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(54) Title: METHOD FOR TREATING INFECTIONS
(57) Abstract: Methods and compositions for detecting, treating, characterizing, and diagnosing infections are described where the composition includes a leukotriene receptor antagonist compound or a pharmaceutically acceptable salt thereof.
TITLE
Method for Treating Infections

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CROSS-REFERENCE TO RELATED APPLICATIONS
[0001] This application claims the benefit of United States Provisional Application Ser. No. 61/545,362 filed October 10, 2011, the entire disclosure of which is expressly incorporated herein by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH
[0002] The invention was made with U.S. Government support under Grant Number 1R15AI089653-01 awarded by the National Institute of Health. The United States Government has certain rights in the invention.

SEQUENCE LISTING
[0003] The instant application contains a Sequence Listing which has been submitted via EFS-web and is hereby incorporated by reference in its entirety. The ASCII copy, created on September 26, 2012, is named 420_53355_SEQ_LIST_D2011-062.txt, and is 488 bytes in size.

TECHNICAL FIELD AND INDUSTRIAL APPLICABILITY OF THE INVENTION
[0004] The present invention relates to methods and compositions for treating, controlling, reducing or inhibiting a bacterial infection in a subject in need thereof. The method includes: administering to the subject an effective amount of a composition comprising a leukotriene receptor antagonist compound or a pharmaceutically acceptable salt thereof.

BACKGROUND OF THE INVENTION
[0005] According to the World Health Organization, 1/3 of the world's population is infected with the bacteria that causes tuberculosis (TB). It is estimated that billions of people around the globe are carriers of TB but do not exhibit overt symptoms of infection. TB is spread through the air and usually attacks the lungs, but may attack other areas of the body as well.
[0006] The typical treatment for TB comes in the form of antibiotics, however, due to the nature and structure of the cell effective TB treatment is difficult. Additionally, drug resistant strains are increasingly common. The standard treatment for tuberculosis is a six-month regimen consisting of four drugs given for two months, followed by two drugs given for four months. Two drugs, given throughout the six-month course of therapy, are isoniazid and rifampin. Although the regimen is straightforward, its administration is quite complicated. Daily ingestion
of eight or nine pills is often required during the first phase of therapy. If the treatment is not continued to completion the patient may experience a relapse, and the relapse rate for patients who do not continue treatment to completion is high.

[0007] What is needed is an effective therapeutic regimen that includes improved treatment protocols. Currently available therapeutics are no longer consistently effective as a result of the problems with treatment compliance, and these compliance problems contribute to the development of drug resistant mycobacterial strains.

[0008] In addition, there are a number of other human and animal diseases caused by mycobacteria, including for example leprosy (Hansen's disease), lymphadenitis, a variety of pulmonary and skin diseases, and wound infection. Although less prevalent, each of these diseases is associated with morbidity, mortality and economic costs such as lost production time and the cost of medical treatment. Resistance to drugs used heretofore to control and treat such diseases is also a current problem, thus raising a further need in this art for more effective drugs against many different Mycobacterium species.

[0009] Because of these new drug resistant strains, the development of new drugs possessing novel anti-tubercular properties are necessary.

[0010] There is also a need in the art for more effective anti-tuberculosis drugs to which M. tuberculosis is not resistant and, most advantageously, drugs having a low resistance development potential.

SUMMARY OF THE INVENTION

[0011] In a first broad aspect, there is provided herein a method for treating, controlling, reducing or inhibiting a bacterial infection in a subject in need thereof. The method includes: administering to the subject an effective amount of a composition comprising a leukotriene receptor antagonist compound or a pharmaceutically acceptable salt thereof.

[0012] In certain embodiments, the leukotriene receptor antagonist compound comprises one or more of:

1. ![Chemical Structure 1]
2. ![Chemical Structure 2]
3. ![Chemical Structure 3]
4. ![Chemical Structure 4]
In certain embodiments, the leukotriene receptor antagonist compound comprises zafirlukast or a pharmacologically active derivative thereof.

In certain embodiments, the leukotriene receptor antagonist compound comprises a metabolite of zafirlukast, or a pharmacologically active derivative thereof. Non-limiting examples include:


2. N-[4-(5-Amino-1-methyl-1H-indol-3-ylmethyl)-3-methoxybenzoyl]-2-methylbenzenesulfonamide.


6. [3-[2-Methoxy-4-(toluene-2-sulfonylaminocarbonyl) benzyl]-l-methyl-1H-indol-5-yl] carbamic acid hydroxycyclopentyl ester.

7. [3-[2-Methoxy-4-(toluene-2-sulfonylaminocarbonyl) benzyl]-1H-indol-5-yl] carbamic acid hydroxycyclopentyl ester.


[0015] In certain embodiments, the infection is a chronic bacterial infection.

[0016] In certain embodiments, the chronic bacterial infection is a mycobacterial infection.

[0017] In certain embodiments, the mycobacterial infection is caused by M. tuberculosis, M. avium-intracellulare, M. kansasii, M. fortuitum, M. chelonae, M. leprae, M. africanum, M. bovis, M. avium, M. microti, M. avium paratuberculosis, M. intracellulare, M. scrofulaceum, M. xenopi, M. marinur or M. ulcerans.

[0018] In certain embodiments, the infection is caused Corynebacterium diphtheriae and Corynebacterium pseudotuberculosis as well as other Corynebacterium sp, including, but not limited to: species Corynebacterium diphtheriae and the nondiphtherial corynebacteria, collectively referred to as diphtheroids; Corynebacterium haemolyticum (Arcanobacterium haemolyticum); Corynebacterium jeikeium; Corynebacterium glutamicum; Corynebacterium pseudodiphtheriticum.

[0019] It is understood that the present invention contemplates treatment instead of and/or in addition to the Current treatment of an infected individual who had not been previously immunized for Corynebacterium infection, which includes treatment with antitoxin produced against the diptheria toxin plus penicillin or erythromycin.

[0020] In certain embodiments, the subject is a mammal. And, in certain embodiments, the mammal is a human.

[0021] In certain embodiments, the subject is a member selected from a human, cattle, goat, pig, sheep, horse, cow, bull, dog, guinea pig, gerbil, rabbit, cat, chicken and turkey.

[0022] In certain embodiments, the effective amount is administered topically, orally, peritoneally, subcutaneously, intramuscularly, intracocularly, intraarterially, intravenously, or locally using an implantable dosage unit.

[0023] In certain embodiments, the compound is administered as a solid, liquid or aerosol.
In certain embodiments, the solid is a pill or an implantable dosage unit.

In certain embodiments, the aerosol comprises an inhaler formulation.

In certain embodiments, the solid, liquid or aerosol comprises a sustained release matrix.

In certain embodiments, the effective amount of the compound comprises from 100 to 0.1 mg per kg of body weight. In certain embodiments, the effective amount of the compound comprises from 50 to 0.2 mg per kg of body weight. In certain embodiments, the effective amount of the compound comprises from 25 to 0.5 mg per kg of body weight. In certain embodiments, the effective amount of the compound comprises from 1 to 1000 mg.

In another broad aspect, there is provided herein a method of inhibiting growth of bacteria, comprising: exposing bacteria to an agent that disrupts inhibits binding of Lsr2 protein to DNA under conditions effective to inhibit growth of the bacteria.

In another broad aspect, there is provided herein a method of treating a bacterial infection in a subject. The method generally comprises: administering to the subject an agent that inhibits binding of Lsr2 protein to DNA under conditions effective to treat the bacterial infection.

In certain embodiments, the method can comprise selecting a subject having a bacterial infection prior to said administering.

In certain embodiments, the agent is administered in combination with one or more antibacterial agents.

In certain embodiments, the one or more antibacterial agents are selected from the group consisting of isoniazid, rifampin, rifabutin, rifapentine, pyrazinamide, ethambutol, kanamycin, erythromycin or other aminoglycoside antibiotics.

In another broad aspect, there is provided herein a method of identifying a compound that inhibits binding of Lsr2 protein to DNA of a pathogen. The method generally comprises:

- providing one or more candidate compounds;
- providing a pathogen, wherein the pathogen comprises a detectable indicator of binding of Lsr2 protein to DNA;
- contacting the pathogen with the one or more candidate compounds; and
- detecting the binding of Lsr2 protein to DNA of the pathogen,

wherein detecting a change in the binding of Lsr2 protein to DNA in the presence of a candidate compound identifies a compound that interferes with the binding of Lsr2 protein to DNA of the pathogen.

In certain embodiments, the pathogen is bacterium. Further, in certain embodiments, the bacterium is Mycobacterium. And, in certain embodiments, the Mycobacterium is Mycobacterium tuberculosis.

In another broad aspect, there is provided herein a method of killing a tuberculosis-
causing microorganism infecting a mammalian cell. The method generally comprises: contacting said cell with a composition comprising a leukotriene receptor antagonist compound or a pharmaceutically acceptable salt thereof.

[0036] In another broad aspect, there is provided herein a method of treating an infection in an animal. The method generally comprises: administering to the animal a therapeutically effective amount of a leukotriene receptor antagonist compound, or a pharmaceutically acceptable salt thereof, sufficient to treat said infection.

[0037] In another broad aspect, there is provided herein a method of treating an animal infected with a disease-causing microorganism of a Mycobacterium species. The method generally comprises: administering to the animal a therapeutically effective amount of a pharmaceutical composition comprising a leukotriene receptor antagonist compound or a pharmaceutically acceptable salt thereof.

[0038] In another broad aspect, there is provided herein a method of killing a microorganism infecting a mammalian cell. The method generally comprises: contacting said cell with the composition comprising a leukotriene receptor antagonist compound or a pharmaceutically acceptable salt thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0039] The patent or application file may contain one or more drawings executed in color and/or one or more photographs. Copies of this patent or patent application publication with color drawing(s) and/or photograph(s) will be provided by the Patent Office upon request and payment of the necessary fee.

[0040] Figure 1. Zafirlukast inhibits Lsr2/DNA complexation in a dose-dependent manner. Increasing amounts of Zafirlukast were titrated into a solution containing a complex of Lsr2 and fluorescently labeled DNA stem-loop. As the concentration of Zafirlukast is increased a decrease in polarization signal is observed indicating a decrease in Lsr2/DNA complex. Analysis of this data indicates a K_i of 2.57 μM ± 0.002 (R^2 = 0.960).

[0041] Figure 2. Kirby-Bauer disk diffusion assay showing growth inhibition of M. smegmatis. Increasing amounts of Zafirlukast were impregnated onto wafers and applied to an agar plate inoculated with M. smegmatis. The sizes of the zones of inhibition are directly proportional to the amount of Zafirlukast in the disk.

[0042] Figure 3. RT-PCR. These experiments exhibit changes in gene expression as a consequence of administering a sublethal concentration of Zafirlukast to a culture of M. tuberculosis. Five genes were chosen to give broad coverage of different classes of genes encoded by M. tb. The sigA gene is a housekeeping gene, sodA is a virulence factor, otsA and fpbC are important for bacterial metabolism and division, and the lsr2 gene that encodes the Lsr2
protein. The only gene whose expression does not increase as a result of administering Zafirlukast was sigA. In particular, the expression of the lsr2 gene has increased 8.8 fold; to compensate for the decreased ability of the Lsr2 protein to bind DNA in the presence of the drug.

[0043] **Figure 4.** 1) Zafirlukast structure. 2) The hypothesized active pharmacophore. 3 & 4) Derivatives that can also offer specific binding to Lsr2.

[0044] **Figure 5.** A plot of polarization versus concentration of Lsr2 demonstrating an increase in polarization corresponding to the Lsr2/DNA complex formation. The concentration of Lsr2 was increased until saturation was reached. From fitting of the normalized data, the $K_d$ for the Lsr2-DNA complexation was determined to be $0.87 \mu M \pm 0.09$ ($R^2 0.961$).

[0045] **Figure 6.** The Kirby-Bauer disc diffusion assay with *E. coli* using wafers impregnated with Kanamycin, Ampicillin and Zafirlukast. Both Kanamycin and Ampicillin exhibited the anticipated zones of inhibition while Zafirlukast produces no zone of inhibition. The lack of promiscuity in prohibiting bacterial growth further supports that Zafirlukast is targeting Lsr2 as opposed to DNA.

[0046] **Figure 7.** Spectra from the Intensity Fading experiment comparing the relative ion intensities of Zafirlukast and Ritonavir. As the concentration of Lsr2 is increased from top to bottom, the relative intensity of Zafirlukast diminishes as compared to the internal standard (Ritonavir). The decrease in relative intensity is attributed to complexation between Lsr2 and Zafirlukast.

[0047] **Figure 8.** Plot of the intensity ratio of Zafirlukast (Z) over Ritonavir (R) versus the concentration of Lsr2. As the concentration of Lsr2 is increased a larger portion of the Zafirlukast is bound to the protein decreasing the amount of free ion to be ionized, thus decreasing the intensity of the Zafirlukast signal.

**DETAILED DESCRIPTION**

[0048] Throughout this disclosure, various publications, patents and published patent specifications are referenced by an identifying citation. The disclosures of these publications, patents and published patent specifications are hereby incorporated by reference into the present disclosure to more fully describe the state of the art to which this invention pertains.

[0049] As used herein, the term "tuberculosis" comprises disease states usually associated with infections caused by mycobacteria species comprising *M. tuberculosis* (*M. tb.*) complex. The term "tuberculosis" is also associated with mycobacterial infections caused by mycobacteria other than *M. tuberculosis* (MOTT). Other mycobacterial species include *M. avium-intracellulare, M. kansarii, M. fortuitum, M. chelonei, M. leprae, M. africanum, and M. microti, M. avium paratuberculosis, M. intracellulare, M. scrofulaceum, M. xenopi, M. marinum, M. ulcerans.*
In certain embodiments, the tuberculosis is multidrug-resistant tuberculosis (MDR tuberculosis) or extensively drug-resistant tuberculosis (XDR tuberculosis). As used herein, "MDR tuberculosis" means a form of tuberculosis that is resistant to two or more of the primary drugs (e.g., isoniazid and rifampicin) used for the treatment of tuberculosis.

The present invention is based, at least in part, on the discovery that Lsr2 binds more than 20% of the genes identified in the *M. tb* genome. Lsr2 is a nucleoid-associated protein found in *M. tb* as well as in other actinobacteria. Lsr2 appears to be functionally analogous to eukaryotic histone proteins as well as the H-NS nucleoid-associated proteins found in *E. coli* and related gammaproteobacteria. Lsr2 binds AT-rich DNA to promote DNA condensation and structural organization of the genome. While not wishing to be bound by theory, the inventors herein now believe that a consequence of this function is that Lsr2 binds promoter regions in the GC-rich mycobacterial genome and thereby acts as a global repressor of transcription.

In addition to identifying the *lsr2* gene as a target for Lsr2 binding, the inventors herein now show that many of the genes bound by Lsr2 are important for the ability of *M. tb* to respond to environmentally induced stress such as the presence of antibiotics, oxidative stress, production of virulence factors, and genes that affect cell proliferation and persistence.

Furthermore, DNA condensation and repression of global transcription by Lsr2 also suggest a role in the progression to latency by *M. tb*. Based on these previous studies, targeting Lsr2 with small molecule inhibitors of DNA binding could severely dysregulate a wide array of important *M. tb* genes, ultimately leading to unhealthy mycobacteria that are unable to control their metabolism and division, or are unable to mount defenses against the human immune system.

In one aspect, there is described herein a high-throughput assay useful to test small-molecule drug and drug-like compounds for their ability to prevent DNA binding by Lsr2. Using this assay, the inventors herein identified a compound that specifically prevents protein-DNA complexation.

The inventors herein now show that this inhibits growth of *M. smegmatis*, a common non-pathogenic surrogate for *M. tb* as well inhibiting the growth of *M. tb*.

In certain embodiments, the infection is caused *Corynebacterium diphtheriae* and Corynebacterium pseudotuberculosis as well as other Corynebacterium sp, including, but not limited to: species *Corynebacterium diphtheriae* and the nondiphtherial corynebacteria, collectively referred to as diphtheroids; *Corynebacterium haemolyticum* (Arcanobacterium haemolyticum); *Corynebacterium jeikeium*; *Corynebacterium glutamicum*; *Corynebacterium pseudodiphtheriticum*.

Nondiphtherial corynebacteria also cause chronic and subclinical diseases in domestic animals and can lead to significant economic losses for farmers. Examples of widespread and difficult-to-control infections include *Corynebacterium pseudotuberculosis*.
caseous lymphadenitis in sheep, goats, and alpacas; *C. pseudotuberculosis* ulcerative dermatitis in cattle; and urinary tract infections and mastitis (affecting milk production) in cattle due to infection with *Corynebacterium renale*, *Corynebacterium cystidis*, *Corynebacterium pilosum*, and *Corynebacterium bovis*. Specific pathogenic groups or species include the following: *Corynebacterium ulcerans*; *C. pseudotuberculosis* (also known as *Corynebacterium ovis*); *Corynebacterium pyogenes*; *A. haemolyticum*; *Corynebacterium aquaticum*; *C. pseudodiphtheriticum* (also known as *Corynebacterium hofmannii*; Group D2 (also known as *Corynebacterium urealyticum*); Group E; and *Cjeikeium* (ie, group JK).

[0058] **EXAMPLES**

[0059] The present invention is further defined in the following Examples, in which all parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. All publications, including patents and non-patent literature, referred to in this specification are expressly incorporated by reference. The following examples are intended to illustrate certain preferred embodiments of the invention and should not be interpreted to limit the scope of the invention as defined in the claims, unless so specified.

[0060] The value of the present invention can thus be seen by reference to the Examples herein.

[0061] **Example 1**

[0062] To aid in better understanding of Lsr2-DNA complexation and as a basis for identifying compounds that can modulate Lsr2 function, a fluorescence polarization (FP) assay was developed. The fluorescein-labeled DNA probe is an AT-rich stem-loop which binds Lsr2. To determine the $K_d$ of the interaction between Lsr2 and DNA, a series of binding experiments were performed where recombinant *M. tb* Lsr2 was titrated into a solution containing the fluorescently labeled probe at a fixed concentration. The resulting equilibrium binding data indicated a $K_d$ of 0.87 μM ± 0.09 (R²=0.961) for the Lsr2/stem-loop complex formation (Figure 5). This information was then used to develop a high-throughput FP assay amenable to library screening for identifying compounds that inhibit Lsr2/DNA complexation.

[0063] The inhibitor screening methodology used a preincubated Lsr2/DNA complex where the Lsr2 concentration was chosen to be above the $K_d$ value to ensure that the assay was both sensitive and discriminating. The assay was used to screen a portion of the NIH Clinical Collection (BioFocus, Saffron Walden, UK). Of the 281 compounds tested, seven compounds were identified as potential leads based on a criterion of an FP signal decrease greater than three
times the standard deviation from the mean polarization measurement. Of these indentified inhibitors, six are known DNA intercalators or groove binders so they were dismissed from further study. Although compounds with these attributes have been previously used to disrupt protein/DNA complex formation they are also known for their lack of specificity and severe side effects when used therapeutically.

[0064] The single remaining lead from this small-scale screening study, Zafirlukast, is a leukotriene receptor antagonist commonly prescribed as a prophylactic treatment for asthma. To determine the inhibitory constant (½) for Zafirlukast, a dose-dependence study using the same FP-based assay was used (Figure 1). The protein and DNA concentrations were held constant at 50 μM and 10 nM, respectively, as the concentration of Zafirlukast was varied. This titration experiment exhibited a consistent dose-dependent inhibition of Lsr2/DNA complexation that yielded a Kᵢ value of 2.57 μM ± 0.002 (R² = 0.960). This assay was also performed using 25 nM of labeled DNA and the same results were obtained, showing that Zafirlukast inhibits complexation by binding Lsr2 and not DNA. This is also supported by the chemical structure of Zafirlukast, in that it is not a polycyclic aromatic compound and that it is anionic at physiological pH. For example, see the structures shown in Figure 4.

[0065] To further show that Zafirlukast is binding Lsr2 and not DNA, an Intensity Fading MALDI-TOF mass spectrometry assay was performed. This methodology is advantageous when attempting to show non-covalent binding of inhibitors possessing limited solubility, like Zafirlukast, to their protein targets. Samples containing fixed concentrations of Zafirlukast and Ritonavir, as an internal standard, were titrated with increasing concentrations of Lsr2. Subsequent MALDI-TOF experiments monitored the intensity of the Zafirlukast and Ritonavir ion peaks (Figure 7).

[0066] Comparing the relative ion abundance of the two drugs from each of these mixtures shows that as the concentration of Lsr2 increases, the ratio of free Zafirlukast to Ritonavir ions decreases (Figure 8). The decrease in Zafirlukast’ s ion intensity is directly attributed to its binding with Lsr2 and its removal from the pool of unbound Zafirlukast.

[0067] One of the primary obstacles in developing successful M. tb therapies is penetrating the mycobacterial outer membrane and cell wall. Therefore, to assess Zafirlukast’ s ability to enter the mycobacterial cell and inhibit cell growth, a Kirby-Bauer disk diffusion assay was performed. An agar plate was inoculated with M. smegmatis, a commonly used surrogate for M. tb. Wafers impregnated with varying amounts of Zafirlukast dissolved in DMSO were placed on the plate along with a control disc that contained only DMSO. Following incubation at 37°C for 18 hours, the plate was evaluated to identify the presence of any zones of inhibition around each disk that would indicate any activity Zafirlukast may have in inhibiting mycobacterial growth (Figure 2).
Zafirlukast does inhibit mycobacterial growth and the radius of the zone of inhibition is directly correlated to the amount of Zafirlukast on the respective discs. In contrast, the same amount of compound in a similar experiment using E. coli exhibited no growth inhibition (Figure 6), showing that the activity of Zafirlukast is specific to bacteria encoding Lsr2. This result also shows that Zafirlukast is interacting with Lsr2 and not binding DNA in a non-specific manner, as E. coli growth would likely have been inhibited by a non-specific DNA intercalator.

Since the growth inhibition studies indicated that Zafirlukast could enter the mycobacterial cell, Real-Time PCR (RT-PCR) was used to examine the effects of administering Zafirlukast on transcription of five M. tb genes bound by Lsr2. Two of the genes, otsA and fbpC, were chosen because they play important roles in synthesizing trehalose and the mycobacterial outer membrane, respectively, which are important for bacterial growth and division. The third gene chosen, sodA, encodes the superoxide dismutase virulence factor. As it is known that Lsr2 represses the transcription of its own gene, lsr2, the mRNA levels of that gene were also tested. Lastly, a constitutively expressed housekeeping gene was examined, sigA. Since Lsr2 is expected to repress transcription of the five test genes, addition of Zafirlukast to a culture of M. tb should produce increased mRNA levels of these genes.

Data from these experiments consistently show that addition of Zafirlukast resulted in sodA having a 4-fold increase in mRNA level, while otsA and fbpC both showed more modest effects with 2.3 and 1.3 fold increases, respectively (Figure 3). The largest increase observed was in the mRNA of lsr2, which displayed an 8.8 fold increase in gene expression. In contrast to that observed for the other genes, the sigA mRNA levels decreased slightly.

As sigA is constitutively expressed at a constant level in cultured bacteria, transcription of this gene is unlikely to respond to only a single signal. In this particular case, the regulatory factors present in response to the stress imparted by administering Zafirlukast likely supersedes the effect of inhibiting Lsr2 function or the subsequent increase in Lsr2 concentration as a consequence of its over expression. Taken together, these data strongly show that Zafirlukast specifically inhibits Lsr2 function in mycobacterial cells.

The present inventors herein identified compounds that inhibit the activity of the highly conserved DNA-binding protein Lsr2. Not only is Zafirlukast now identified as an inhibitor of Lsr2 function, but the application of this drug also alters gene expression levels in M. tb and leads to growth inhibition of mycobacteria presumably by profoundly dysregulating protein expression levels in the bacterial cell.

While not wishing to be bound by theory, the inventors herein now believe that Zafirlukast has synergistic effects with other first-line drugs since Lsr2 expression increases during the administration of known anti-tubercular drugs presumably to help M. tb survive during pharmacologically-induced stress. Second, since Lsr2 is clearly important for the mycobacterial response to environmental stress, the efficacy of Zafirlukast may be greater in the
host by disrupting defenses evolved in *M. tb.* to evade or disrupt the human immune response. This may promote killing of *M. tb* by the human immune system. Third, Zafirlukast does not appear to function through the same mechanism of any current TB drugs, so it is likely to be effective against XDR-TB. In addition, since Zafirlukast is an approved, safe, orally available drug that is readily transported to lung tissue, repurposing it for the treatment of TB could have an immediate impact on global health.

[0074] **Experimental Data:**

[0075] All polarization experiments were performed using a Biotek Synergy H4 Plate reader and analyzed with Gen5 software. The tested compounds for the library screening were from the National Institute of Health Clinical Collection (NCC) and purchased from BioFocus. Zafirlukast was purchased from Sigma-Aldrich.

[0076] 5’-fluorescein labeled AT-rich stem-loop DNA with the sequence 5’

CCTAATTATAACGAAGTTAATAGG-3’ [SEQ ID NO:1] was purchased from Integrated DNA Technologies (Coralville, IA) and dissolved in deionized water at a concentration of 100 µM, heated to 95°C for 15 minutes, cooled on ice, and stored at -20°C until needed. All mass spectrometry experiments were carried out on a Bruker ultrafleXtreme MALDI TOF/TOF.

[0077] **Lsr2 expression and purification.**

[0078] Lsr2 from recombinant DNA was expressed in *E. coli* BL21 (DE3) using Luria-Bertani (LB) media. The culture was grown at 37°C until reaching an O.D.₆₅₀ between 0.6 and 0.8 followed by induction with IPTG at 16°C for 36 hours. Lsr2 was purified using immobilized metal affinity chromatography followed by cation exchange chromatography.

[0079] **Determination of Lsr2/DNA K₆.**

[0080] The K₆ was obtained using a 384-well microplate format fluorescence polarization (FP) assay performed at 37°C. Each well contained 1 µL of fluorescently labeled DNA (10 nM) and concentrations of Lsr2 ranging from 0 to 200 µM. Additional buffer solution (20 mM Tris pH 7.5, 50 mM NaCl) was added as needed to reach a final volume of 25 µL.

[0081] **Screening of NCC.**

[0082] The NCC was screened in a corning 384-well microplate at 37°C with an excitation of 485 nm and an emission of 528 nm. In each well 22.75 µL of Lsr2 (15 µM) in 20 mM Tris pH 7.5, 50 mM NaCl, 1.25 µL of fluorescently labeled DNA (5 µM) and 1 µL of compound (10 mM) in DMSO. Controls were also performed by adding 1 µL of DMSO lacking drug. The FP signals were evaluated for each tested drug. Any compound showing a decrease in the FP signal that was greater than three times the standard deviation was considered a hit.

[0083] **Determination of K₆.**

[0084] The ¼ was obtained using the same FP assay format. Each well contained 1 µE of fluorescently labeled DNA (10 nM), 20 µE of Lsr2 (50 µM) and the concentration of Zafirlukast
was varied by adding a constant volume (1 µE) with increasing concentrations, from 0 to 300 µM. K₁ was calculated with Prism (GraphPad software) using the equation

\[ K_\text{r} = \frac{IC_{50}}{(1+Lsr2/K_\text{s})} \]

[0085] **Kirby-Bauer disc diffusion Assay.**

[0086] *M. smegmatis* with Ampicillin was grown at 37°C for 72 hours in Middlebrook 7H9 Broth. A carbenicillin agar plate was inoculated with 250 µL of the *M. smegmatis* solution. Once the media had been allowed to stand for 3 min wafers that had been impregnated with increasing concentrations Zafirlukast (392 µg, 196 µg, 95 µg, 50 µg, 0 µg in 20 µL of DMSO) were placed on the agar plate. The agar plate was incubated for 14 hours at 37°C then the zones of inhibition were examined. The analogous experiment using *E. coli* in LB media was performed using the same protocol. The Kirby-Bauer disk diffusion assays were performed in a similar manner using *M. tb*. However, growth at 37°C was for 10 days because of the much longer doubling time exhibited by *M. tb*.

[0087] **Intensity Fading MALDI-TOF mass spectrometry assay.**

[0088] The nonbinding internal standard, Ritonavir, was diluted to a concentration that gave a MALDI MS ion signal of intensity that resembled that of the Zafirlukast. The compounds were both dissolved in DMSO, combined and diluted with a 20 mM Tris pH 8.5 solution. From this solution 2 µE were added to each of the solutions containing increasing concentration of Lsr2 in 20 mM Tris pH 8.5. The solutions were then incubated at 4 °C for 12 hours. The sample was mixed using the dried droplet method with a matrix solution (1:2 v/v) of sinapinic acid (10mg/mL) containing 30% acetonitrile (v/v) diluted in deionized water.

[0089] **Example 2**

[0090] **Formulations**

[0091] Therapeutics, including compositions containing the leukotriene receptor antagonist compounds, can be prepared in physiologically acceptable formulations, such as in pharmaceutically acceptable carriers, using known techniques. For example, the compound can be combined with a pharmaceutically acceptable excipient to form a therapeutic composition.

[0092] The compositions may be administered in the form of a solid, liquid or aerosol. Examples of solid compositions include pills, creams, soaps and implantable dosage units. Pills may be administered orally. Therapeutic creams and anti-mycobacteria soaps may be administered topically. Implantable dosage units may be administered locally, for example, in the lungs, or may be implanted for systematic release of the therapeutic composition, for example, subcutaneously. Examples of liquid compositions include formulations adapted for injection intramuscularly, subcutaneously, intravenously, intraarterially, and formulations for topical and intraocular administration. Examples of aerosol formulations include inhaler formulations for administration to the lungs.
A sustained release matrix, as used herein, is a matrix made of materials, usually polymers, which are degradable by enzymatic or acid/base hydrolysis, or by dissolution. Once inserted into the body, the matrix is acted upon by enzymes and body fluids. The sustained release matrix is chosen desirably from biocompatible materials, including, but not limited to, liposomes, polylactides, polyglycolide (polymer of glycolic acid), polylactide co-glycolide (copolymers of lactic and glycolic acid), poly(anhydrides), poly(ortho)esters, polypeptides, hyaluronic acid, collagen, chondroitin sulfate, carboxylic acids, fatty acids, phospholipids, polysaccharides, nucleic acids, polyamino acids, amino acids such as phenylalanine, tyrosine, isoleucine, polynucleotides, polyvinyl propylene, polyvinylpyrrolidone and silicone. Biodegradable matrix include, for example, a matrix of one of either polylactide, polyglycolide, or polylactide co-glycolide.

The dosage of the composition will depend on the condition being treated, the particular composition used, and other clinical factors, such as weight and condition of the patient, and the route of administration. One suitable dosage may range from 100 to 0.1 mg/kg. Another dosage may range from 50 to 0.2 mg/kg. Still another dosage may range from 25 to 0.5 mg/kg.

Tablets or other forms of media may contain from 1 to 1000 mg of the leukotriene receptor antagonist. Dosage ranges and schedules of administration similar to ethambutol or other anti-tuberculosis drugs may be used.

The composition may be administered in combination with other compositions and procedures for the treatment of other disorders occurring in combination with mycobacterial disease. For example, tuberculosis frequently occurs as a secondary complication associated with acquired immunodeficiency syndrome (AIDS). Patients undergoing AIDS treatment, which includes procedures such as surgery, radiation or chemotherapy, may benefit from the therapeutic methods and compositions described herein.

It will be appreciated by those skilled in the art that the amount of active ingredients in the combinations required for use in treatment will vary according to a variety of factors, including the nature of the condition being treated and the age and condition of the patient, and will ultimately be at the discretion of the attending physician or health care practitioner. The factors to be considered include the route of administration and nature of the formulation, the animal's body weight, age and general condition and the nature and severity of the disease to be treated.

Other systems, methods, features, and advantages of the present invention will be or will become apparent to one with skill in the art upon examination of the following drawings and detailed description. It is intended that all such additional systems, methods, features, and advantages be included within this description, be within the scope of the present invention, and be protected by the accompanying claims.
While the invention has been described with reference to various and preferred embodiments, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted for elements thereof without departing from the essential scope of the invention. In addition, many modifications may be made to adapt a particular situation or material to the teachings of the invention without departing from the essential scope thereof.

Therefore, it is intended that the invention not be limited to the particular embodiment disclosed herein contemplated for carrying out this invention, but that the invention will include all embodiments falling within the scope of the claims.

The publication and other material used herein to illuminate the invention or provide additional details respecting the practice of the invention, are incorporated be reference herein, and for convenience are provided in the following bibliography.

Citation of the any of the documents recited herein is not intended as an admission that any of the foregoing is pertinent prior art. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicant and does not constitute any admission as to the correctness of the dates or contents of these documents.
CLAIMS

What is claimed is:

1. A method for treating, controlling, reducing or inhibiting a bacterial infection in a subject in need thereof, comprising:
   administering to the subject an effective amount of a composition comprising a leukotriene receptor antagonist compound or a pharmaceutically acceptable salt thereof, the leukotriene receptor antagonist compound comprising one or more of:

2. The method of claim 1, wherein the leukotriene receptor antagonist compound comprises zafirlukast or a pharmacologically active derivative thereof.

3. The method of claim 1, wherein the leukotriene receptor antagonist compound comprises a metabolite of zafirlukast, or a pharmacologically active derivative thereof, comprising one or more of:
where the metabolites are:

1) Zafirlukast: \([3-[2-\text{Methoxy}-(\text{toluene-2-sulfonylaminocarbonyl})\text{benzyl}]-1\text{-methyl-1H-indol-5-yl}]\text{carbamic acid cyclopentyl ester)}\);

2) N-[4-(5-Amino-1-methyl-1H-indol-3-ylmethyl)-3-methoxybenzoyl]-2-methylbenzenesulfonamide ;

3) N-\([3-[2-\text{Methoxy}-(\text{toluene-2-sulfonylaminocarbonyl})\text{benzyl}]-1\text{-methyl-1H-indol-5-yl}]\text{acetamide};

4) N-[1-Hydroxymethyl-3-[2-methoxy-(\text{toluene-2-sulfonylaminocarbonyl})-benzyl]-1H-indol-5-yl]acetamide ;

5) N-[3-[2-\text{Methoxy}-(\text{toluene-2-sulfonylaminocarbonyl})\text{benzyl}]-1H-indol-5-yl]acetamide;

6) \([3-[2-\text{Methoxy}-(\text{toluene-2-sulfonylaminocarbonyl})\text{benzyl}]-1\text{-methyl-1H-indol-5-yl}]\text{carbamic acid hydroxy cyclopentyl ester;}

7) \([3-[2-\text{Methoxy}-(\text{toluene-2-sulfonylaminocarbonyl})\text{benzyl}]-1H-indol-5-yl]\text{carbamic acid hydroxycyclopentyl ester;}

8) \([1\text{-Hydroxymethyl}-3-[2-\text{Methoxy}-(\text{toluene-2-sulfonylaminocarbonyl})\text{-benzyl}]-1\text{H-indol-5-yl}]\text{carbamic acid cyclopentyl ester; and,}

9) \([3-[2-\text{Methoxy}-(\text{toluene-2-sulfonylaminocarbonyl})\text{ benzyl}]-1H-indol-5-yl]\text{carbamic acid cyclopentyl ester.}
4. The method of claim 1, wherein the infection is a chronic bacterial infection.

5. The method of claim 4, wherein the chronic bacterial infection is a mycobacterial infection.

6. The method of claim 4, wherein the mycobacterial infection is caused by one or more of: *M. tuberculosis*, *M. avium-intracellulare*, *M. kansasii*, *M. fortuitum*, *M. chelonae*, *M. leprae*, *M. africanum*, *M. bovis*, *M. avium*, *M. microti*, *M. avium paratuberculosis*, *M. intracellular*, *M. scrofulaceum*, *M. xenopi*, *M. marinnur*, or *M. ulcerans*.

7. The method of claim 4, wherein the infection is caused one or more of: *Corynebacterium diphtheriae* and *Corynebacterium pseudotuberculosis* as well as other *Corynebacterium* sp, including, but not limited to: species *Corynebacterium diphtheriae* and the nondiphtherial corynebacteria, collectively referred to as diphtheroids; *Corynebacterium haemolyticum* (Arcanobacterium haemolyticum); *Corynebacterium jeikeium*; *Corynebacterium glutamicum*; *Corynebacterium pseudodiphtheriticum*.

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

9. The method of claim 1, wherein the subject is a member selected from a human, cattle, goat, pig, sheep, horse, cow, bull, dog, guinea pig, gerbil, rabbit, cat, chicken and turkey.

10. The method of claim 1, wherein the mammal is a human.

11. The method of claim 1, wherein the effective amount is administered topically, orally, peritoneally, subcutaneously, intramuscularly, intraocularly, intraarterially, intravenously, or locally using an implantable dosage unit.

12. The method of claim 11, wherein the compound is administered as a solid, liquid or aerosol.

13. The method of claim 12, wherein the solid is a pill or an implantable dosage unit.

14. The method of claim 12, wherein the aerosol comprises an inhaler formulation.

15. The method of claim 12, wherein the solid, liquid or aerosol comprises a sustained release matrix.
16. The method of claim 1, wherein the effective amount of the compound comprises from 100 to 0.1 mg per kg of body weight.

17. The method of claim 1, wherein the effective amount of the compound comprises from 50 to 0.2 mg per kg of body weight.

18. The method of claim 1, wherein the effective amount of the compound comprises from 25 to 0.5 mg per kg of body weight.

19. The method of claim 1, wherein the effective amount of the compound comprises from 1 to 1000 mg.

20. A method of inhibiting growth of a bacterium, comprising:
   exposing the bacterium to an agent that disrupts inhibits binding of Lsr2 protein to DNA under conditions effective to inhibit growth of the bacterium.

21. The method of claim 20, wherein the bacterium is a *Mycobacterium* species.

22. The method of claim 21, wherein the *Mycobacterium* is *Mycobacterium tuberculosis*.

23. The method of claim 21, wherein the *Mycobacterium* species is one or more of: *M. tuberculosis*, *M. avium-intracellulare*, *M. kansasii*, *M. fortuitum*, *M. chelonei*, *M. leprae*, *M. africanum*, *M. bovis*, *M. avium*, *M. microti*, *M. avium paratuberculosis*, *M. intracellulare*, *M. scrofulaceum*, *M. xenopi*, *M. marinnur*, or *M. ulcerans*.

24. The method of claim 20, wherein the bacterium is one or more of: *Corynebacterium diphtheriae* and *Corynebacterium pseudotuberculosis* as well as other *Corynebacterium* sp, including, but not limited to: species *Corynebacterium diphtheriae* and the nondiphtherial corynebacteria, collectively referred to as diphtheroids; *Corynebacterium haemolyticum* (*Arcanobacterium haemolyticum*); *Corynebacterium jeikeium*; *Corynebacterium glutamicum*; *Corynebacterium pseudodiphtheriticum*.

25. The method of claim 20, wherein the agent is a leukotriene receptor antagonist compound or a pharmaceutically acceptable salt thereof.
26. The method of claim 20, wherein the leukotriene receptor antagonist compound comprises one or more of:

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27. The method of claim 21, wherein the leukotriene receptor antagonist compound comprises zafirlukast or a pharmacologically active derivative thereof.

28. The method of claim 21, wherein the leukotriene receptor antagonist compound comprises a metabolite of zafirlukast, or a pharmacologically active derivative thereof, comprising one or more of:

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where the metabolites are:

1) Zafirlukast; [3-[2-Methoxy-4-(toluene-2-sulfonylaminocarbonyl)benzyl]-1-methyl-1H-indol-5-yl]carbamic acid cyclopentyl ester;

2) N-[4-(5-Amino-1-methyl-1H-indol-3-ylmethyl)-3-methoxybenzoyl]-2-methylbenzenesulfonamide;

3) N-[3-[2-Methoxy-4-(toluene-2-sulfonylaminocarbonyl)benzyl]-1-methyl-1H-indol-5-yl]acetamide;

4) N-[1-Hydroxymethyl-3-[2-methoxy-4-(toluene-2-sulfonylaminocarbonyl)benzyl]-1H-indol-5-yl]acetamide;

5) N-[3-[2-Methoxy-4-(toluene-2-sulfonylaminocarbonyl)benzyl]-1H-indol-5-yl]acetamide;

6) [3-[2-Methoxy-4-(toluene-2-sulfonylaminocarbonyl)benzyl]-1-methyl-1H-indol-5-yl]carbamic acid hydroxycyclopentyl ester;

7) [3-[2-Methoxy-4-(toluene-2-sulfonylaminocarbonyl)benzyl]-1H-indol-5-yl]carbamic acid hydroxycyclopentyl ester;

8) [1-Hydroxymethyl-3-[2-methoxy-4-(toluene-2-sulfonylaminocarbonyl)benzyl]-1H-indol-5-yl]carbamic acid cyclopentyl ester; and,

9) [3-[2-Methoxy-4-(toluene-2-sulfonylaminocarbonyl) benzyl]-1H-indol-5-yl]carbamic acid cyclopentyl ester.

29. A method of treating a bacterial infection in a subject, comprising:
administering to the subject an agent that inhibits binding of Lsr2 protein to DNA under conditions effective to treat the bacterial infection.

30. The method of claim 29, wherein the bacterial infection is caused by a Mycobacterium species.
31. The method of claim 29, wherein the bacterium is a *Mycobacterium* species.

32. The method of claim 31, wherein the *Mycobacterium* is *Mycobacterium tuberculosis*.

33. The method of claim 31, wherein the *Mycobacterium* species is one or more of: *M. tuberculosis*, *M. avium-intracellulare*, *M. kansasi*, *M. fortuitum*, *M. chelonei*, *M. leprae*, *M. africanum*, *M. bovis*, *M. avium*, *M. microti*, *M. avium paratuberculosis*, *M. intracellulare*, *M. scrofulaceum*, *M. xenopi*, *M. marinum*, or *M. ulcerans*.

34. The method of claim 29, wherein the bacterial infection is caused by one or more of: *Corynebacterium diphtheriae* and *Corynebacterium pseudotuberculosis* as well as other *Corynebacterium* sp, including, but not limited to: species *Corynebacterium diphtheriae* and the nondiphtherial corynebacteria, collectively referred to as diphtheroids; *Corynebacterium haemolyticum* (*Arcanobacterium haemolyticum*); *Corynebacterium jeikeium*; *Corynebacterium glutamicum*; *Corynebacterium pseudodiphtheriticum*.

35. The method of claim 29, wherein the agent is a leukotriene receptor antagonist compound or a pharmaceutically acceptable salt thereof.

36. The method of claim 29, wherein the leukotriene receptor antagonist compound comprises one or more of:

1. ![Chemical Structure 1]
2. ![Chemical Structure 2]
3. ![Chemical Structure 3]
4. ![Chemical Structure 4]

37. The method of claim 29, wherein the leukotriene receptor antagonist compound comprises zafirlukast or a pharmacologically active derivative thereof.
38. The method of claim 29, wherein the leukotriene receptor antagonist compound comprises a metabolite of zafirlukast, or a pharmacologically active derivative thereof, comprising one or more of:

where the metabolites are:

1) Zafirlukast; [3-[2-Methoxy-4-(toluene-2-sulfonylaminocarbonyl)benzyl] -1-methyl-1H-indol-5-yl]carbamic acid cyclopentyl ester;

2) N-[4-(5-Amino-1-methyl-1H-indol-3-ylmethyl)-3-methoxybenzoyl]-2-methylbenzenesulfonamide ;

3) N-[3-[2-Methoxy-4-(toluene-2-sulfonylaminocarbonyl)benzyl] -1-methyl-1H-indol-5-yl]acetamide;

4) N-[1-Hydroxymethyl-3-[2-methoxy-4-(toluene-2-sulfonylaminocarbonyl)-benzyl] -1H-indol-5-yl]acetamide ;
5) N-[3-[2-Methoxy-4-(toluene-2-sulfonylaminocarbonyl)benzyl]-1H-indol-5-yl]acetamide;
6) [3-[2-Methoxy-4-(toluene-2-sulfonylaminocarbonyl)benzyl]-1-methyl-1H-indol-5-yl]carbamic acid hydroxycyclopentyl ester;
7) [3-[2-Methoxy-4-(toluene-2-sulfonylaminocarbonyl)benzyl]-1H-indol-5-yl]carbamic acid hydroxycyclopentyl ester;
8) [1-Hydroxymethyl-3-[2-methoxy-4-(toluene-2-sulfonylaminocarbonyl)-benzyl]-1H-indol-5-yl]carbamic acid cyclopentyl ester; and,
9) [3-[2-Methoxy-4-(toluene-2-sulfonylaminocarbonyl) benzyl]-1H-indol-5-yl]carbamic acid cyclopentyl ester.

39. The method of claim 29, further comprising selecting a subject having a bacterial infection prior to said administering.

40. The method of claim 29, wherein the agent is administered in combination with one or more antibacterial agents.

41. The method of claim 40, wherein the one or more antibacterial agents are selected from the group consisting of isoniazid, rifampin, rifabutin, rifapentine, pyrazinamide, ethambutol, kanamycin, erythromycin, or other aminoglycoside antibiotics.

42. A method of identifying a compound that inhibits binding of Lsr2 protein to DNA of a pathogen, comprising:
   providing one or more candidate compounds;
   providing a pathogen, wherein the pathogen comprises a detectable indicator of binding of Lsr2 protein to DNA;
   contacting the pathogen with the one or more candidate compounds; and
   detecting the binding of Lsr2 protein to DNA of the pathogen,
   wherein detecting a change in the binding of Lsr2 protein to DNA in the presence of a candidate compound identifies a compound that interferes with the binding of Lsr2 protein to DNA of the pathogen.

43. The method of claim 42, wherein the pathogen is bacterium.

44. The method of claim 43, wherein the bacterium is Mycobacterium.
45. The method of claim 44, wherein the Mycobacterium is *Mycobacterium tuberculosis*.

46. The method of claim 44, wherein the *Mycobacterium* species is one or more of: *M. tuberculosis*, *M. avium-intracellulare*, *M. kansasii*, *M. fortuitum*, *M. chelonei*, *M. africanum*, *M. bovis*, *M. avium*, *M. microti*, *M. avium paratuberculosis*, *M. intracellulare*, *M. scrofulaceum*, *M. xenopi*, *M. marinum*, or *M. ulcerans*.

47. The method of claim 42, wherein the pathogen is one or more of: *Corynebacterium diphtheriae* and *Corynebacterium pseudotuberculosis* as well as other *Corynebacterium* sp, including, but not limited to: species *Corynebacterium diphtheriae* and the nondiphtherial corynebacteria, collectively referred to as diphtheroids; *Corynebacterium haemolyticum* (*Arcanobacterium haemolyticum*); *Corynebacterium jeikeium*; *Corynebacterium glutamicum*; *Corynebacterium pseudodiphtheriticum*.

48. A method of killing a tuberculosis-causing microorganism infecting a mammalian cell, comprising:
   contacting said cell with a composition comprising a leukotriene receptor antagonist compound or a pharmaceutically acceptable salt thereof.

49. A method of treating an infection in an animal, comprising:
   administering to the animal a therapeutically effective amount of a leukotriene receptor antagonist compound, or a pharmaceutically acceptable salt thereof, sufficient to treat said infection.

50. A method of treating an animal infected with a disease-causing microorganism of a *Mycobacterium* species, comprising:
   administering to the animal a therapeutically effective amount of a pharmaceutical composition comprising a leukotriene receptor antagonist compound or a pharmaceutically acceptable salt thereof.

51. A method of killing a microorganism infecting a mammalian cell, comprising:
   contacting said cell with the composition comprising a leukotriene receptor antagonist compound or a pharmaceutically acceptable salt thereof.
Figure 1

Figure 2
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**Figure 3**

1

![Chemical structure 1](image1)

2

![Chemical structure 2](image2)

3

![Chemical structure 3](image3)

4

![Chemical structure 4](image4)

**Figure 4**
Figure 5

Figure 6
Figure 8

Intensity Fading

Intensity Ratio $Z/R$

[μM of Lsr2]
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01N 43/38; A61K 31/405; A61K 31/44 (2012.01)

USPC - 514/415

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)

USPC: 514/415

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

USPC: 514/291, 514/392, 548/510 (keyword limited; terms below)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<td>Y</td>
<td>WO2006/067392 A2 (Hulikal et al.) 29 June 2006 (29.06.2006) pg 2, ln 21-25, ln 25-29, pg 4, ln 8-10, pg 15, ln 7-14, ln 19-26, pg 18, ln 18-27, pg 19, ln 3-10</td>
<td>1-51</td>
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<tr>
<td>Y</td>
<td>Colangeli et al. 'Transcriptional Regulation of Multi-Drug Tolerance and Antibiotic-Induced Responses by the Histone-Like Protein Lsr2 in M. tuberculosis' PLoS Pathogens vol 3 e87 pg 0780-0792; 22 June 2007 (22.06.2007) abstract, pg 0786, col 1, para 2 to col 2, para 1, pg 0787, col 2, para 2, pg 0788, col 2, para 2</td>
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Date of the actual completion of the international search

19 December 2012 (19.12.2012)

Date of mailing of the international search report

23 JAN 2013

Name and mailing address of the ISA/US

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