any and all embodiments of the invention described herein can be combined with one or more other embodiments of the invention, including those described only under one aspect of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a simulated PK /PD profile for a hypothetical drug administered to a host, and the resulting microorganism titer change over time.

FIG. 2 shows the results of a bacterial killing kinetics and growth recovery experiment with an LpxC inhibitor and the organism *P. aeruginosa*. The inset box reports the fold-concentration relative to the compound MIC (minimal inhibition concentration) that the compound was incubated with the bacteria for during the time period between -1 and 0 hours. The “+” symbols are experimental results. The fitted curves (solid lines) were calculated with Equation (III) and returned the parameter values listed at the top of the plot. Units for the y-axis are in $\log_{10}$ CFU (colony forming units). Units for the x-axis are in hours. Returned parameters have units as follows: C - unitless, equal to the ratio $K_M/[S]$; k_5 - hr^{-1} ; k_6 - hr^{-1} ; Kpi, nM; lambda (λ), hr^{-1} ; eps (ϵ), hr^{-1} .

DETAILED DESCRIPTION OF THE INVENTION

1. Overview

The methods of the invention described herein are based at least in part on the discovery that the relationship between the various parameters associated with microorganism infection of a host (e.g., a human or an animal) can be described by a comprehensive equation, based on established pharmacokinetic (PK), pharmacodynamic (PD), and enzyme kinetic principals, such as the *Michaelis–Menten* equation.

For any given microorganism infection in a host, applying the equation allows one to isolate or choose a particular parameter of interest, and to predict how changing the remaining

parameters can affect the behavior of the particular parameter of interest. In practice, this approach enables the methods of the invention to address numerous practical problems associated with microorganism infection, including, but not limited to: determining the time course of a given treatment after taking into consideration any PAE (Post Antibiotic Effect) such that the minimal effective drug dose is administered; designing or adjusting dosing regimens based on a desired outcome of treatment; screening for an optimal drug with the desired combination of characteristics, such as efficacy, PAE, toxicity, side effect; *etc.*

Definitions for certain terms as used herein are provided below, followed by a detailed description of the equation that describes the relationship among the various parameters associated with microorganism infection. Exemplary methods of the invention applying the equation are provided further below. Specific non-limiting examples are also provided to further illustrate the various aspects of the invention.

2. Definitions

“Compound” includes known and novel molecules that are active against an enzymatic target. The compound may be naturally existing, synthetic, or semi-synthetic, and can be any molecule in nature, such as small molecule (*e.g.*, less than or about 5,000 Da, 1000 Da, 500 Da, 250 Da, or 100 Da), peptide, peptidomimetic, steroid, lipid, carbohydrate, nucleic acid, nucleotide, nucleoside, vitamin, prodrugs, or metabolic precursor thereof. In certain embodiments, the compound is an antibiotic or antibacterial agent.

“Antibiotics” and “antibacterial” both refer to a compound or substance that kills or slows down the growth of bacteria. In the broad sense, antibiotic(s) also include a broader range of antimicrobial compounds, including antifungals and other compounds. Antibiotic includes any natural substance produced by a microorganism that is antagonistic to the growth of other microorganisms, usually even in high dilution (*e.g.*, aminoglycosides). Antibiotic may also include synthetic or semisynthetic (*e.g.*, with modifications to various natural compounds) antimicrobial (*e.g.*, antibacterial) compounds. Semisynthetic antimicrobial (*e.g.*, antibacterial) compounds include, for example, the beta-lactam antibacterials (*e.g.*, penicillin produced by fungi in the genus *Penicillium*), the cephalosporins, and the carbapenems. Synthetic antimicrobial (*e.g.*, antibacterial) compounds include, for example, the sulfonamides, the quinolones, and the oxazolidinones. Many antimicrobial (*e.g.*, antibacterial) compounds are

relatively small molecules with a molecular weight of less than 2000 atomic mass units. The term antibiotic includes both agents that kill microbes (*e.g.*, bactericidal agents that kill bacteria), and agents that slow down or stall microbial growth (*e.g.*, bacteriostatic agents).

A representative but non-limiting list of antibiotics that can be used with the methods of the invention include: **Aminoglycosides** (*e.g.*, Amikacin, Gentamicin, Kanamycin, Neomycin, Netilmicin, Tobramycin, Paromomycin); **Ansamycins** (*e.g.*, Geldanamycin, Herbimycin); **Carbacephem** (*e.g.*, Loracarbef); **Carbapenems** (*e.g.*, Ertapenem, Doripenem, Imipenem/Cilastatin, Meropenem); **Cephalosporins** (*e.g.*, Cefadroxil, Cefazolin, Cefalotin or Cefalothin, Cefalexin, Cefaclor, Cefamandole, Cefoxitin, Cefprozil, Cefuroxime, Cefixime, Cefdinir, Cefditoren, Cefoperazone, Cefotaxime, Cefpodoxime, Ceftazidime, Ceftibuten, Ceftizoxime, Ceftriaxone, Cefepime, Ceftaroline fosamil, Ceftobiprole); **Glycopeptides** (*e.g.*, Teicoplanin, Vancomycin, Telavancin); **Lincosamides** (*e.g.*, Clindamycin, Lincomycin); **Lipopeptide** (*e.g.*, Daptomycin); **Macrolides** (*e.g.*, Azithromycin, Clarithromycin, Dirithromycin, Erythromycin, Roxithromycin, Troleandomycin, Telithromycin, Spectinomycin, Spiramycin); **Monobactams** (*e.g.*, Aztreonam); **Nitrofurans** (*e.g.*, Furazolidone, Nitrofurantoin); **Penicillins** (*e.g.*, Amoxicillin, Ampicillin, Azlocillin, Carbenicillin, Cloxacillin, Dicloxacillin, Flucloxacillin, Mezlocillin, Methicillin, Nafcillin, Oxacillin, Penicillin G, Penicillin V, Piperacillin, Temocillin, Ticarcillin); **Penicillin combinations** (*e.g.*, Amoxicillin/clavulanate, Ampicillin/sulbactam, Piperacillin/tazobactam, Ticarcillin/clavulanate); **Polypeptides** (*e.g.*, Bacitracin, Colistin, Polymyxin B); **Quinolones** (*e.g.*, Ciprofloxacin, Enoxacin, Gatifloxacin, Levofloxacin, Lomefloxacin, Moxifloxacin, Nalidixic acid, Norfloxacin, Ofloxacin, Trovafloxacin, Grepafloxacin, Sparfloxacin, Terafloxacin); **Sulfonamides** (*e.g.*, Mafenide, Sulfonamidochrysoidine (archaic), Sulfacetamide, Sulfadiazine, Silver sulfadiazine, Sulfamethizole, Sulfamethoxazole, Sulfanilimide (archaic), Sulfasalazine, Sulfisoxazole, Trimethoprim-Sulfamethoxazole (Co-trimoxazole) (TMP-SMX)); **Tetracyclines** (*e.g.*, Demeclocycline, Doxycycline, Minocycline, Oxytetracycline, Tetracycline); **Antibiotics against mycobacteria** (*e.g.*, Clofazimine, Dapsone, Capreomycin, Cycloserine, Ethambutol, Ethionamide, Isoniazid, Pyrazinamide, Rifampicin (Rifampin in US), Rifabutin, Rifapentine, Streptomycin); **and other antibiotics** (*e.g.*, Arsphenamine, Chloramphenicol, Fosfomycin; Fusidic acid, Linezolid, Metronidazole, Mupirocin, Platensimycin, Quinupristin/Dalfopristin, Rifaximin, Thiamphenicol, Tigecycline, Tinidazole, Trimethoprim). In some embodiments, the

antibiotic is an LpxC1 inhibitor (*e.g.*, a compound that inhibits the enzyme LpxC1).

“Inhibits an enzyme” includes inhibiting at least one function or activity of the enzyme. Preferably, the function or activity inhibited (by the compound) is required or essential for the survival, growth, and/or proliferation of the microorganism. The function or activity of the enzyme may be partially inhibited, or nearly completely inhibited. In certain embodiments, the function or activity of the enzyme is inhibited to such an extent that the enzyme is no more than 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5%, 1%, 0.1%, 0.01%, 0.001% active compared to the enzyme without inhibition. In certain embodiments, the function or activity of the enzyme is inhibited to such an extent that survival, growth, and/or proliferation of the microorganism is abolished or impaired.

“Inhibits proliferation of the microorganism” as used herein includes killing the microorganism, or inhibiting / stalling / retarding the proliferation (*e.g.*, increase in number) and/or growth (*e.g.*, increase in size or volume) of the microorganism, or both.

“Infectious disease,” “transmissible diseases,” and “communicable diseases” are used interchangeably to include clinically evident illness (*i.e.*, characteristic medical signs and/or symptoms of disease) resulting from the infection, presence, and/or growth of pathogenic biological agents in an individual host organism. In certain cases, infectious diseases may be asymptomatic for much or even all of their courses in a given host. In the latter case, the disease may be defined as a disease in hosts who secondarily become ill after contact with an asymptomatic carrier.

Infectious diseases are sometimes called “contagious” when they are easily transmitted by contact with an ill person or their secretions (*e.g.*, influenza). Thus, a “contagious disease” is a subset of infectious disease that is especially infective or easily transmitted. Other types of infectious / transmissible / communicable diseases with more specialized routes of infection, such as vector transmission or sexual transmission, are usually not regarded as “contagious,” and often do not require medical isolation or quarantine.

“Infectious microorganisms” refer to pathogens including, but not limited to, certain viruses, bacteria, fungi, protozoa, and multicellular parasites that are the cause of disease epidemics, in the sense that without the pathogen, no infectious epidemic occurs. In certain embodiments, an infectious microorganism refers to bacteria that cause an infectious disease. In

certain embodiments, the infectious microorganism is antibiotic resistant, antifungal resistant or antiviral resistant.

A representative but non-limiting list of infectious diseases and the responsible bacteria causing the diseases, to which the subject invention can be applied, is provided below:

Acinetobacter infections (*Acinetobacter baumannii*), Actinomycosis (*Actinomyces israelii*, *Actinomyces gerencseriae* and *Propionibacterium propionicus*), African sleeping sickness or African trypanosomiasis (*Trypanosoma brucei*), AIDS or Acquired immunodeficiency syndrome (HIV or Human immunodeficiency virus), Amebiasis (*Entamoeba histolytica*), Anaplasmosis (*Anaplasma* genus), Anthrax (*Bacillus anthracis*), *Arcanobacterium haemolyticum* infection (*Arcanobacterium haemolyticum*), Argentine hemorrhagic fever (Junin virus), Ascariasis (*Ascaris lumbricoides*), Aspergillosis (*Aspergillus* genus), Astrovirus infection (*Astroviridae* family), Babesiosis (*Babesia* genus), *Bacillus cereus* infection (*Bacillus cereus*), Bacterial pneumonia (multiple bacteria), Bacterial vaginosis or BV (multiple bacteria), *Bacteroides* infection (*Bacteroides* genus), Balantidiasis (*Balantidium coli*), *Baylisascaris* infection (*Baylisascaris* genus), BK virus infection (BK virus), Black piedra (*Piedraia hortae*), *Blastocystis hominis* infection (*Blastocystis hominis*), Blastomycosis (*Blastomyces dermatitidis*), Bolivian hemorrhagic fever (Machupo virus), *Borrelia* infection (*Borrelia* genus), Botulism and Infant botulism (*Clostridium botulinum*), Brazilian hemorrhagic fever (Sabia), Brucellosis (*Brucella* genus), *Burkholderia* infection (usually *Burkholderia cepacia* and other *Burkholderia* species), Buruli ulcer (*Mycobacterium ulcerans*), Calicivirus infection, Norovirus and Sapovirus (*Caliciviridae* family), Campylobacteriosis (*Campylobacter* genus), Candidiasis (Moniliasis; Thrush) (usually *Candida albicans* and other *Candida* species), Cat-scratch disease (*Bartonella henselae*), Cellulitis (usually Group A *Streptococcus* and *Staphylococcus*), Chagas Disease (American trypanosomiasis) (*Trypanosoma cruzi*), Chancroid (*Haemophilus ducreyi*), Chickenpox (Varicella zoster virus (VZV)), Chlamydia (*Chlamydia trachomatis*), *Chlamydophila pneumoniae* infection (*Chlamydophila pneumoniae*), Cholera (*Vibrio cholerae*), Chromoblastomycosis (usually *Fonsecaea pedrosoi*), Clonorchiasis (*Clonorchis sinensis*), *Clostridium difficile* infection (*Clostridium difficile*), Coccidioidomycosis (*Coccidioides immitis* and *Coccidioides posadasii*), Colorado tick fever (CTF) (Colorado tick fever virus (CTFV)), Common cold (Acute viral rhinopharyngitis; Acute coryza) (usually rhinoviruses and coronaviruses), Crimean-Congo hemorrhagic fever ((CCHF) Crimean-Congo hemorrhagic fever

virus), Cryptococcosis (*Cryptococcus neoformans*), Cryptosporidiosis (*Cryptosporidium* genus), Cutaneous larva migrans (CLM) (usually *Ancylostoma braziliense*; multiple other parasites), Cyclosporiasis (*Cyclospora cayetanensis*), Cysticercosis (*Taenia solium*), Cytomegalovirus infection (Cytomegalovirus), Dengue fever (Dengue viruses (DEN-1, DEN-2, DEN-3 and DEN-4) - Flaviviruses), Dientamoebiasis (*Dientamoeba fragilis*), Diphtheria (*Corynebacterium diphtheriae*), Diphyllbothriasis (*Diphyllbothrium*), Dracunculiasis (*Dracunculus medinensis*), Ebola hemorrhagic fever (Ebola virus (EBOV)), Echinococcosis (*Echinococcus* genus), Ehrlichiosis (*Ehrlichia* genus), Enterobiasis (Pinworm infection) (*Enterobius vermicularis*), *Enterococcus* infection (*Enterococcus* genus), Enterovirus infection (Enterovirus genus), Epidemic typhus (*Rickettsia prowazekii*), Erythema infectiosum (Fifth disease) (Parvovirus B19), Exanthem subitum (Sixth disease) (Human herpesvirus 6 (HHV-6) and Human herpesvirus 7 (HHV-7)), Fasciolopsiasis (*Fasciolopsis buski*), Fasciolosis (*Fasciola hepatica* and *Fasciola gigantica*), Filariasis (*Filarioidea* superfamily), Food poisoning by *Clostridium perfringens* (*Clostridium perfringens*), Free-living amebic infection multiple (*Fusobacterium* infection *Fusobacterium* genus), Gas gangrene (Clostridial myonecrosis) (usually *Clostridium perfringens*; other *Clostridium* species), Geotrichosis (*Geotrichum candidum*), Giardiasis (*Giardia intestinalis*), Glanders (*Burkholderia mallei*), Gnathostomiasis (*Gnathostoma spinigerum* and *Gnathostoma hispidum*), Gonorrhea (*Neisseria gonorrhoeae*), Granuloma inguinale (Donovanosis) (*Klebsiella granulomatis*), Group A streptococcal infection (*Streptococcus pyogenes*), Group B streptococcal infection (*Streptococcus agalactiae*), *Haemophilus influenzae* infection (*Haemophilus influenzae*), Hand, foot and mouth disease (HFMD) (Enteroviruses, mainly Coxsackie A virus and Enterovirus 71 (EV71)), Hantavirus Pulmonary Syndrome (HPS) (Sin Nombre virus), *Helicobacter pylori* infection (*Helicobacter pylori*), Hemolytic-uremic syndrome (HUS) (*Escherichia coli* O157:H7, O111 and O104:H4), Hemorrhagic fever with renal syndrome (HFRS) (Bunyaviridae family), Hepatitis A (Hepatitis A Virus), Hepatitis B (Hepatitis B Virus), Hepatitis C (Hepatitis C Virus), Hepatitis D (Hepatitis D Virus), Hepatitis E (Hepatitis E Virus), Herpes simplex Herpes simplex virus 1 and 2 (HSV-1 and HSV-2) (Histoplasmosis *Histoplasma capsulatum*), Hookworm infection (*Ancylostoma duodenale* and *Necator americanus*), Human bocavirus infection (Human bocavirus (HBoV)), Human ewingii ehrlichiosis (*Ehrlichia ewingii*), Human granulocytic anaplasmosis (HGA) (*Anaplasma phagocytophilum*), Human metapneumovirus infection (Human metapneumovirus

(hMPV)), Human monocytic ehrlichiosis (*Ehrlichia chaffeensis*), Human papillomavirus (HPV) infection (Human papillomavirus (HPV)), Human parainfluenza virus infection (Human parainfluenza viruses (HPIV)), Hymenolepiasis (*Hymenolepis nana* and *Hymenolepis diminuta*), Epstein-Barr Virus Infectious Mononucleosis (Mono) (Epstein-Barr Virus (EBV)), Influenza (flu) (Orthomyxoviridae family), Isosporiasis (*Isospora belli*), Keratitis (multiple), *Kingella kingae* infection (*Kingella kingae*), Lassa fever (Lassa virus), Legionellosis (Legionnaires' disease) (*Legionella pneumophila*), Legionellosis (Pontiac fever) (*Legionella pneumophila*), Leishmaniasis (*Leishmania* genus), Leprosy (*Mycobacterium leprae* and *Mycobacterium lepromatosis*), Leptospirosis (*Leptospira* genus), Listeriosis (*Listeria monocytogenes*), Lyme disease (Lyme borreliosis) (usually *Borrelia burgdorferi* and other *Borrelia* species), Lymphatic filariasis (Elephantiasis) (*Wuchereria bancrofti* and *Brugia malayi*), Lymphocytic choriomeningitis (Lymphocytic choriomeningitis virus (LCMV)), Malaria (*Plasmodium* genus), Marburg hemorrhagic fever (MHF) (Marburg virus), Measles (Measles virus), Melioidosis (Whitmore's disease) (*Burkholderia pseudomallei*), Meningitis (multiple), Meningococcal disease (*Neisseria meningitidis*), Metagonimiasis (usually *Metagonimus yokagawai*), Microsporidiosis (Microsporidia phylum), Molluscum contagiosum (MC) (Molluscum contagiosum virus (MCV)), Mumps (Mumps virus), Murine typhus (Endemic typhus) (*Rickettsia typhi*), Mycoplasma pneumonia (*Mycoplasma pneumoniae*), Mycetoma (numerous species of bacteria (Actinomycetoma) and fungi (Eumycetoma)), Myiasis (parasitic dipterous fly larvae), Neonatal conjunctivitis (Ophthalmia neonatorum) (most commonly *Chlamydia trachomatis* and *Neisseria gonorrhoeae*), Nocardiosis (usually *Nocardia asteroides* and other *Nocardia* species), Onchocerciasis (River blindness) (*Onchocerca volvulus*), Paracoccidioidomycosis (South American blastomycosis) (*Paracoccidioides brasiliensis*), Paragonimiasis (usually *Paragonimus westermani* and other *Paragonimus*), species (Pasteurellosis *Pasteurella* genus), Pediculosis capitis (Head lice) (*Pediculus humanus capitis*), Pediculosis corporis (Body lice) (*Pediculus humanus corporis*), Pediculosis pubis (Pubic lice, Crab lice) (*Phthirus pubis*), Pelvic inflammatory disease (PID) (multiple), Pertussis (Whooping cough) (*Bordetella pertussis*), Plague (*Yersinia pestis*), Pneumococcal infection (*Streptococcus pneumoniae*), Pneumocystis pneumonia (PCP) (*Pneumocystis jirovecii*), Pneumonia (multiple), Poliomyelitis Poliovirus *Prevotella* infection (*Prevotella* genus), Primary amoebic meningoencephalitis (PAM) (usually *Naegleria fowleri*), Progressive multifocal leukoencephalopathy (JC virus), Psittacosis

(*Chlamydophila psittaci*), Q fever (*Coxiella burnetii*), Rabies (Rabies virus), Rat-bite fever (*Streptobacillus moniliformis* and *Spirillum minus*), Respiratory syncytial virus infection (Respiratory syncytial virus (RSV)), Rhinosporidiosis (*Rhinosporidium seeberi*), Rhinovirus infection (Rhinovirus), Rickettsial infection (*Rickettsia* genus), Rickettsialpox (*Rickettsia akari*), Rift Valley fever (RVF) (Rift Valley fever virus), Rocky mountain spotted fever (RMSF) (*Rickettsia rickettsii*), Rotavirus infection (Rotavirus), Rubella (Rubella virus), Salmonellosis (*Salmonella* genus), SARS (Severe Acute Respiratory Syndrome) (SARS coronavirus), Scabies (*Sarcoptes scabiei*), Schistosomiasis (*Schistosoma* genus), Sepsis (multiple), Shigellosis (Bacillary dysentery) (*Shigella* genus), Shingles (Herpes zoster) (Varicella zoster virus (VZV)), Smallpox (Variola) (Variola major or Variola minor), Sporotrichosis (*Sporothrix schenckii*), Staphylococcal food poisoning (*Staphylococcus* genus), Staphylococcal infection (*Staphylococcus* genus), Strongyloidiasis (*Strongyloides stercoralis*), Syphilis (*Treponema pallidum*), Taeniasis (*Taenia* genus), Tetanus (Lockjaw) (*Clostridium tetani*), Tinea barbae (Barber's itch) (usually *Trichophyton* genus), Tinea capitis (Ringworm of the Scalp) (usually *Trichophyton tonsurans*), Tinea corporis (Ringworm of the Body) (usually *Trichophyton* genus), Tinea cruris (Jock itch) (usually *Epidermophyton floccosum*, *Trichophyton rubrum*, and *Trichophyton mentagrophytes*), Tinea manuum (Ringworm of the Hand) (*Trichophyton rubrum*), Tinea nigra (usually *Hortaea werneckii*), Tinea pedis (Athlete's foot) (usually *Trichophyton* genus), Tinea unguium (Onychomycosis) (usually *Trichophyton* genus), Tinea versicolor (Pityriasis versicolor) (*Malassezia* genus), Toxocariasis (Ocular Larva Migrants (OLM)) (*Toxocara canis* or *Toxocara cati*), Toxocariasis (Visceral Larva Migrants (VLM)) (*Toxocara canis* or *Toxocara cati*), Toxoplasmosis (*Toxoplasma gondii*), Trichinellosis (*Trichinella spiralis*), Trichomoniasis (*Trichomonas vaginalis*), Trichuriasis (Whipworm infection) (*Trichuris trichiura*), Tuberculosis (usually *Mycobacterium tuberculosis*), Tularemia (*Francisella tularensis*), *Ureaplasma urealyticum* infection (*Ureaplasma urealyticum*), Venezuelan equine encephalitis (Venezuelan equine encephalitis virus), Venezuelan hemorrhagic fever (Guanarito virus), Viral pneumonia (multiple viruses), West Nile Fever (West Nile virus), White piedra (Tinea blanca) (*Trichosporon beigeli*), *Yersinia pseudotuberculosis* infection (*Yersinia pseudotuberculosis*), Yersiniosis (*Yersinia enterocolitica*), Yellow fever (Yellow fever virus), Zygomycosis (Mucorales order (Mucormycosis) and Entomophthorales order (Entomophthoramycosis)).

A representative but non-limiting list of bacterial genus and typical species that may cause infectious diseases, and to which the subject invention can be applied, is provided below: *Bacillus* (*Bacillus anthracis*); *Bordetella* (*Bordetella pertussis*); *Borrelia* (*Borrelia burgdorferi*); *Brucella* (*Brucella abortus*, *Brucella canis*, *Brucella melitensis*, *Brucella suis*); *Campylobacter* (*Campylobacter jejuni*); *Chlamydia* and *Chlamydophila* (*Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Chlamydophila psittaci*); *Clostridium* (*Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Clostridium tetani*); *Corynebacterium* (*Corynebacterium diphtheriae*); *Enterococcus* (*Enterococcus faecalis*, *Enterococcus faecium*); *Escherichia* (*Escherichia coli*, including Enteropathogenic, Enterotoxigenic, and *E. coli* O157:H7); *Francisella* (*Francisella tularensis*); *Haemophilus* (*Haemophilus influenzae*); *Helicobacter* (*Helicobacter pylori*); *Legionella* (*Legionella pneumophila*); *Leptospira* (*Leptospira interrogans*); *Listeria* (*Listeria monocytogenes*); *Mycobacterium* (*Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycobacterium ulcerans*); *Mycoplasma* (*Mycoplasma pneumoniae*); *Neisseria* (*Neisseria gonorrhoeae*, *Neisseria meningitidis*); *Pseudomonas* (*Pseudomonas aeruginosa*); *Rickettsia* (*Rickettsia rickettsii*); *Salmonella* (*Salmonella typhi*, *Salmonella typhimurium*); *Shigella* (*Shigella sonnei*); *Staphylococcus* (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*); *Streptococcus* (*Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*); *Treponema* (*Treponema pallidum*); *Vibrio* (*Vibrio cholerae*); and *Yersinia* (*Yersinia pestis*).

“Microorganism” or “microbe,” in the broadest sense, includes pathogenic unicellular or microscopic (e.g., invisible to naked eye) multicellular organisms and prions that infect human and other non-human animals and cause infectious disease, such as bacteria, fungi, algae, virus, prions, and protozoa. In certain embodiments, microorganism refers to the above pathogens that do not include prion. In certain embodiments, microorganism refers to the above pathogens that do not include virus. In certain embodiments, microorganism refers to the above pathogens that do not include eukaryotes such as fungi, parasites, and protists.

“Microorganism infection” includes any disease or pathological condition, with or without manifested or detectable symptoms, that is caused by or results from infection by one or more microorganisms as defined herein. In certain embodiments, microorganism infection includes bacterial infection that may or may not be contagious.

“Dosing regimen” include a schedule and the related parameters for administration of one or more drugs, compounds, medicaments, or pharmaceutical compositions. A dosing regimen may specify some or all of the following non-limiting aspects: the amount / dose for a specific drug, applicable age group (*e.g.*, adult vs. children under certain age or weight class), patient sub-population (*e.g.*, women that is not pregnant, pre-existing conditions, prior treatment history, on-going treatment with other drugs), timing (*e.g.*, morning, evening, upon waking up, before sleep, before or after meal, *etc.*), frequency (*e.g.*, once or twice daily, weekly, *etc.*), and route (*e.g.*, *i.v.*, *i.p.*, *s.c.*, *i.m.*, oral, topical, *etc.*) of administration, *etc.*

“Intrinsic logarithmic growth rate of a microorganism” describes the growth rate of a microorganism of interest under a set of conditions pertinent to microorganism growth. For example, any given microorganism may have a growth rate (*e.g.*, measurable by population doubling time, or CFU increase over unit time, *etc.*) under a given circumstance, such as culture with defined components, or under the physiological condition of an infection site when the microorganism is growing *in vivo*. Thus intrinsic logarithmic growth rate of a microorganism refers to the growth rate under the condition present, such as the physiological condition of an infection site, in the absence of an added antibiotic agent.

Likewise, “maximum kill rate of the microorganism by a compound” describes the rate the microorganism is killed or growth inhibited by the compound under ideal conditions, usually measurable by comparing growth (or death) rate of the microorganism in the absence (or presence) of the compound.

“Replenishment rate of the enzyme” refers to the rate of new enzyme (*i.e.*, the target of the compound / drug) added to the system. This mostly relates to the rate any new enzyme is being synthesized by the microorganism under the conditions (such as the physiological condition at the infection site).

“Concentration of a compound at the site of enzyme function” refers to compound concentration at the immediate vicinity of the enzyme to which the compound binds and inhibits. Thus if the enzyme is in the cytosol, concentration of a compound at the site of enzyme function is the concentration of the compound inside the microorganism, *e.g.*, the compound concentration in the cytosol, or the specific cytosolic compartment in which the enzyme predominantly resides and functions. If the enzyme is on cell surface, concentration of a

compound at the site of enzyme function refers to the compound concentration immediately surrounding the enzyme, which may be partly outside the microorganism.

In the context of the instant invention, concentration of a compound / inhibitor at the site of enzyme function may be represented as $[I]$, which may be accounted for with the combined parameters p_m/K_i for certain intracellular enzymes (see below), which may also be equal to $p_m \times [I]_0$ (where $[I]_0$ is the initial compound / inhibitor concentration within the time period of interest). For free compound or inhibitor in PK or *in vitro* tests, $[I]$ is $[I]_0$ for the duration of the test, where compound concentration at the site of enzyme function does not substantially deviate from the initial value $[I]_0$.

“Pharmacokinetics (PK)” refers to a branch of pharmacology dedicated to the determination of the fate of substances administered to a living organism. It describes how the body affects a specific substance or drug after administration. Pharmacokinetic properties of drugs may be affected by elements such as the site of administration and the dose of administered drug, which may both affect the absorption rate. The substances of interest include, without limitation, pharmaceutical agents, antibiotics, hormones, nutrients, and toxins, *etc.* The core aspects of pharmacokinetics may include Absorption (the process of a substance entering the blood circulation); Distribution (the dispersion or dissemination of substances throughout the fluids and tissues of the body); Metabolism or Biotransformation (the irreversible transformation of parent compounds into daughter metabolites); and Excretion (the removal of the substances from the body), or the “ADME” scheme. Sometimes, a fifth process, Liberation (the process of release of drug from the formulation) has also been highlighted as playing an important role in pharmacokinetics. Hence “LADME” may sometimes be used in place of “ADME” in reference to the core aspects of pharmacokinetics.

“Pharmacokinetic (PK) Profile” is sometimes used to describe the relationship between drug concentration and time course after drug administration. Drug concentration can be expressed as log concentration, and may represent drug concentration at the site of microorganism infection. Alternatively, drug concentration may be represented by concentration in a bodily fluid, such as serum or plasma concentration. Such systemic drug concentration can be converted to drug concentration at the site of microorganism infection based on pharmacodynamic (PD) properties of the drug in the host.

“Pharmacodynamics (PD)” is the study of the biochemical and physiological effects of drugs on the body or on microorganisms or parasites within or on the body, and the mechanisms of drug action and the relationship between drug concentration and effect.

“Post Antibiotic Effect (PAE)” refers to the phenomenon of persistent suppression of bacterial growth, after a brief exposure (*e.g.*, 1 or 2 hours) of bacteria to an antibiotic even in the absence of minimal inhibitory concentration (MIC) of the antibiotic and other host defense mechanisms. Factors that affect the duration of the post antibiotic effect include duration of antibiotic exposure, bacterial species, culture medium, and class of antibiotic, *etc.* The ability of an antibiotic to induce a PAE is an attractive property of the antibiotic, since antibiotic concentrations could fall below the MIC for the bacterium yet retain their effectiveness in their ability to suppress the growth.

“Subject in need of treatment” includes subjects (human or non-human patients) that may or may not have a symptom, a detectable symptom, or a symptom that is causing discomfort in the subject. It also includes subjects (human or non-human patients) that may or may not have developed a symptom, a detectable symptom, or a symptom that is causing discomfort in the subject. In certain embodiments, treatment to such subject may be necessary to prevent the development or re-development of a symptom, or to prevent the transmission of the disease to a healthy individual, *e.g.*, one in contact with the subject.

“Treating” includes administration of one or more compounds, drugs, or therapeutic agents to a subject, for the purpose of alleviating, reducing, or eliminating one or more symptoms or causes for the symptoms, or for the purpose of retard the progression or advancement of certain worsening conditions. In certain embodiments, treating does not include prevention, or administration of any compounds, drugs, or therapeutic agents in anticipation of a microorganism infection or the development of a symptom as the result of such infection.

“Administering” includes any suitable means or art-recognized methods for contacting, introducing, dispensing, delivering, or applying compounds, drugs, or therapeutic agents to a subject, via any suitable routes to any desired location in the subject, including but not limited to, parenteral, oral, intramuscular injection, subcutaneous / intradermal injection, intravenous injection, buccal administration, transdermal delivery, and administration by the intranasal or respiratory tract route.

“Initiating a time course of treatment” includes commencing the treatment by administering the compound to the subject. Preferably, the time (*e.g.*, the day) the compound is first administered to the subject marks the beginning of the time course of treatment, which may be subdivided into multiple smaller time intervals (*e.g.*, minutes, hours, etc.), the first of which smaller time intervals is used to calculate γ according to Equation (IV) below.

“Microorganism titer” includes the number of the microorganism in a unit volume, preferably viable microorganism and/or microorganism capable of proliferation. Microorganism titer may be measured using different art-recognized methods, so long as the measurement is consistent or compatible when used in the equations described herein. One common way to measure microorganism titer is CFU or colony forming units, which essentially measures, within a defined volume, the number of microorganisms capable of proliferating and forming colonies.

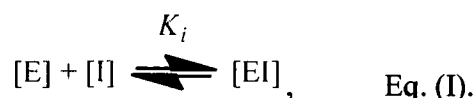
“Substantially constant” includes small variations or deviations from a constant, such as an average, a maximum value, or a minimum value. The variations or deviations may be no more than 20%, 15%, 10%, 5%, 3%, 1% or less of the constant.

The “enzymes” that are bound and inhibited by the compounds or antibiotics of the invention are those that mediate one or more critical functions required for microbial (*e.g.*, bacterial) survival and/or growth and/or proliferation. The antibiotics may inhibit the enzymes in the following biological pathways through one or more specified mechanisms: cell wall synthesis (*e.g.*, inhibit peptidoglycan synthesis, disrupt peptidoglycan cross-linkage, disrupt movement of peptidoglycan precursors, or disrupt mycolic acid or arabinoglycan synthesis), protein synthesis or translation (*e.g.*, irreversibly bind 30S ribosomal proteins, block tRNA binding to 30S ribosome-mRNA complex, bind peptidyl transferase component of 50S ribosome, block peptide elongation, reversibly bind 50S ribosome, inhibit peptidyl transferase by interfering with binding of amino acid-acyl-tRNA complex), maintaining cell membrane integrity and other critical functions of the cell membrane (*e.g.*, disrupt cytoplasmic membranes), nucleic acid synthesis (*e.g.*, inhibit DNA gyrases or topoisomerases required for supercoiling of DNA, generate metabolic cytotoxic byproducts that disrupt DNA, bind to DNA-dependent RNA polymerase inhibiting initiation & Rifabutin of RNA synthesis, inhibit RNA transcription), and synthesis of key metabolic compounds (*e.g.*, compete with *p*-aminobenzoic acid (PABA) to prevent synthesis of folic acid, inhibit dihydrofolate reductase to prevent synthesis of folic acid).

3. Relationships among the Various Parameters of Microorganism Infection

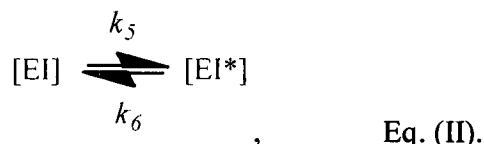
When a specific type of microorganism, such as an infectious bacterium, infects a host, the bacterium proliferates under the physiological conditions of the infection site within the host to increase its total population. Meanwhile, host defense mechanisms (innate or acquired immune systems) engage the expanding population of bacteria to reduce the population increase. This balance is sometimes shifted by the administration of drug compounds designed to combat the specific microorganism infection, such as one or more antibiotics designed to kill the microorganism, or to inhibit the growth of the microorganism. These drug compounds usually selectively target one or more key enzymes of the infecting microorganism (such as an essential metabolic enzyme required for microorganism survival or proliferation), and it is the impairment of the function(s) of the key enzymes that either kills the microorganism or retards the growth of the microorganism or expansion of the microorganism population.

The binding of a drug compound to its target enzyme *in vivo* promotes the formation of a compound-enzyme complex (or “enzyme-inhibitor” complex, or simply “EI”), which process can be described with a binding constant K_i , according to Equation (I):

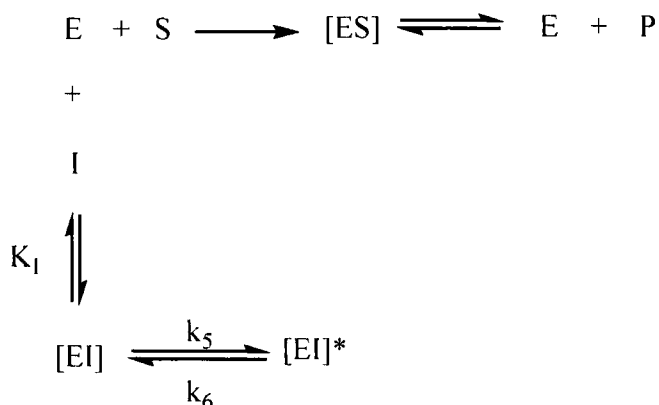


In this equation, $[E]$ represents the concentration of the bacterial target enzyme, $[I]$ represents the concentration of the compound drug or enzyme inhibitor, and $[EI]$ represents the concentration of the compound-enzyme complex. It can generally be assumed that the enzyme, the compound, and the complex are in fast equilibrium, such that any change in concentration of one of E, I and EI will almost instantly be reflected in concentration changes of the other two, based on the K_i .

In some cases, however, the compound-enzyme complex EI is in further equilibrium with a tight compound-enzyme complex (“EI*”), with an on-rate constant k_5 and an off-rate constant k_6 , according to Equation (II):



Thus the relationship amongst the enzyme (E), its substrate (S), its inhibitor (I), the product (P), the enzyme-substrate complex (ES), the enzyme-inhibitor complexes (EI and EI*), and the associated kinetic parameters K_i and k_5 , k_6 can be represented in the diagram below.



At least in theory, the on-rate constant k_5 and the off-rate constant k_6 can both be very fast such that EI and EI* are in fast equilibrium based on k_5 and k_6 . However, at least in certain instances, the off-rate constant k_6 can be quite slow, such that when the concentration of at least one of EI and EI* changes, a significant “lag” is observed before a new equilibrium can be reached.

This phenomenon correlates somewhat to the Post-Antibiotic Effect (PAE) observed in the treatment of certain bacterial infections with certain antibiotics. The PAE is a term used to describe the persistent suppression of bacterial growth that can occur after limited exposure to an antimicrobial agent. See McDonald, Craig & Kunin, *Persistent effect of antibiotics on Staphylococcus aureus after exposure for limited periods of time*. Journal of Infectious Diseases 135: 217-223, 1977 (the entire contents of which is expressly incorporated herein by reference). Also see, Craig, *The Role of Pharmacodynamics in Effective Treatment of Community-Acquired Pathogens*, Proceedings of the 39th Annual Meeting of the Infectious Disease Society of America, Vol. 2, No. 4, 2002 (the entire contents of which is expressly incorporated herein by reference); and Craig, *Post-Antibiotic Effects in Experimental Infection Models: Relationship to in vitro Phenomena and to Treatment of Infections in Man*, J. Antimicrobial Chemotherapy, 31, Supp. D, pp. 149-158, 1993 (the entire contents of which is expressly incorporated herein by reference).

Briefly, when certain antibiotics are administered to treat a bacterial infection, bacterial

growth is inhibited so long as the concentration of the administered antibiotics is at or above a minimum inhibition concentration (MIC). As soon as the drug concentration falls below the MIC, due to factors such as drug metabolism or excretion by the host, bacterial growth resumes without discernible delay. That is, no PAE is observed when using the specific antibiotics to treat the specific bacterial infection. In other situations, however, bacterial growth is still inhibited or significantly retarded even after the drug concentration falls below the MIC. It is only after a set period of time after the drug concentration has fallen below the MIC that bacterial growth resumes at full strength. The presence of a prolonged *in vivo* PAE may be beneficial since it allows wider dosing intervals without sacrificing therapeutic efficacy.

Without wishing to be bound by any particular theory, Applicants believe that at least part of the mechanism for the observed PAE is that the drug compound binds to its target enzyme and locks the enzyme in a stable EI* complex, such that even after the withdrawal or reduction of the drug compound in the host system, a significant portion of the EI* complex is slow to convert back to the EI complex which is in rapid equilibrium with free enzyme E. This slow conversion, as partly dictated by the slow off rate constant k_6 of Equation (II), helps to prevent the target enzyme from facilitating recovery of the infectious bacteria, at least during the initial phase after the drug concentration falls below the MIC. In practice, by having the knowledge of the existence of PAE for a particular antibiotic used to treat a specific infection, one can design a better dosing regimen that takes into consideration the existence and extent of PAE, such that a minimally effective drug concentration is used to treat a bacterial infection.

Concerning antibiotic dosing regimens, historically, they have been determined based upon the pharmacokinetic (PK) parameters of the particular antibiotic. Recent experience suggests, however, that pharmacodynamic (PD) parameters should also play an important role, because drug concentrations in the tissues or body fluids as well as at the site of infection are considered. Drug concentration in the tissues or body fluids to the large extent determines the pharmacological and toxicological effects of the drug, while drug concentration at the site of microbial infection determines the antimicrobial effect.

Once a dosing regimen has been determined, drug compounds are administered to the host at a given dose or doses, and at specified intervals according to the dosing regimen. As a result of the PK properties of the drug compound, a pharmacokinetic (PK) profile of the

compound in the subject, particularly at the site of the microorganism infection, may be produced. The PK profile provides a time course of the relevant drug concentration (*e.g.*, drug concentration at the site of infection and/or site of bacterial growth) over time.

It has been discovered that the relationship between several key parameters associated with a particular microorganism infection can be expressed with Equation (III) below:

$$[N] = [N_0] \exp([\lambda + \varepsilon(\sigma - 1)]t + \varepsilon\rho \left(\frac{k_6}{2kb} - \frac{1}{2} \right) t^2 + \varepsilon \frac{\gamma}{k} (1 - e^{-kt})] \quad \text{Eq. (III)}$$

wherein:

N_0 is microorganism titer or concentration (that may be measured by CFU per unit volume) at the beginning of a period of time during which drug compound concentration does not substantially change (*e.g.*, drug compound concentration does not deviate from average drug concentration during the period by more than 20%, 15%, 10%, or 5%);

N is microorganism titer or concentration (that may be measured by CFU per unit volume) at the end of the period of time;

t is the duration of time for the period;

λ is intrinsic logarithmic growth rate of the microorganism, under the physiological conditions at the site of infection or growth;

ε is maximum kill rate of the microorganism by the compound, under the physiological conditions at the site of infection or growth;

ρ is replenishment rate of the enzyme (*e.g.*, the rate at which new enzymes are produced by the microorganism or otherwise made available to the microorganism at the site of infection or growth), under the physiological conditions at the site of infection or growth;

$[I]$ is concentration of the compound at the site of enzyme function (*e.g.*, inside the microorganism for an intracellular enzyme), which, if necessary, can be converted from the known drug compound concentration outside the microorganism, and a correlation between drug concentration outside the microorganism and drug concentration at the site of enzyme function;

K_i is the binding constant defined in Eq. (I);

k_5 and k_6 are on- and off-rates defined in Eq. (II);

$$\beta = 1 + K_M / [S] + (K_M / [S]) \times ([I] / K_i);$$

$$k = k_6 + (k_5 / \beta) \times (K_M / [S]) \times ([I] / K_i);$$

$$\sigma = (k \times (k_6 + \rho) / \beta - k_6 \times \rho / \beta) / k^2;$$

K_M is the Michaelis-Menten constant of the enzyme;

$[S]$ is concentration of the substrate of the enzyme at the site of enzyme function (e.g., inside the microorganism).

Parameters σ , β , k above are auxiliary parameters introduced into the equation to simplify the equation.

The remaining symbol γ is a constant that can be determined by the initial value of the enzyme substrate complex for the period of time.

Although Equation (III) above requires the inhibitor concentration $[I]$ to remain substantially constant during a period of time in question, the practical applicability of the Equation is not so limited. This is because for any PK profile of the drug compound, the time course of interest can be sub-divided into a series of successive intervals, wherein the drug compound concentration does not substantially change within each of the small intervals. Thus Equation (III) can be applied for each of such intervals to determine microorganism titer at the end of each interval (N), based on the microorganism titer at the beginning of each interval (N_0). Once N is determined for one particular interval, it can serve as the N_0 for the immediate following interval, in which Equation (III) can still be applied to determine the N at the end of the immediate following interval. By following this procedure, microorganism titer change over time can be determined based on a successive series of calculations according to Equation (III).

It should be noted that, during the series of calculations above, a unique/critical point of the computation is the determination of the value γ . The magnitude of this value is dependent on whether $[EI^*]$ is increasing or depleting. Additionally, the value of γ varies with time, and is calculated or approximated in successive small intervals. For the first interval (during which drug compound concentration does not substantially change) γ can be determined using the following Equation (IV), taking into consideration the reasonable assumption that there is no EI^* complex at the beginning of the period (i.e., $[EI^*] = 0$):

$$\gamma = 1 / \beta + (k \times (k_6 + \rho) / \beta - k_6 \times \rho / \beta) / k^2, \text{ Eq. (IV).}$$

The various parameters in Equation (IV) are as defined above.

Once constant γ is determined for the first interval, for the second interval and beyond, constant γ for each interval can be determined using the constant γ value from the immediate preceding interval and current drug compound concentration and other parameters above. More specifically, to iterate between successive time intervals, the following specific steps can be used for any “current” interval:

1. Compute the value of [ES] at the end of the immediate preceding interval (*e.g.*, for the second interval as the current interval, the value of [ES] at the end of the first interval) according to the following Equation (VI):

$$[ES] = \frac{bk-h}{k^2} + \frac{h}{k}t + \gamma e^{-kt}, \text{ Eq. (VI).}$$

Wherein $b = (k_6 + \rho) / \beta$; $h = k_6 \times \rho / \beta$; $\beta (\beta = 1 + K_M / [S] + (K_M / [S]) \times ([I] / K_i))$ and $k (k = k_6 + (k_5 / \beta) \times (K_M / [S]) \times ([I] / K_i))$ are as defined above, and γ is the constant for the immediate preceding interval (*e.g.*, the 1st interval for the 2nd interval as the current interval). For the 2nd interval, for example, constant γ for the immediate preceding interval (*i.e.*, the 1st interval) can be determined based on Eq. (IV) above. For the 3rd interval and beyond, constant γ for the immediate preceding interval (*e.g.*, 2nd interval and beyond) can be determined using the procedure being described here in steps 1-3.

2. Compute constant β for the current interval, using the new inhibitor level [I] for the current interval. Note that:

$$\beta = 1 + K_M / [S] + (K_M / [S]) \times ([I] / K_i).$$

3. Compute constant γ for the current interval using the following Equation (V):

$$\gamma = \frac{\beta_{old}[ES]_{end-of-last-interval}}{\beta_{new}} - \sigma_{new}, \text{ Eq. (V);}$$

wherein in Eq. (V):

β_{old} is β defined above for the immediate preceding interval;

β_{new} and σ_{new} are β and $\sigma (\sigma = (k \times (k_6 + \rho) / \beta - k_6 \times \rho / \beta) / k^2)$ defined above for the current interval;

$[ES]_{end}$ is concentration of enzyme-substrate complex at the end of the immediate preceding interval, and is calculated according to Equation (VI) in step

1 above.

Once the values of constants γ and β for the current interval (e.g., the 2nd interval) are determined using the above steps 1-3, they can be used to calculate [ES] at the end of the current interval (e.g., the 2nd interval) as in step 1 above, and β_{old} , which [ES] and β_{old} values are then in turn used in calculating constant γ for the 3rd interval according to steps 1-3. So on and so forth.

With the above procedure, the model enables one to determine or simulate the titer of a infecting microorganism in a host from beginning to end, given the known, calculated, and/or measured parameters in the equations, and knowledge of the pharmacokinetics of the inhibitor.

Equation (III) above may be further simplified under certain conditions, as described in more detail below.

First, when k_6 is 0 or is nearly 0, the conversion from EI to EI* becomes irreversible, such that EI* does not revert back to EI in any significant way, if at all. Under this condition, Equation (III) becomes Equation (III)a below:

$$[N] = \exp(\lambda t - \varepsilon \left(\frac{\rho}{k_5 C \frac{[I]}{K_t}} - 1 \right) t - \frac{\varepsilon \rho}{2} t^2 - \varepsilon \frac{1 - \frac{\rho}{k_5 C \frac{[I]}{K_t}} (1 + C + C \frac{[I]}{K_t})}{k_5 C \frac{[I]}{K_t}} \left(1 - e^{-\frac{k_5 C \frac{[I]}{K_t}}{(1 + C + C \frac{[I]}{K_t})} t} \right)) N_0$$

Eq. (III)a

Second, when $k_6 \gg k_5$, or when k_5 / k_6 is 0 or is nearly 0, any EI* (if any at all) almost instantly reverts back to EI, and Equation (III) becomes Equation (III)b:

$$[N] = \exp(\lambda t + \varepsilon \left(\frac{1}{\beta} - 1 \right) (t + \rho t^2 / 2)) N_0$$

Eq. (III)b

Third, in some systems where enzyme replenishment rate ρ is very small or negligible, the total enzyme concentration can be considered as a constant in the process. Under this reasonable approximation, Equation (III) becomes Equation (III)c:

$$[N] = \exp\left(\left[\lambda + \varepsilon\left(\frac{k_6}{k_6(1 + C + C\frac{[II]}{K_t}) + k_5C\frac{[II]}{K_t}} - 1\right)\right]t + \varepsilon \frac{k_5C\frac{[II]}{K_t}}{(k_6(1 + C + C\frac{[II]}{K_t}) + k_5C\frac{[II]}{K_t})^2} (1 - e^{-(k_6 + \frac{k_5C\frac{[II]}{K_t}}{1 + C + C\frac{[II]}{K_t}})t})\right)N_0$$

Eq. (III)c

Fourth, when both k_6 and ρ are both 0 or both near 0, *i.e.*, irreversible EI to EI* conversion, and no enzyme replenishment, Equation (III) can be further simplified into Equation (III)d:

$$[N] = \exp((\lambda - \varepsilon)t + \varepsilon \frac{1}{k_5C\frac{[II]}{K_t}} \left(1 - e^{-\frac{k_5C\frac{[II]}{K_t}}{(1 + C + C\frac{[II]}{K_t})}t}\right)) N_0$$

Eq. (III)d

Finally, if the EI* to EI conversion is reversible ($k_6 \gg k_5$), and if there is no enzyme replenishment ($\rho = 0$), Equation (III) can be further simplified into Equation (III)e:

$$[N] = \exp\left(\lambda t - \varepsilon t \frac{1 + \frac{[II]}{K_t}}{\frac{[S]}{K_m} + 1 + \frac{[II]}{K_t}}\right) N_0$$

Eq. (III)e

In Eq. (III)e, $[S] / K_m$ is EC_{50} .

Note that in Equation (III), the linear term $[\lambda + \varepsilon(\sigma - 1)]$ largely determines whether the titer N will be decreased or increased compared to N_0 , wherein a positive $[\lambda + \varepsilon(\sigma - 1)]$ value increases titer, and a negative $[\lambda + \varepsilon(\sigma - 1)]$ value decreases titer.

Also note that the non-linear terms in Equation (III) add a dip / valley or a bump / peak to the titer curve, and the depth or height of such dips and bumps is equal to γ / k^2 . Since γ is computed as $(k_6[EI^*] - k_5[EI]) / \beta$, to increase the depth of the dip, more $[EI^*]$ excess is desired,

which can be achieved either by increasing the incubation time or with a higher inhibitor concentration.

On the other hand, the time that takes to erase or ease the dip or bump is:

$$t = \frac{\gamma}{k^2(\lambda - \epsilon(1 + b/k))}$$

Equation (III) also suggests that at certain inhibitor concentration [I], N neither increases nor decreases compared to N_0 ("stasis"). That is, when:

$$\lambda - \epsilon(1 - \frac{b}{k} + \frac{\gamma}{k} e^{-kt}) = 0$$

When the time frame is long term (*i.e.*, t is large), the above equation can be further simplified into:

$$\lambda - \epsilon(1 - \frac{b}{k}) = 0, \text{ or } \lambda - \epsilon(1 - \frac{k^6}{k^6(1+C+C[I]/K_i) + k_5C[I]/K_i}) = 0$$

Thus the concentration that induces stasis is:

$$[I] = \frac{k_6K_i((C+1)\lambda - C\epsilon)}{C(\epsilon - \lambda)(k_6 + k_5)}$$

The utility of Equation (III) is not limited to calculating / predicting / simulating bacterial titer (N) change over time. In fact, given the established relationship among the above parameters, Equation (III) enables one to simulate any of the given parameters provided that the other parameters are fixed, or provided by experimental data.

Specifically, for simulation in general, the following parameters may be needed: k_5 , k_6 , K_i , $C = K_M / [S]$, λ , ϵ , and ρ . A graphic user interface (GUI) can and has been built to dynamically change the values of these parameters (except for the one of interest) to show how such parameter changes will affect the behavior of the parameter of interest. Given the way Equation (III) is applied to overcome the limitation of requiring substantial constant inhibitor concentration, a specified range of time that includes time zero is provided (*i.e.*, when the process is just started). In addition, the concentration level of the drug compound or inhibitor can be provided as a time related vector, or as the output of a PK model.

All parameters other than K_i can be readily fitted as regular parameters, as could be done

for any standard curve-fitting algorithm. For K_i , however, a potential complication may arise due to a hidden parameter p_m (as defined below). In Equation (III), K_i is always associated with the drug compound concentration $[I]$ at the target site, which is often inside a cell when, for example, the enzyme target of the drug compound is a cytosolic enzyme. Meanwhile, inhibitor concentration provided in a typical PK profile usually reflects drug compound concentration in an extracellular space, such as in plasma or at a local infection site. Thus, under this circumstance, the drug compound concentration provided by the PK profile may need to be adjusted or converted to intracellular drug compound concentration $[I]$.

Under typical circumstances, the intracellular drug compound concentration $[I]$ is much less than the extracellular drug compound concentration, due to factors such as microorganism's ability to defend against drug compound permeation into the cell. This type of microorganism defense may be partially or completely impaired in mutants that are unable to pump intracellular drug compounds outside the cell. The ratio between the inside concentration level and outside concentration level may be correlated to and determined by so-called pump mutant coefficient, denoted as p_m . Thus, $[I]$ in Equation (III) may actually be $p_m \times [I]_o$, wherein $[I]_o$ is the inhibitor concentration outside the microorganism.

Consequently, K_i and p_m cannot be fitted independently. Instead, these two parameters can be combined into a new fitable parameter as K_{pi} , which represents the ratio of K_i and p_m .

Merely to illustrate, FIG. 1 shows a simulated PK profile for a hypothetical drug administered to a host. A GUI interface is provided such that a user can change one or more parameters to simulate different resulting PK profiles. The parameters included in the GUI interface includes drug dose R_0 (0.5 mcg), dose / day (twice a day, 12 hrs apart), route of administration (*i.v.* infusion), drug half-life $t_{1/2}$ (2.5 hrs), V_d (volume of distribution - the apparent volume in which a drug is distributed in vivo, 6 mL), *etc.* The simulated PK profile shows that drug concentration rapidly increases after the first dose at time 0, and peaks around 1 hr after *i.v.* infusion. The concentration of the drug compound then steadily decreases according to a drug half-life $t_{1/2}$ of 2.5 hours, reaching nadir after about 12 hrs, at which point a second dose is administered to repeat the process. Here, U_b and L_b refer to "Upper bound" and "Lower bound" respectively.

The lower panel of FIG. 1 shows microorganism cell count (in log scale) over the same

time period in response to drug compound concentration change, as determined by applying Equation (III). In this simulation, k_5 is set at 0.5, k_6 is set at 0.3, K_i is set at 30, p_m is 0.001, ρ is set at 0.25, C is set at 1.5. The terms lmd and eps refer to lambda (λ) and epsilon (ϵ) respectively. It is apparent that MIC is about 1×10^4 mcg/mL, such that microorganism cell count begins to recover as soon as drug compound concentration falls below the MIC at about 7.5 hrs after the first administration, and again at just over 20 hrs after the first administration.

Conversely, using any standard curve-fitting algorithm, the parameters can be determined by “fitting” Equation (III) using the measured microorganism titer change over time. Such fitted parameter values may be further compared with the corresponding experimentally determined values to show the close matches between the values. See Examples below and FIG. 2. Such model fitting typically requires information about microorganism count at different time points, and inhibitor concentrations over time.

In general, the greater the number of time points and concentration values are available, the higher the quality and/or confidence level the fitted parameter values will have.

4. *Method of Treatment*

The invention described herein provides a valuable means to control a course of treatment for microorganism infection.

In one embodiment, the invention provides a method of treating a microorganism infection in a subject in need of treatment, such that treatment according to an established dosing regimen can be precisely controlled, and drug administration can be timely terminated once the microorganism titer falls below a pre-determined level.

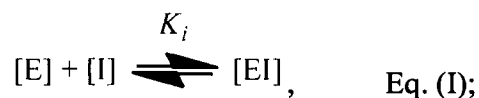
Thus in one aspect, the invention provides a method of treating a microorganism infection in a subject in need of treatment, the method comprising:

- (1) providing a compound that binds to and inhibits an enzyme of the microorganism, and inhibits proliferation of the microorganism;
- (2) providing a dosing regimen for administering the compound to the subject;
- (3) administering the compound to the subject according to the dosing regimen, and initiating a time course of treatment;

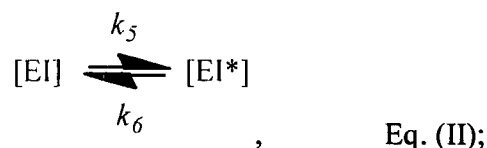
- (4) dividing the time course of treatment into a series of successive intervals, wherein within each said intervals, compound concentration at a site of microorganism infection remains substantially constant (*e.g.*, less than 5% deviation from the average concentration);
- (5) beginning from the first of said series of successive intervals, and for each said series of successive intervals, constantly determining, according to Equation (III), microorganism titer N at the end of each said series of successive intervals, using microorganism titer N_0 at the beginning of each said series of successive intervals, wherein N for one interval is N_0 for the immediate following interval; and,
- (6) terminating treatment according to the dosing regimen when microorganism titer N at a given interval falls below a pre-determined / target microorganism titer, thereby treating the microorganism infection;

wherein:

- (a) the compound binds to the enzyme *in vivo* to form a compound-enzyme complex ("EI"), with a binding constant K_i , according to Equation (I):



- (b) the compound-enzyme complex EI is in equilibrium with a tight compound-enzyme complex ("EI*"), with an on-rate constant k_5 and an off-rate constant k_6 , according to Equation (II):



- (c) the dosing regimen produces a pharmacokinetic (PK) profile of the compound in the subject (at the site of the microorganism infection);
- (d) Equation (III) is:

$$[N] = [N_0] \exp([\lambda + \varepsilon(\sigma - 1)]t + \varepsilon p \left(\frac{k_6}{2kb} - \frac{1}{2} \right) t^2 + \varepsilon \frac{\gamma}{k} (1 - e^{-kt})$$

wherein:

N_0 is microorganism titer at the beginning of each said series of successive

intervals;

N is microorganism titer at the end of each said series of successive intervals;

t is the duration of time for a given interval associated with N_0 ;

λ is intrinsic logarithmic growth rate of the microorganism;

ε is maximum kill rate of the microorganism by the compound;

ρ is replenishment rate of the enzyme;

$[I]$ is concentration of the compound at the site of enzyme (*e.g.*, inside the microorganism);

K_i is the binding constant defined in Eq. (I);

k_5 and k_6 are on- and off-rates defined in Eq. (II);

$\beta = 1 + K_M / [S] + (K_M / [S]) \times ([I] / K_i)$;

$k = k_6 + (k_5 / \beta) \times (K_M / [S]) \times ([I] / K_i)$;

$\sigma = (k \times (k_6 + \rho) / \beta - k_6 \times \rho / \beta) / k^2$;

K_M is the Michaelis-Menten constant of the enzyme;

$[S]$ is concentration of the substrate of the enzyme at the site of the enzyme function (*e.g.*, inside the microorganism);

for the first of said series of successive intervals, γ is a constant determined by Equation (IV):

$$\gamma = 1 / \beta + (k \times (k_6 + \rho) / \beta - k_6 \times \rho / \beta) / k^2, \quad \text{Eq. (IV);}$$

for each subsequent said series of successive intervals, γ is a constant determined by Equation (V):

$$\gamma = \frac{\beta_{old}[ES]_{\text{end-of-last-interval}}}{\beta_{new}} - \sigma_{new}, \quad \text{Eq. (V);}$$

wherein in Eq. (V):

β_{old} is β defined above for the immediate preceding interval;

β_{new} and σ_{new} are β and σ defined above for the current interval;

$[ES]_{\text{end}}$ is concentration of enzyme-substrate complex at the end of the immediate preceding interval, and is calculated according to Equation (VI), wherein $b = (k_6 + \rho) / \beta$; $h = k_6 \times \rho / \beta$; and γ is as defined above for the immediate preceding interval:

$$[ES] = \frac{bk-h}{k^2} + \frac{h}{k} t + \gamma e^{-kt}, \text{ Eq. (VI).}$$

According to this aspect of the invention, a patient (*e.g.*, human or a non-human animal) infected by a microorganism (*e.g.*, a species of infectious bacteria) may be administered a drug compound (*e.g.*, an antibiotic effective in treating the bacterial infection) according to an established dosing regimen (*e.g.*, 2-4 times daily, preferably equal interval oral, *i.v.*, or *i.m.* administration). The initial microorganism load at the site of infection (*e.g.*, CFU count in the blood of the patient if it is a systemic infection) may be determined at the beginning of the treatment, when the first dose of the drug compound is administered according to the dosing regimen. This initial load serves as N_0 in Equation (III). Since the PK/PD profile of the drug in the given host is usually known or can be readily predicted or measured (such as periodic measurement of drug concentration within the host system), Equation (III) may be used to determine at what point in time microorganism titer falls below a pre-determined safe level, such as a reduction of 2-4 logs depending on the initial N_0 value. The determined time point may be the time to terminate treatment according to the dosing regimen.

For example, when a host or patient is following a fixed dose three times daily dosing regimen to treat a microorganism infection, the relevant PK/PD profile of the drug in the host (for example, one that reflects the profile at the site of infection) can be readily determined or predicted or estimated. Based on the PK/PD profile and the associated intracellular drug concentration $[I]$, Equation (III) can be used to determine microorganism titer N based on a successive series of calculations for a series of small time intervals, during which $[I]$ can be considered substantially invariable. Once the determined N is low enough, *e.g.*, 2-4 logs lower than the starting titer N_0 , several days into the dosing regimen, treatment of infection can be considered complete, and the host or patient no longer needs to follow the dosing regimen from that point on.

This method is useful, for example, for treating infections in which obvious symptoms of the infection disappear long before a safely low microorganism titer is reached, such that dosing regimens should still be rigorously followed for an additional period of time even after the first disappearance of certain symptoms, but for no longer than necessary.

In certain embodiments, the microorganism is a bacterium, such as *P. aeruginosa*, *E. coli*,

K. pneumonia, *A. baumannii*, *M. tuberculosis*, *S. aureus*, *S. pneumonia*, *H. influenzae*, *Citrobacter spp.*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *S. marcescens*, *A. baumannii*, and *S. maltophilia*.

In certain embodiments, the bacterium is *P. aeruginosa*, *E. coli*, *K. pneumonia*, *A. baumannii*, *M. tuberculosis*, *S. aureus*, *S. pneumonia*, or *H. influenzae*.

In certain embodiments, the infection is systemic, such as sepsis. In other embodiment, the infection is local, *e.g.*, limited to local sites, such as cuts and wounds limited to local tissues.

In certain embodiments, the subject is a human, or a non-human mammal (*e.g.*, a mammal; a primate; a livestock animal including beef cattle, dairy cow, water buffalo, bison, yak, camel, llama, pig, horse, donkey, mule, sheep, goat, deer, rabbit, poultry (chicken, duck, goose, turkey); a pet or a lab animal including cat, dog, mouse, rat, guinea pig, hamster, *etc.*).

In certain embodiments, the compound is an antibiotic, such as Ampicillin, Cloxacillin, Methicillin, Cefotaxime, Ceftriaxone, Moxalactam, Imipenem, or Meropenem; vancomycin, erythromycin, Gentamicin, Amikacin, Tobramycin, Rifampin, Trimethoprim, Ciprofloxacin, and any other antibiotics known to have PAEs.

In certain embodiments, the antibiotic exhibits a post-antibiotic effect (PAE) in treating infection by the microorganism.

In certain embodiments, k_6 is less than about 10 hr^{-1} , 5 hr^{-1} , 3 hr^{-1} , 1 hr^{-1} , 0.5 hr^{-1} , 0.3 hr^{-1} , 0.1 hr^{-1} or less. In other embodiments, k_6 is within the range between any of the two recited values above.

In certain embodiments, the series of successive intervals are of identical duration.

In related embodiments, at least two of the series of successive intervals are of different duration. The latter situation may be helpful in that narrower intervals may be beneficial for a steep increase or decrease in [I], while wider intervals may be sufficient for a shallow or gradual increase or decrease in [I].

In certain embodiments, [I] is calculated / determined based on the PK or PK/PD profile of the compound. Alternatively, [I] may be based on direct measurement of systemic inhibitor compound concentration, which is in turn converted to [I] for use in Equation (III).

Alternatively, in a related embodiment, the invention provides a method of treating a

microorganism infection in a subject in need of treatment, the method comprising first determining a specific dosing regimen that would produce a desired treatment result based on calculations according to Equation (III), and then administering the drug to the host or patient according to the selected dosing regimen.

For example, for a specific microorganism infection, if it is desirable (*e.g.*, in a particular clinical situation) that microorganism titer drops over a course of 2 days to below a desired level (*e.g.*, 2-4 logs reduction compared to the starting titer N_0), Equation (III) may be used to screen a number of potential dosing regimens for treating the infection, in order to select the dosing regimen that possesses the optimal combination of fast reduction of microorganism titer and tolerable toxicity and/or side effects (due to, for example, the magnitude of the highest drug concentration in the host).

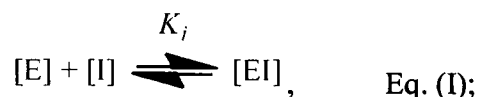
Thus in another aspect, the invention provides a method of screening or selecting for a dosing regimen for treating a microorganism infection in a subject in need of treatment, the method comprising:

- (1) providing a compound that binds to and inhibits an enzyme of the microorganism, and inhibits proliferation of the microorganism;
- (2) providing a candidate dosing regimen for administering the compound to the subject;
- (3) providing a time course of treatment beginning from the time the compound is first administered to the subject according to the dosing regimen;
- (4) dividing the time course of treatment into a series of successive intervals, wherein within each said intervals, compound concentration at a site of microorganism infection remains substantially constant (*e.g.*, less than 5% deviation from the average concentration);
- (5) beginning from the first of said series of successive intervals, and for each said series of successive intervals, constantly determining, according to Equation (III), microorganism titer N at the end of each said series of successive intervals, using microorganism titer N_0 at the beginning of each of said series of successive intervals, wherein N for one interval is N_0 for the immediate following interval; and,

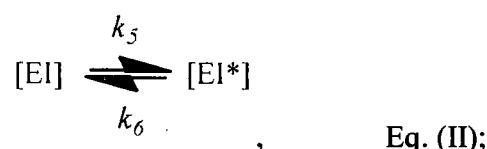
- (6) determining microorganism titer at the end of a pre-determined end point time, and comparing with a pre-determined end point titer, wherein the candidate dosing regimen is selected when the microorganism titer at the end of the pre-determined end point time is no more than the pre-determined end point titer;

wherein:

- (a) the compound binds to the enzyme *in vivo* to form a compound-enzyme complex ("EI"), with a binding constant K_i , according to Equation (I):



- (b) the compound-enzyme complex EI is in equilibrium with a tight compound-enzyme complex ("EI*"), with an on-rate constant k_5 and an off-rate constant k_6 , according to Equation (II):



- (c) the dosing regimen produces a pharmacokinetic (PK) profile of the compound in the subject (at the site of the microorganism infection);
- (d) Equation (III) is:

$$[N] = [N_0] \exp([\lambda + \varepsilon(\sigma - 1)]t + \varepsilon\rho \left(\frac{k_6}{2kb} - \frac{1}{2} \right) t^2 + \varepsilon \frac{\gamma}{k} (1 - e^{-kt}))$$

wherein:

N_0 is microorganism titer at the beginning of each said series of successive intervals;

N is microorganism titer at the end of each said series of successive intervals;

t is the duration of time for a given interval associated with N_0 ;

λ is intrinsic logarithmic growth rate of the microorganism;

ε is maximum kill rate of the microorganism by the compound;

ρ is replenishment rate of the enzyme;

$[I]$ is concentration of the compound at the site of enzyme function (e.g., inside the microorganism);

K_i is the binding constant defined in Eq. (I);

k_5 and k_6 are on- and off-rates defined in Eq. (II);

$$\beta = 1 + K_M / [S] + (K_M / [S]) \times ([I] / K_i);$$

$$k = k_6 + (k_5 / \beta) \times (K_M / [S]) \times ([I] / K_i);$$

$$\sigma = (k \times (k_6 + \rho) / \beta - k_6 \times \rho / \beta) / k^2;$$

K_M is the Michaelis-Menten constant of the enzyme;

$[S]$ is concentration of the substrate of the enzyme at the site of enzyme function (e.g., inside the microorganism);

for the first of said series of successive intervals, γ is a constant determined by Equation (IV):

$$\gamma = 1 / \beta + (k \times (k_6 + \rho) / \beta - k_6 \times \rho / \beta) / k^2, \quad \text{Eq. (IV);}$$

for each subsequent said series of successive intervals, γ is a constant determined by Equation (V):

$$\gamma = \frac{\beta_{old}[ES]_{end-of-last-interval}}{\beta_{new}} - \sigma_{new}, \quad \text{Eq. (V);}$$

wherein in Eq. (V):

β_{old} is β defined above for the immediate preceding interval;

β_{new} and σ_{new} are β and σ defined above for the current interval;

$[ES]_{end}$ is concentration of enzyme-substrate complex at the end of the immediate preceding interval, and is calculated according to Equation (VI), wherein $b = (k_6 + \rho) / \beta$; $h = k_6 \times \rho / \beta$; and γ is as defined above for the immediate preceding interval:

$$[ES] = \frac{bk-h}{k^2} + \frac{h}{k} t + \gamma e^{-kt}, \quad \text{Eq. (VI).}$$

In a related aspect, the invention provides a method of treating a microorganism infection in a subject in need of treatment, the method comprising: using the method immediately above to screen for and select a dosing regimen that produces the desired treatment (e.g., a 2-4 logs reduction in microorganism titer in relation to starting titer), and further comprising administering the compound to the host according to the selected dosing regimen.

Any of the specific embodiments described above apply to all aspects of the invention.

5. *Compound Screening / Design*

The invention described herein further provides a method for screening inhibitor compounds that may be used to achieve desired treatment time course and efficacy, while preferably avoiding or minimizing associated side effects or toxicological effects.

Related or unrelated inhibitor compounds may share similar biological functions, *e.g.*, inhibiting the function of an essential metabolic enzyme of the infectious microorganism. Each inhibitor may have different yet determinable K_i , k_5 , k_6 , PK/PD profile, and maximum kill rate ε . Given the relationship among the parameters in Equation (III), the time course of treatment using any of the several possible inhibitors can be determined based on Equation (III), and the results compared to identify the optimum balance between efficacy and side effects.

Alternatively, the invention also allows one to “design” a drug based on a series of fixed parameters in Equation (III), such that one or more remaining parameters can be calculated / determined accordingly, and compounds having such calculated / determined parameters can be designed or screened for. For example, if it is desired to administer an inhibitor compound to treat a microorganism infection according to a pre-determined dosing regimen and/or a pre-determined time course of treatment, certain parameters such as N_0 , end titer N , duration of treatment, intrinsic logarithmic growth rate of the microorganism (λ), replenishment rate of the enzyme (ρ), and concentration of the compound at the site of enzyme function (*e.g.*, inside the microorganism) can be fixed or pre-determined. Other parameters, such as K_i , k_5 , k_6 , and PK/PD profile, may be fitted using Equation (III), either collectively or individually. The results become screening criteria as to what the parameters for a “designer compound” should be, thus guiding future screening efforts for compounds meeting such design criteria.

In certain embodiments, some of the Equation (III) parameters that are related to an inherent property or characteristic of the inhibitor such as K_i , k_5 , and k_6 , as well as PK/PD profile, may also be fixed or pre-determined, and Equation (III) may be used to determine what the remaining parameters should be. This may be useful when a lead drug compound is identified, which lead compound may possess some but not all of the desired parameters (*e.g.*, the lead compound may have desired K_i and k_5 , but the value of k_6 for the lead compound is not ideal). Thus further designing or screening may be carried out to identify second generation compounds that possess some of the desired parameters.

6. *Methods of Efficacy Determination*

The invention described herein further provides a method for determining or predicting efficacy of a compound administered according to a set dosing regimen, in the context of treating a specific microorganism infection in a specific host. That is, whether the specific compound in question will be efficacious under a given dosing regimen for treating a given host for a given disease.

The invention described herein further provides a method for determining or predicting efficacy of a dosing regimen for a pre-determined compound, in the context of treating a specific microorganism infection in a specific host. That is, whether the specific dosing regimen in question for the selected compound will be efficacious for treating a given host for a given disease.

Likewise, the invention further provides a method for determining or predicting efficacy of a pre-determined compound used according to a pre-determined dosing regimen, for treating a specific host with a given initial microorganism load. That is, whether the host can be successfully treated by the compound under the dosing regimen, and if so, how long the treatment should be.

EXAMPLES

Example 1: LpxC Assay

Lipid A is the hydrophobic anchor of lipopolysaccharide (LPS), and forms the major lipid component of the outer monolayer of the outer membrane of Gram-negative bacteria, such as *Escherichia coli*. Lipid A is required for bacterial growth and virulence, and inhibition of its biosynthesis is lethal to bacteria. UDP-3-*O*-(*R*-3-hydroxymyristoyl)-*N*-acetylglucosamine deacetylase (LpxC) is a metalloenzyme that catalyzes the second step in the biosynthesis of lipid A. Consequently, inhibitors of LpxC have been shown to have antibiotic activities, and can be the inhibitor compound of the invention with respect to the essential metabolic enzyme target LpxC.

The LpxC enzyme assay described herein may be used to determine enzyme (LpxC) activity under various experimental conditions, such as with or without the presence of significant inhibitors. More specifically, inhibitor binding kinetics can be measured using an LC/MS based assay of product formation.

Briefly, at the beginning of the experiment, a given amount of the enzyme and the inhibitor are incubated for a period of time at appropriate temperature (*e.g.*, 2 hrs at room temperature) to allow the formation of enzyme-inhibitor complex EI and EI*. Then an aliquot of the EI/EI* complex is rapidly diluted with excess substrate, such as a 1000-fold dilution with excess substrate S, such that substantially all enzymes in the EI complex (which are in fast equilibrium with free form enzyme E) becomes free form enzyme available to convert the substrate to the product P. The speed with which product P is produced, as measurable by LC/MS, is indicative of the amount of free form enzyme in the reaction mixture. Meanwhile, if there is any enzyme locked in the EI* complex, and thus not available as free form enzyme in the 1000-fold dilution mixture, the amount of product P is reduced accordingly. See Example 2.

All LpxC enzyme assays were performed using a buffer containing 50 mM NaH₂PO₄ pH 7.4, 0.2 mg/ml Bovine serum albumin (BSA), and 0.5 M sucrose. The LpxC substrate used was typically UDP-3-*O*-(*R*-3-decanoyl)-*N*-acetylglucosamine. A limited number of assays with *E. coli* LpxC alternately used the 3-Hydroxymyristoyl version of this substrate.

Wild type *E. coli* and *P. aeruginosa* LpxC were prepared following procedures similar to those published previously (Barb *et al.*, Biochemistry 46: 3793-3802, 2007; and Coggins *et al.*, Biochemistry 44, 1114-1126, 2005). Deacetylated product was detected and quantified by LC/MS/MS using an AB/Sciex 4000 triple quadrupole mass spectrometer interfaced with a Shimadzu LC20 HPLC system. Assay samples were subjected to HPLC using a buffer system comprising 10 mM ammonium acetate, pH 6.5 (Buffer A), with reaction product eluting over a 1 minute gradient from 5% to 90% acetonitrile (Buffer B) at a flow rate of 0.5 ml/minute. Deacetylated product was detected by LC/MS/MS using an AB/Sciex 4000 triple quadrupole mass spectrometer. The mass spectrometer was operated in negative ion, MRM mode where quantification was accomplished by monitoring peak areas corresponding to the parent ion to *m/z* 79 fragmentation reaction.

Example 2: Enzyme Activity Recovery Assay

To estimate the effective k_{off} (or k_6) of compounds from *P. aeruginosa* or *E. coli* LpxC, enzyme was incubated with a slight excess of compound (typically 50 nM compound with 30 nM enzyme) for 3 hours, then the resultant enzyme-inhibitor complex was diluted 1000-fold in 2 steps. Compounds for incubation were diluted from 10 mM to 10 μ M in $10 \times$ steps in fresh DMSO, then diluted to 1 μ M in 1:1 DMSO:assay buffer prior to incubation with enzyme. Enzyme was diluted to 32 nM in assay buffer, after which compound was added to a final concentration of 50 nM. After incubation for 3 hours to allow formation of the enzyme-inhibitor complex, samples were diluted 20-fold in assay buffer, followed by another 50-fold dilution in buffer containing 25 μ M LpxC substrate. Reaction aliquots were collected at measured time intervals following dilution, quenched with a 2-fold volume of 2% acetic acid, and assayed by LC/MS/MS.

To estimate the enzyme recovery rate constant, product formation as a function of time were fit using Excel/XLFit to equation 1, where V_0 was set to 0 (no background product accumulation was observed) and V_s was set to the linear enzyme velocity observed in the absence of inhibitor. The inverse of the k value obtained, multiplied by the natural logarithm of 2, was taken as the residence time, $t_{1/2}$.

Equation 1

$$P = v_s t + (v_0 - v_s)(1 - e^{-kt}) / k$$

K_i^* and K_i Estimation. Estimation of K_i^* and K_i was accomplished using an IC_{50} assay format using respective long or short pre-incubation of compounds with enzyme.

To measure K_i^* , compounds were titrated to achieve final assay concentrations from 20 nM to 39 pM in a 2-fold dilution series. Compounds were incubated overnight (18 hours) in assay buffer containing 30 pM *E. coli* or *P. aeruginosa* LpxC. Reactions were then initiated by addition of substrate to reach final substrate and enzyme concentrations of 5 μ M and 15 pM respectively. Reactions proceeded for 60 minutes before quenching with 2-fold volume of 2% acetic acid and quantification of reaction product by LC/MS/MS. Positive and negative inhibition controls used N-[(1S,2R)-2-hydroxy-1-(hydroxycarbamoyl)propyl]-4-[2-[4-(morpholinomethyl)phenyl]ethynyl]benzamide (10 μ M) and DMSO (2% final) respectively. An IC_{50} was calculated (Excel/XLFit - 4 parameter dose-response model) from the measured percent inhibition as a function of inhibitor concentration.

For K_i estimation, an IC_{50} assay format was used where reactions were run for a minimum length of time with no enzyme pre-incubation. In these assays compounds were titrated to reach assay concentrations from 40 nM to 78 pM. Compounds were first pre-incubated for 20 minutes in assay buffer containing 10 μ M substrate, then reactions were initiated with enzyme to reach final enzyme and substrate concentrations of 100 pM and 5 μ M respectively. Reactions were run for 3 minutes, quenched with a 2-fold volume of 2% acetic acid, and assayed by LC/MS/MS.

An IC_{50} was estimated from percent inhibition as a function of inhibitor concentration and taken as K_i .

Example 3: Killing Kinetics Assay

Media for each bacteria species used in the killing kinetics assay were prepared based on Table 1 below. Culture conditions for each bacteria species are also listed.

Table 1

Bacteria	Media Requirements		Incubation	
	Liquid media	Solid media	Temperature	Environment
<i>E. coli</i>	MHBII	Blood agar	37 °C	O ₂
<i>H. influenzae</i>	HTM	Chocolate Blood agar	37 °C	5% CO ₂
<i>K. pneumoniae</i>	MHBII	Blood agar	37 °C	O ₂
<i>P. aeruginosa</i>	MHBII	Blood agar	37 °C	O ₂
<i>S. aureus</i>	MHBII	Blood agar	37 °C	O ₂
<i>S. pyogenes</i>	2.5% LHB	Blood agar	37 °C	5% CO ₂
<i>S. pneumoniae</i>	2.5% LHB	Blood agar	37 °C	5% CO ₂

Two days before the assay, bacteria were streaked / inoculated onto appropriate agar plates, which were then incubated overnight at about 37 °C, with or without CO₂, depending on bacterial strain used (Chart 1).

One day before the assay, a 6-well plate with 6 mLs of the bacteria-specific media per well was prepared. The bacteria was inoculated in the 1st well, and serial dilutions were made by taking and mixing an aliquot of about 20-50 μ L from the 1st well to the 2nd well, and repeating

the process for each of the remaining wells (*e.g.*, an aliquot of about 20-50 μL was transferred from the 2nd well to the 3rd well, and mixed, *etc*). The 6-well plate was then incubated overnight at 37 °C (with or without CO_2). Meanwhile, several flasks were each filled with 200 mL of the bacteria-specific media and warmed overnight at 37 °C. Serial dilution plates for each time point were also prepared.

On the day of the assay, bacterial growth in each well were determined by measuring OD_{600} , and wells with bacterial OD_{600} of about 0.3-0.6 were selected for the assay. The selected culture was first diluted to a predicted 0.001 OD_{600} in the pre-warmed 200 mL flask with appropriate media, and 20 mL of the diluted culture were transferred to 7 flasks, one for each concentration of the compound. The compounds were then added in appropriate volume to achieve the desired multitudes of the MIC concentration within the flask. For convenience, seven compound concentrations were tested, *e.g.*, 0 $\times$, 0.5 $\times$, 1 $\times$, 2 $\times$, 4 $\times$, 8 $\times$, and 16 $\times$ of MIC. See Table 2. For the negative control (0 $\times$), the solvent DMSO was added in the same amount (200 μL) as the highest compound volume.

The culture and the various concentrations of compounds were incubated for a pre-determined amount of time to allow the formation of any enzyme-inhibitor complex.

Table 2

MIC values	Linezolid ($\mu\text{g/mL}$)
Sau516	2

Linezolid Concentration	Flask concentration ($\mu\text{g/mL}$)	3.2 mg/mL cmp added to 20 mL culture
0 $\times$	0	200 μL DMSO
1/2 $\times$	1	6.25 μL
1 $\times$	2	12.5 μL
2 $\times$	4	25 μL
4 $\times$	8	50 μL
8 $\times$	16	100 μL
16 $\times$	32	200 μL

Once the pre-determined incubation period was over for a specific time point, for each flask (compound concentration), aliquots were added into column 1 of the dilution plate according to Chart #3, and successive 10-fold dilutions were made according to Table 3. Once all the flask cultures have been added to the dilution plate, the flasks were returned to the shaker in the 37 °C incubator (with or without CO₂ depending on the strain) until the next time point, at which time the above process was repeated until all the time points were covered.

Table 3: Culture Dilution Plate Setup

		Culture Serial Dilutions											
		U -1 -2 -3 -4 -5 -6 -7											
Compound Dilutions		1	2	3	4	5	6	7	8	9	10	11	12
	0×	A											
	1/2×	B											
	1×	C											
	2×	D											
	4×	E											
	8×	F											
	16×	G											
		H											

U = Undiluted

For the bacterial culture in the dilution plates, aliquots of between 5-100 µL were plated on agar plates for each compound concentration at each dilution, and the plates were kept at 37 °C overnight (with or without CO₂ depending on the strain).

On the next day, bacterial growth in each plate was determined by counting CFU/mL, and the data was used to plot growth curve under each condition (compound concentrations over different time points). See Table 4, which shows a representative result for one of the test compounds with about 3 logs of killing in about 6 hours.

Table 4

Averages

Time Point:	0	2 hours	4 hours	6 hours	8 hours	24 hours
0×	3.00E+06	1.40E+07	2.10E+08	1.80E+09	4.20E+09	1.10E+10
1/2×	3.10E+06	5.10E+06	4.80E+06	7.50E+06	1.00E+07	1.80E+09
1×	2.90E+06	4.20E+06	3.90E+06	4.70E+06	6.90E+06	3.50E+07
2×	3.70E+06	3.40E+06	3.70E+06	3.30E+06	2.40E+06	8.50E+05
4×	3.20E+06	3.20E+06	2.40E+06	2.10E+06	1.50E+06	1.00E+06
8×	3.30E+06	3.20E+06	3.60E+06	2.30E+06	1.90E+06	3.10E+05
16×	3.90E+06	3.30E+06	3.20E+06	2.10E+06	1.50E+06	3.40E+05

CLAIMS

We claim:

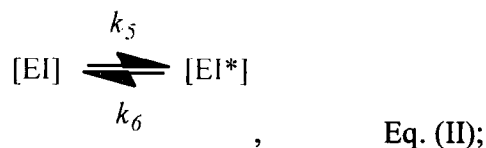
1. A method of treating a microorganism infection in a subject in need of treatment, the method comprises:
 - (1) providing a compound that binds to and inhibits an enzyme of the microorganism, thereby inhibiting proliferation of the microorganism;
 - (2) providing a dosing regimen for administering the compound to the subject;
 - (3) administering the compound to the subject according to the dosing regimen, and initiating a time course of treatment;
 - (4) dividing the time course of treatment into a series of successive intervals, wherein within each said interval, compound concentration at a site of microorganism infection remains substantially constant;
 - (5) beginning from the first of said series of successive intervals, and for each said series of successive intervals, constantly determining, according to Equation (III), microorganism titer N at the end of each said series of successive intervals, using microorganism titer N_0 at the beginning of each said series of successive intervals, wherein N for one interval is N_0 for the immediate following interval; and,
 - (6) terminating treatment according to the dosing regimen when microorganism titer N at a given interval falls below a pre-determined / target microorganism titer, thereby treating the microorganism infection;

wherein:

- (a) the compound binds to the enzyme *in vivo* to form a compound-enzyme complex ("EI"), with a binding constant K_i , according to Equation (I):



- (b) the compound-enzyme complex EI is in equilibrium with a tight compound-enzyme complex ("EI*"), with an on-rate constant k_5 and an off-rate constant k_6 , according to Equation (II):



- (c) the dosing regimen produces a pharmacokinetic (PK) profile of the compound in the subject;
- (d) Equation (III) is:

$$[N] = [N_0] \exp([\lambda + \varepsilon(\sigma - 1)]t + \varepsilon\rho \left(\frac{k_6}{2kb} - \frac{1}{2} \right) t^2 + \varepsilon \frac{\gamma}{k} (1 - e^{-kt}))$$

wherein:

N_0 is microorganism titer at the beginning of each said series of successive intervals;

N is microorganism titer at the end of each said series of successive intervals;

t is the duration of time for a given interval associated with N_0 ;

λ is intrinsic logarithmic growth rate of the microorganism;

ε is maximum kill rate of the microorganism by the compound;

ρ is replenishment rate of the enzyme;

$[I]$ is concentration of the compound at the site of enzyme function (*e.g.*, inside the microorganism);

K_i is the binding constant defined in Eq. (I);

k_5 and k_6 are on- and off-rates defined in Eq. (II);

$\beta = 1 + K_M / [S] + (K_M / [S]) \times ([I] / K_i)$;

$k = k_6 + (k_5 / \beta) \times (K_M / [S]) \times ([I] / K_i)$;

$\sigma = (k \times (k_6 + \rho) / \beta - k_6 \times \rho / \beta) / k^2$;

K_M is the Michaelis-Menten constant of the enzyme;

$[S]$ is concentration of the substrate of the enzyme at the site of enzyme function (*e.g.*, inside the microorganism);

for the first of said series of successive intervals, γ is a constant determined by Equation (IV):

$$\gamma = 1 / \beta + (k \times (k_6 + \rho) / \beta - k_6 \times \rho / \beta) / k^2, \quad \text{Eq. (IV);}$$

for each subsequent said series of successive intervals, γ is a constant determined by Equation (V):

$$\gamma = \frac{\beta_{old}[ES]_{end-of-last-interval}}{\beta_{new}} - \sigma_{new}, \quad \text{Eq. (V);}$$

wherein in Eq. (V):

β_{old} is β defined above for the immediate preceding interval;

β_{new} and σ_{new} are β and σ defined above for the current interval;

$[ES]_{end}$ is concentration of enzyme-substrate complex at the end of the immediate preceding interval, and is calculated according to Equation (VI), wherein $b = (k_6 + \rho) / \beta$; $h = k_6 \times \rho / \beta$; and γ is as defined above for the immediate preceding interval:

$$[ES] = \frac{bk-h}{k^2} + \frac{h}{k} t + \gamma e^{-kt}, \quad \text{Eq. (VI).}$$

2. The method of claim 1, wherein the microorganism is a bacterium.
3. The method of claim 2, wherein the bacterium is *P. aeruginosa*, *E. coli*, *K. pneumonia*, *A. baumannii*, *M. tuberculosis*, *S. aureus*, *S. pneumonia*, or *H. influenzae*.
4. The method of claim 1, wherein the infection is systemic.
5. The method of claim 1, wherein the infection is local.
6. The method of claim 1, wherein the subject is a human, or a non-human mammal (*e.g.*, a livestock animal, a pet, a lab animal *etc.*).
7. The method of claim 1, wherein the compound is an antibiotic.
8. The method of claim 7, wherein the antibiotic is Ampicillin, Cloxacillin, Methicillin, Cefotaxime, Ceftriaxone, Moxalactam, Imipenem, or Meropenem; vancomycin, erythromycin, Gentamicin, Amikacin, Tobramycin, Rifampin, Trimethoprim, or Ciprofloxacin.
9. The method of claim 7, wherein the antibiotic exhibits a post-antibiotic effect (PAE) in treating infection by the microorganism.
10. The method of claim 1, wherein k_6 is less than about 1 hr^{-1} .

11. The method of claim 1, wherein said series of successive intervals are of identical duration.
12. The method of claim 1, wherein at least two of said series of successive intervals are of different duration.
13. The method of claim 1, wherein [I] is calculated / determined based on the PK profile of the compound.

FIG. 1

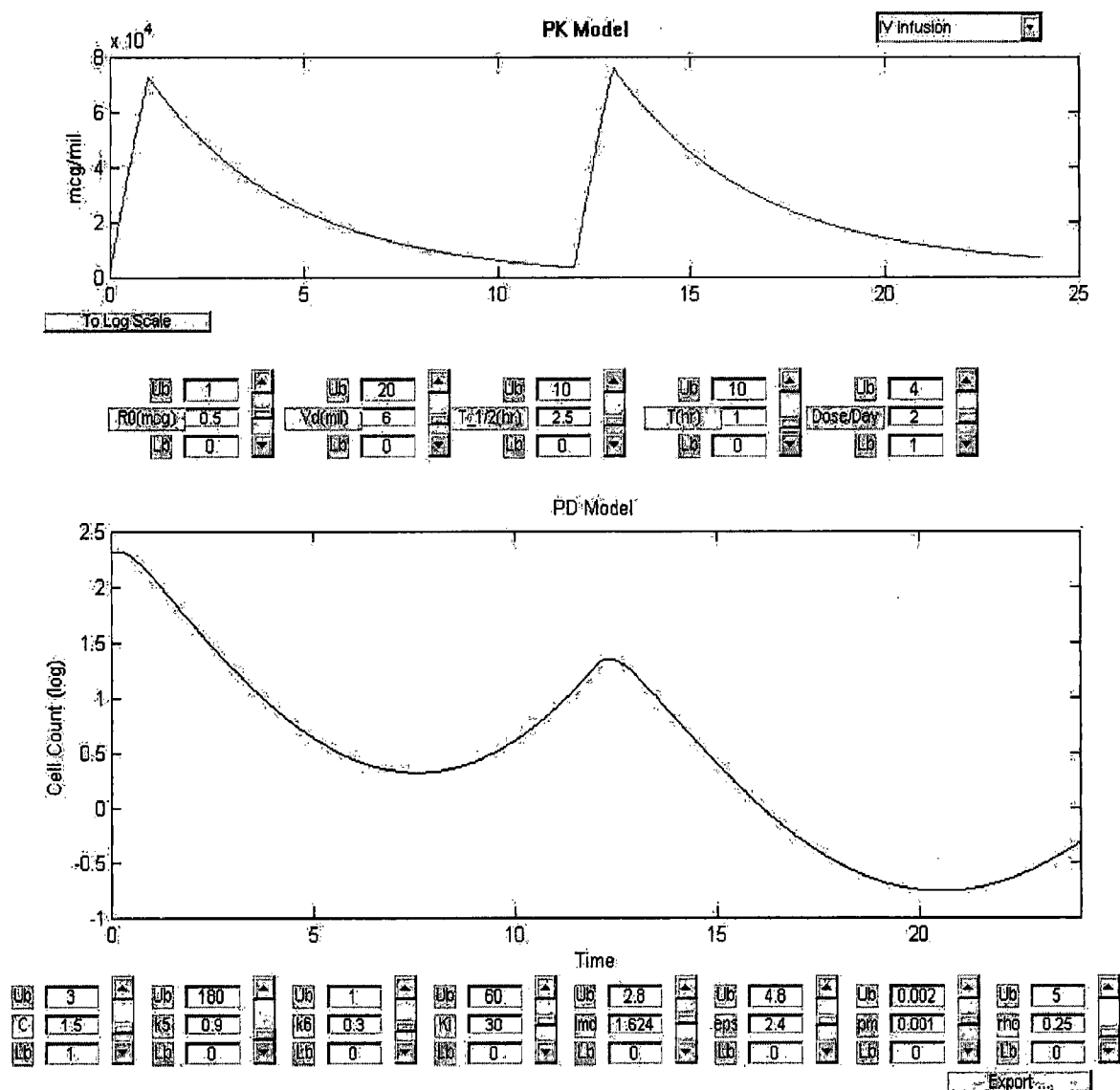
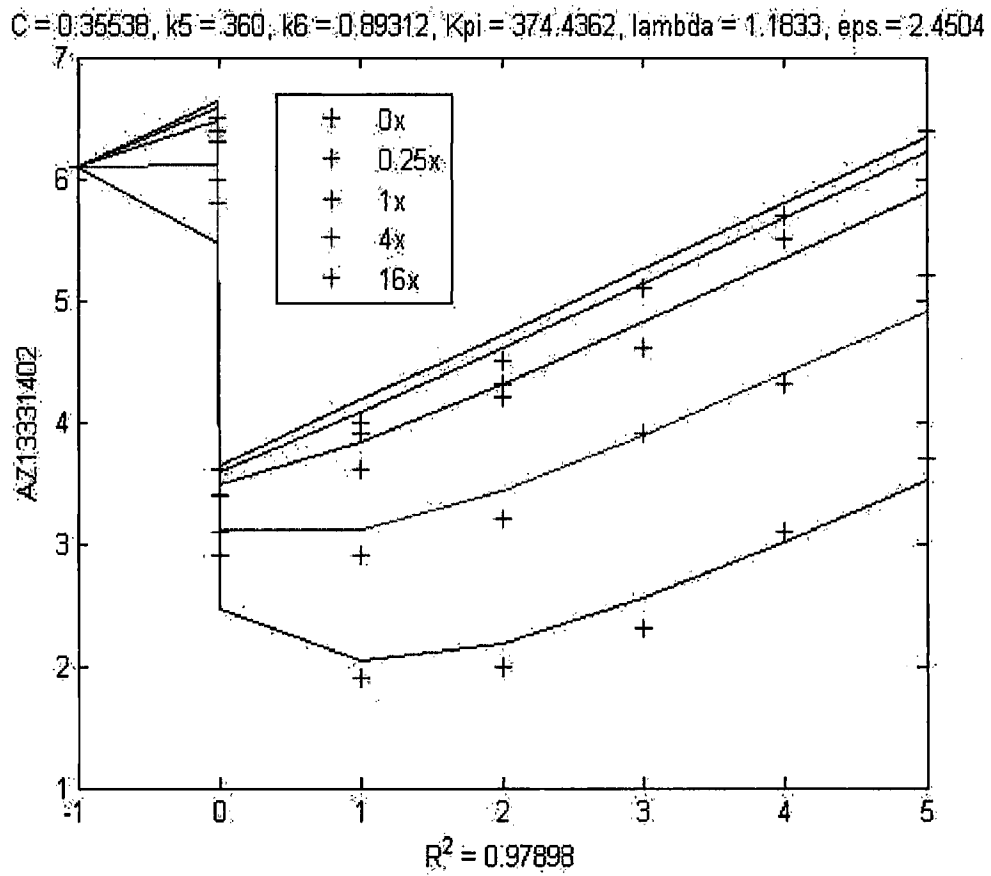


FIG. 2



INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2013/051850

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/00 A61P31/00
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

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, INSPEC, COMPENDEX, 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	<p>TUNKEL ALLAN R ET AL: "Practice guidelines for the management of bacterial meningitis", CLINICAL INFECTIOUS DISEASES, vol. 39, no. 9, 1 November 2004 (2004-11-01), pages 1267-1284, XP002715683, ISSN: 1058-4838 in particular Table 8; tables</p> <p style="text-align: center;">----- -/--</p>	1-13



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

6 November 2013

Date of mailing of the international search report

26/11/2013

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INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2013/051850

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WARREN JOHN W ET AL: "Guidelines for antimicrobial treatment of uncomplicated acute bacterial cystitis and acute pyelonephritis in women", CLINICAL INFECTIOUS DISEASES, vol. 29, no. 4, October 1999 (1999-10), pages 745-758, XP002715684, ISSN: 1058-4838 tables	1-13
X	----- SCHAUB NORA ET AL: "Addressing unmet clinical needs in the early diagnosis of sepsis", SWISS MEDICAL WEEKLY, vol. 141, July 2011 (2011-07), XP002715685, the whole document	1-13
X	----- VIOLA LUIGI ET AL: "Serious Pseudomonas aeruginosa infection in healthy children: case report and review of the literature", PEDIATRICS INTERNATIONAL, vol. 48, no. 3, June 2006 (2006-06), pages 330-333, XP002715686, ISSN: 1328-8067 the whole document -----	1-13