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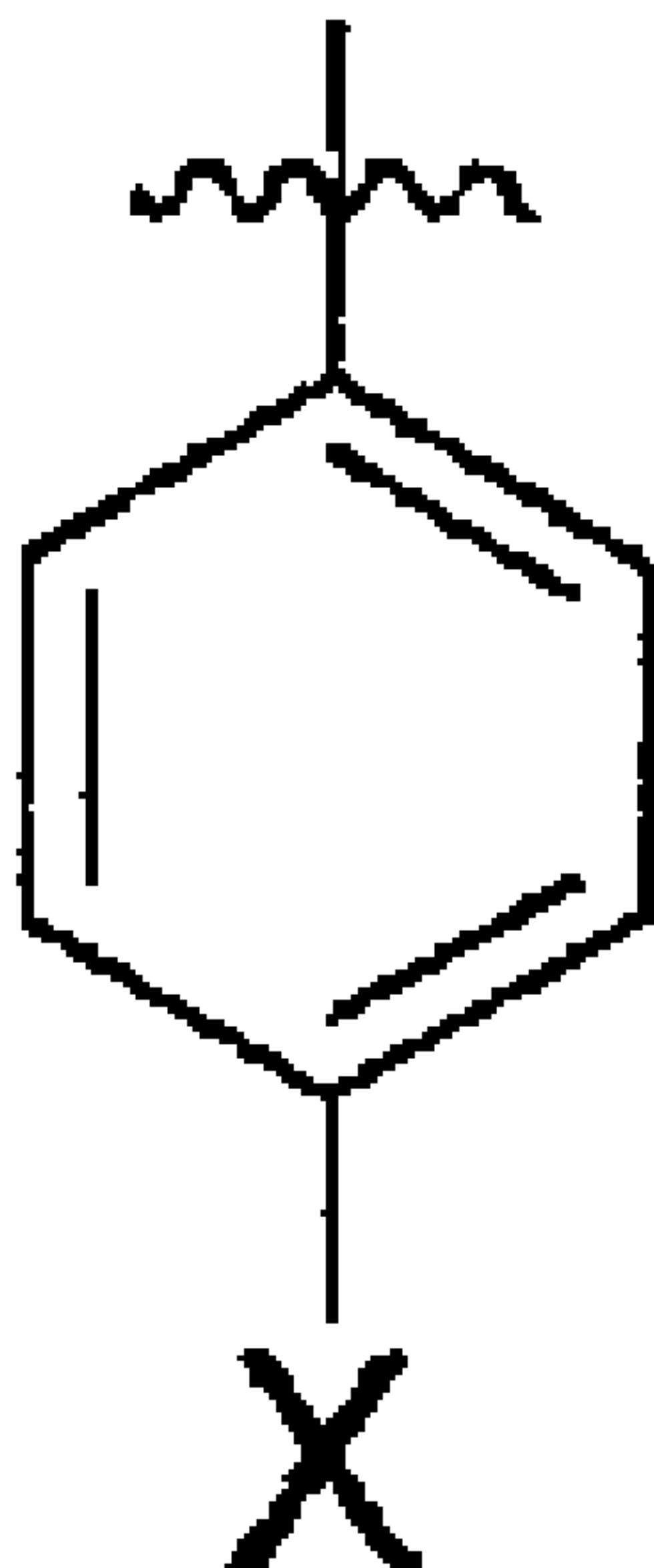
(71) Demandeurs/Applicants:
JAYARAMAN, ARUMUGAM, CA;
STERENBERG, BRIAN T., CA;
STAVRINIDES, JOHN, CA

(72) Inventeurs/Inventors:
JAYARAMAN, ARUMUGAM, CA;
STERENBERG, BRIAN T., CA;
STAVRINIDES, JOHN, CA

(74) Agent: ADE & COMPANY INC.

(54) Titre : UN ANTIBACTERIEN INORGANIQUE SYNTHETIQUE POTENT AYANT UNE ACTIVITE CONTRE LES
PATHOGENES RESISTANTS AUX MEDICAMENTS

(54) Title: A POTENT SYNTHETIC INORGANIC ANTIBACTERIAL WITH ACTIVITY AGAINST DRUG-RESISTANT
PATHOGENS



**X = H, OMe,
NEt₂, CH₃,
Ph**

(57) Abrégé/Abstract:

A novel inorganic antibiotic, phosphopyricin, has antibiotic activity against the Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium* (VRE). We show that this potent antibiotic is bactericidal, and exhibits low toxicity in an acute dose assay in mice. As a synthetic compound that does not occur naturally, phosphopyricin would be evolutionarily foreign to microbes, thereby slowing the evolution of resistance. In addition, it degrades in light, and will break down in the general environment where strong selective pressures imposed by antibiotic residuals are known to accelerate antibiotic resistance. Phosphopyricin represents an innovation in antimicrobials, having a synthetic core, and a photosensitive chemical architecture that would reduce accumulation in the environment.

ABSTRACT

A novel inorganic antibiotic, phosphopyricin, has antibiotic activity against the Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium* (VRE). We show that this potent antibiotic is bactericidal, and exhibits low toxicity in an acute dose assay in mice. As a synthetic compound that does not occur naturally, phosphopyricin would be evolutionarily foreign to microbes, thereby slowing the evolution of resistance. In addition, it degrades in light, and will break down in the general environment where strong selective pressures imposed by antibiotic residuals are known to accelerate antibiotic resistance. Phosphopyricin represents an innovation in antimicrobials, having a synthetic core, and a photosensitive chemical architecture that would reduce accumulation in the environment.

A POTENT SYNTHETIC INORGANIC ANTIBACTERIAL WITH ACTIVITY AGAINST DRUG-RESISTANT PATHOGENS

BACKGROUND OF THE INVENTION

Widespread and increasing antibiotic resistance among the “ESKAPE”
5 pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*,
Acinetobacter baumannii, *Pseudomonas aeruginosa*, and *Enterobacter spp.*)¹ has
become a critical problem for healthcare. Organic natural products, which have
provided the core set of current therapeutics, have been central to our ability to
control these multi-drug resistant pathogens; however, microbes have been
10 harnessing antibiotics for competition for billions of years, and as a result,
mechanisms for resisting and tolerating antibiotics have been evolving for just as
long^{3,4}. For each new antimicrobial natural product discovered, at least one
mechanism of resistance is already present in the general environment, greatly
accelerating the emergence of antibiotic resistance⁵. In fact, over evolutionary time,
15 microbes have likely encountered most naturally occurring organic and inorganic
molecules in the environment, and have evolved specific strategies to contend with
those that are immediately toxic³. Compounding the antibiotic resistance problem are
the high concentrations of anthropogenically-generated antibiotic residuals that are
deposited into the environment, resulting in strong selective pressures for the
20 evolution of resistance^{6,7}.

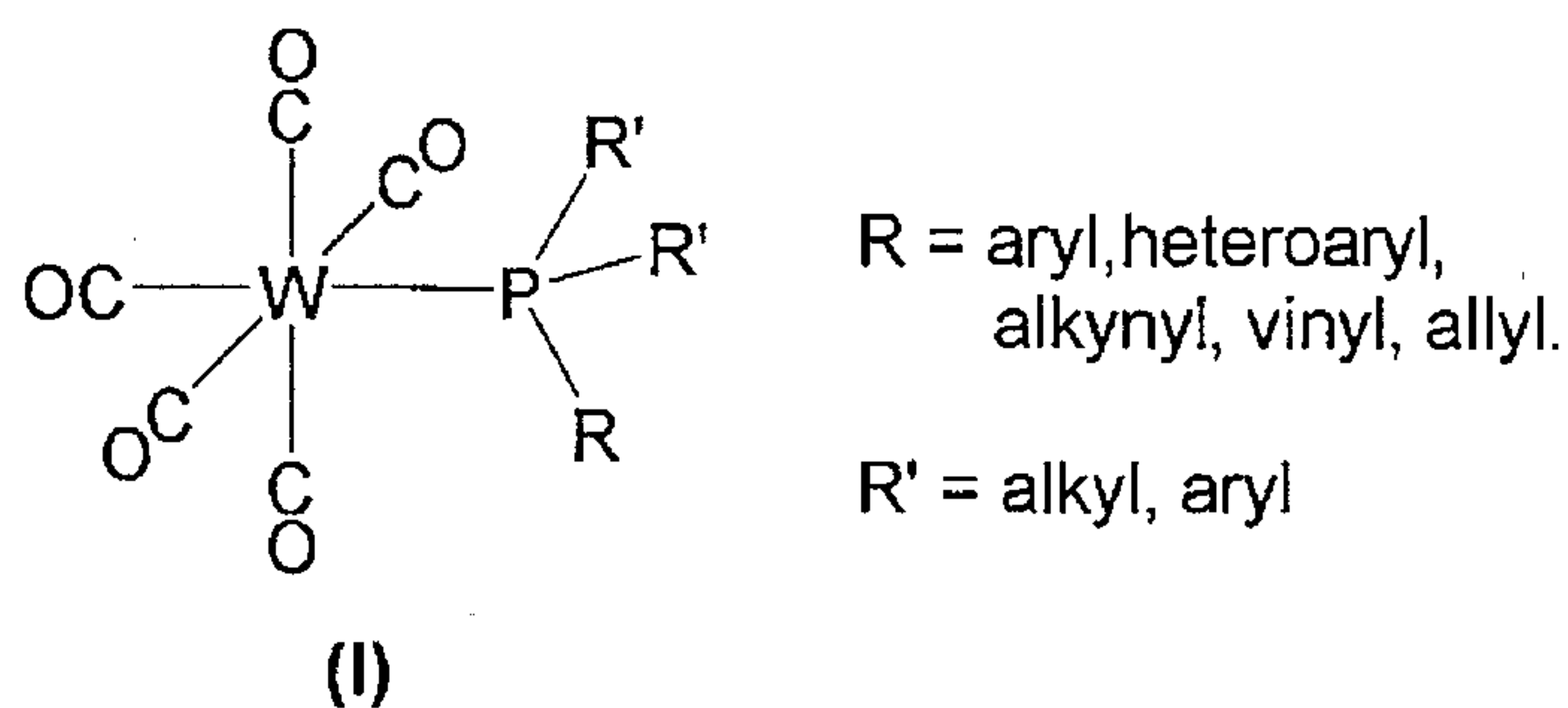
One possible strategy for overcoming these obstacles and regaining some
ground on the antibiotic resistance problem is to develop synthetic antimicrobials
whose chemical architectures do not occur naturally, and would thus be evolutionarily
foreign to bacteria. In addition, if such antimicrobials degraded rapidly in the general
25 environment, strong selective pressure for the evolution of resistance would be
reduced significantly. Organophosphorus compounds remain an untapped pool of
chemical architectures that could possess novel chemistries and differential binding
affinities to diverse microbiological targets. Also, because phosphorus has a similar
electronegativity to carbon, the chemistry of low valent phosphorus often resembles
30 that of carbon, making it highly amenable for syntheses of compounds for biological

applications⁸. Phosphorus-containing antibiotics have been synthesized previously, including fosfomicin⁹, clindamycin¹⁰, and torezolid¹¹; however, these molecules are organophosphates that may be susceptible to existing antibiotic resistance mechanisms.

5 In contrast to organophosphates, phosphine derivatives remain underexplored as potential antimicrobials^{12,13}. These compounds provide a large pool of potentially biologically active molecules with vast structural diversity, mainly due to the ease with which they can be modified chemically¹⁴. Phosphines also provide a simple means of introducing metal complexes into biologically active molecules, via coordination of the
10 lone pair, further expanding the range of inorganic groups that can be explored. Phosphine derivatives have been shown to have potential as anti-cancer drugs¹³, and even to have potential as antibiotics¹². Some have been developed into resistance protein inhibitors, including avibactam, clavulanic acid, tazobactam, and sulbactam¹⁵⁻¹⁸. One recent study synthesized phosphine derivatives of ciprofloxacin and
15 norfloxacin, yielding an additional class of broad-spectrum antibiotics effective against *P. aeruginosa*, *S. aureus*, *K. pneumoniae*, and *E. coli*¹⁹. The use of phosphorus-containing functional groups can yield compounds that bind strongly to certain enzymes where a carbon or nitrogen analog would not. Non-phosphate, organophosphorus compounds therefore have enormous capacity to provide new
20 chemical architectures for the development of next-generation antibiotics.

SUMMARY OF THE INVENTION

According to a first aspect of the invention, there is provided a compound comprising the structure of Formula (I):



wherein:

"W" is tungsten;

"C" is carbon;

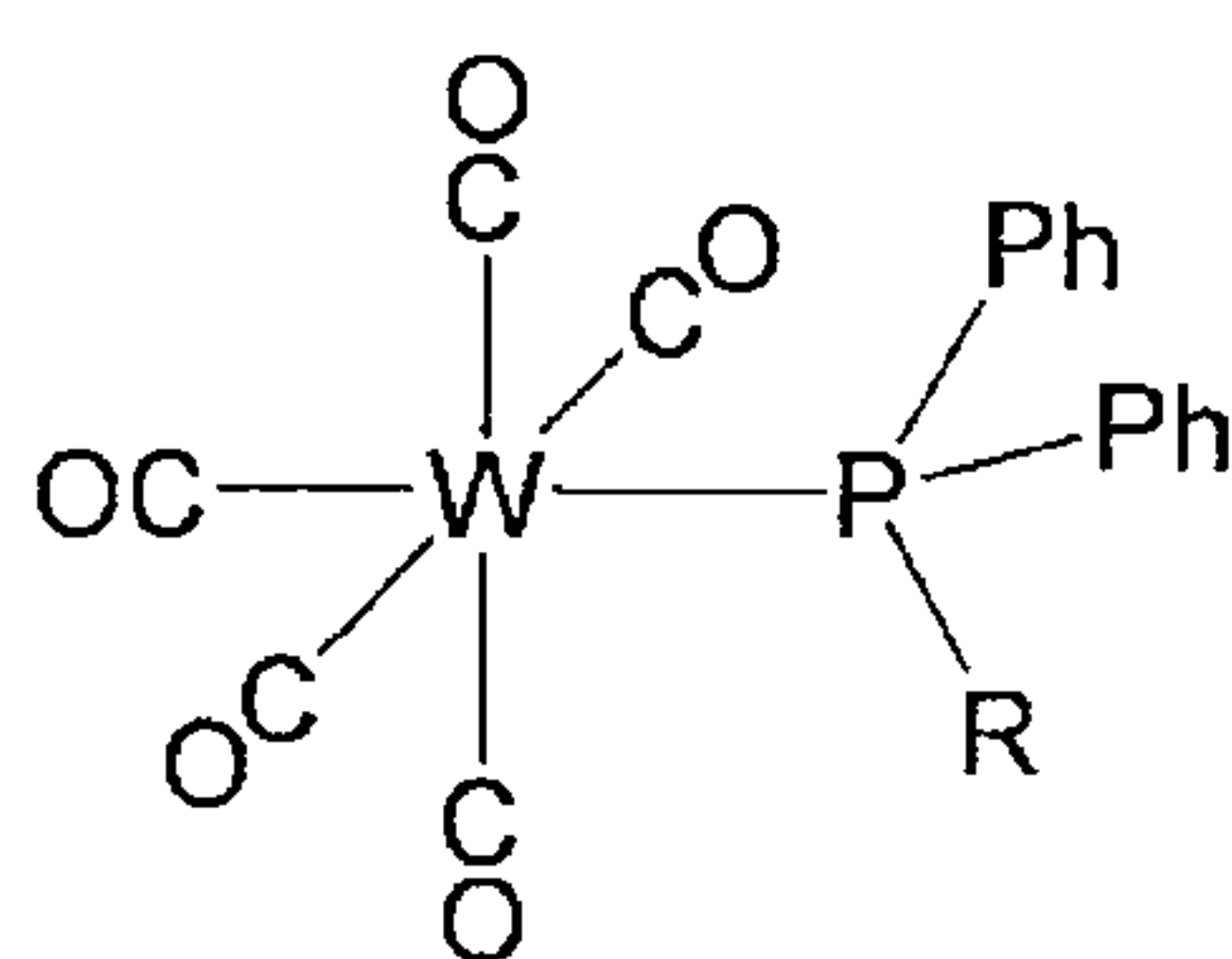
"O" is oxygen;

5 "P" is phosphorus;

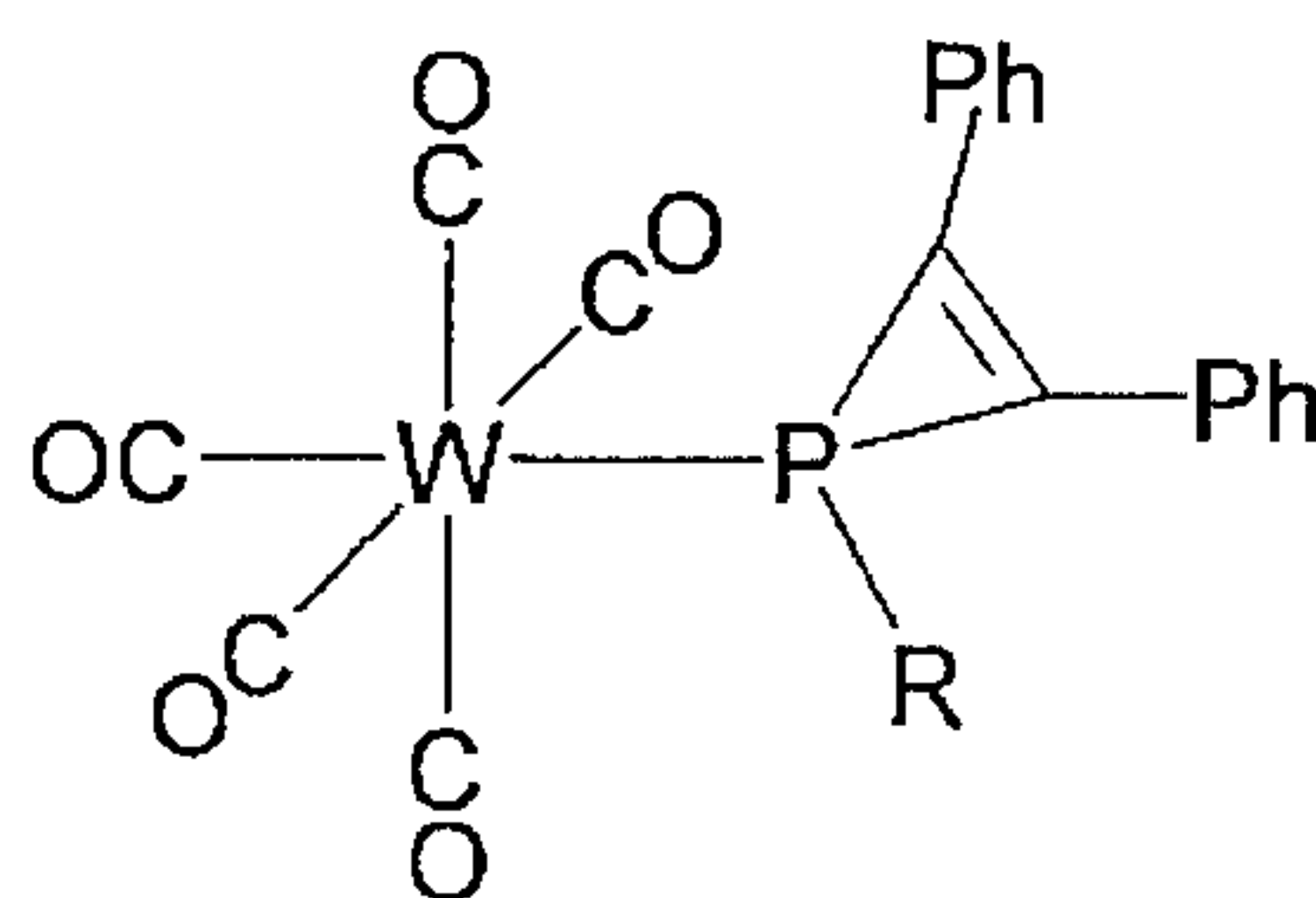
R is selected from substituted aryl, alkynyl, vinyl, cyclic vinyl, substituted vinyl, allyl, cyclic allyl and substituted allyl; and

R' is alkyl or aryl.

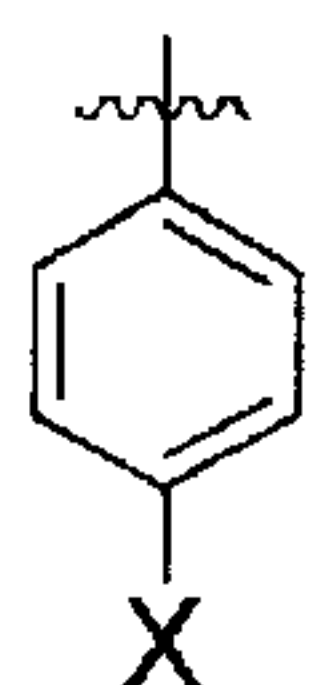
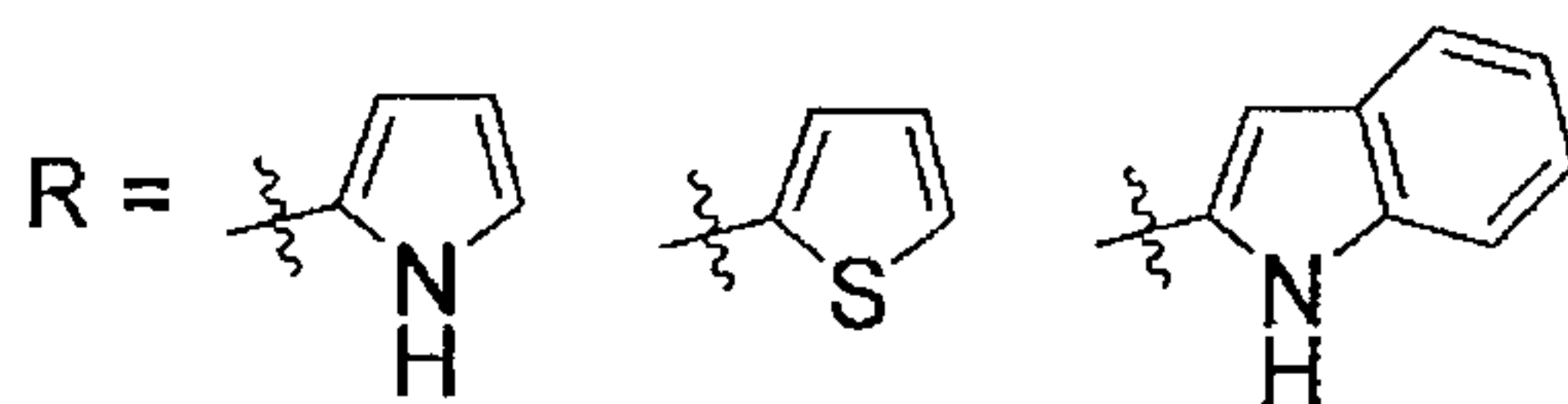
The compound may comprise the structure of Formula (IIa) or (IIb):



(IIa)



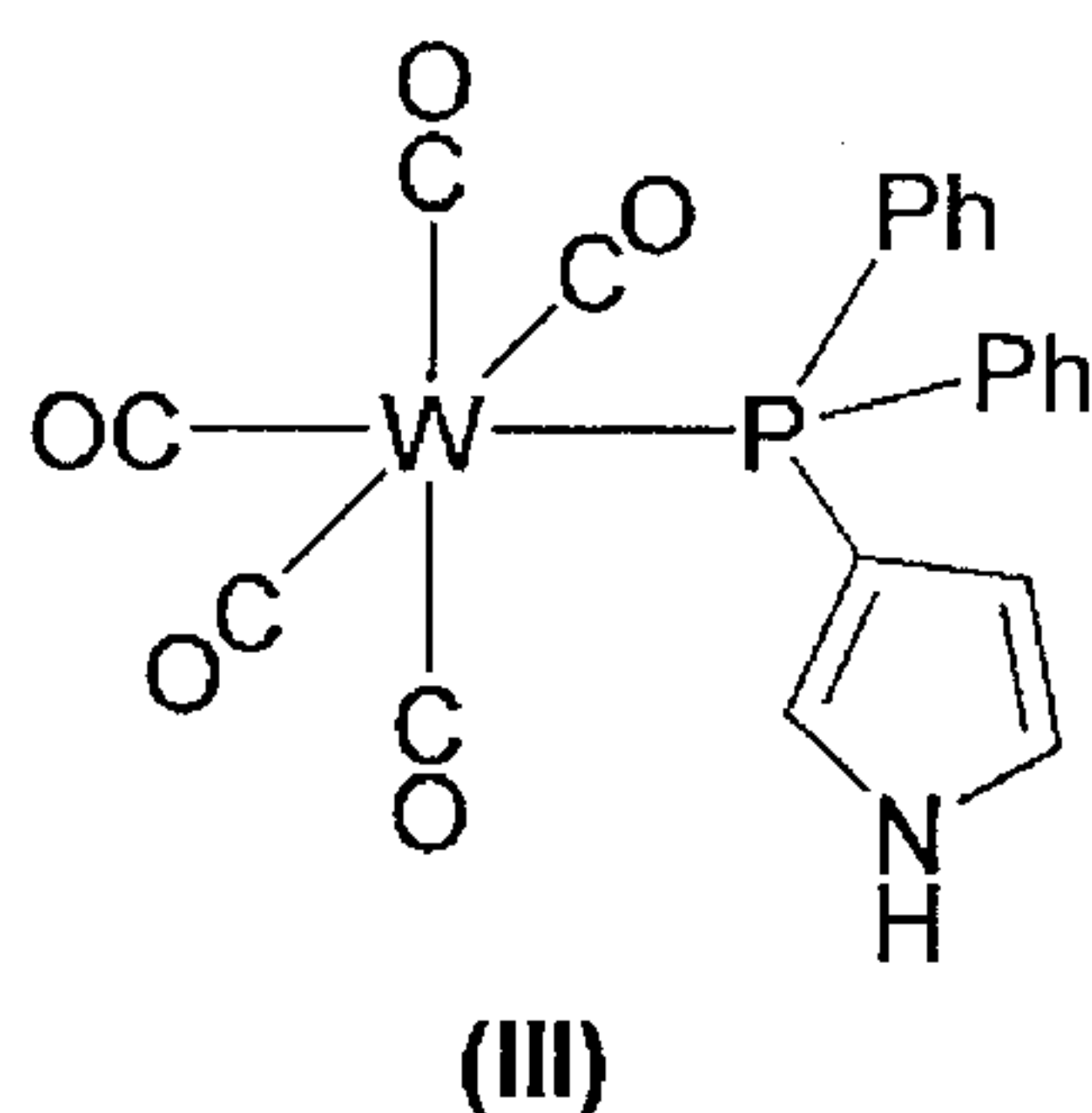
(IIb)



X = H, OMe,
NEt₂, CH₃,
Ph

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The compound may comprise the structure of Formula (III):



According to another aspect of the invention, there is provided use of a compound as described above for treating a bacterial infection.

5 According to another aspect of the invention, there is provided a method of treating a bacterial infection comprising administering to an individual in need of such treatment an effective amount of a compound as described above.

According to a further aspect of the invention, there is provided a method of preparing a medicament for treating a bacterial infection comprising admixing a
10 compound as described above with a suitable excipient.

According to yet another aspect of the invention, there is provided a compound as described above for treating a bacterial infection.

The individual may be an animal, for example a mammal, for example, a human, although as will be appreciated by one of skill in the art, uses such as for
15 example veterinary uses are also contemplated and are within the scope of the invention.

Furthermore, as discussed herein, given the wide range of bacterial targets sensitive to the compounds of the invention, the compounds may also be used for the treatment of bacterial infections of plants, that is, for the treatment or elimination of
20 plant pathogens.

According to another aspect of the invention, there is provided an antibacterial composition comprising a compound as described above.

According to another aspect of the invention, there is provided the use of a compound as described herein in an antibacterial composition.

25 According to another aspect of the invention, there is provided a method of

preparing an antibacterial composition comprising admixing a compound as described herein with a suitable excipient.

As will be appreciated by one of skill in the art, the antibacterial composition may be a pharmaceutical composition or a medicament for treatment of an animal, for example, a mammal, for example a human. Alternatively, the antibacterial composition may be used for the treatment of a non-human animal, for example but by no means limited to a livestock animal, a domestic animal or a wild animal.

The antibacterial composition may be formulated for agricultural use, that is, as an anti-bacterial agent against plant pathogens.

The antibacterial composition may be a disinfectant, for example but by no means limited to a spray, a gel, a powder or a solution for use in the environment, that is, ex vivo, for example, on surfaces that are to be cleaned, for example, kitchen or food preparation surfaces, floors, exterior body surfaces such as hands and the like, that is, anything that may need or benefit from disinfection prior to human contact.

The antibacterial composition may be used as a general disinfectant for surfaces, for example, on medical devices and/or medical instruments prior to contact with or insertion into patients.

As discussed herein, the compounds of the invention will break down under direct light in between about 8-16 hours. As discussed herein, that is sufficient time for the compounds of the invention to exert their effects on bacteria. Also, if it's maintained in the dark during the disinfection process, it maintains its activity.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Development of phosphopyricin (9bWC3) by derivatization.

Elimination of the phosphirene ring of antimicrobial compound 9aWC2 yielded compound 9bWC2, both of which had similar minimum inhibitory concentrations against *Staphylococcus aureus* K1-7. Substitution of the C2-bonded pyrrole of 9bWC2 with a thiophene, phenyl or indole reduced activity. Replacement of the C2-bonded pyrrole of 9bWC2 with a C3-bonded pyrrole resulted in phosphopyricin (9bWC3), and increased potency against *S. aureus*. Phosphopyricin had similar activity against the

Gram-positive *Enterococcus faecium* K02G0810 and *S. mutans*. Substitution of the tungsten pentacarbonyl group of phosphopyricin with either a molybdenum pentacarbonyl group or an oxide abolished activity ($< 1024 \mu\text{g/ml}$).

Figure 2. Properties of phosphopyricin. **a.** Minimum inhibitory concentration of phosphopyricin against *Staphylococcus aureus* K1-7. **b.** Activity of phosphopyricin against *S. aureus* K1-7 following exposure to 24 hours of light, as compared to dark and isopropanol (iso) controls. **c.** Recovery of *S. aureus* K1-7 from culture exposed to phosphopyricin or 9bWC2, relative to the antibiotic-free control. * $p < 10^{-5}$; ** $p < 10^{-6}$. **d.** Optical density of *S. enterica* incubated in the presence of either phosphopyricin with EDTA (PP+EDTA), EDTA alone (EDTA), phosphopyricin alone (PP), or with neither (culture control). Letters above error bars (s.d.) indicate statistically significantly different groups ($p < 0.005$).

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned hereunder are incorporated herein by reference.

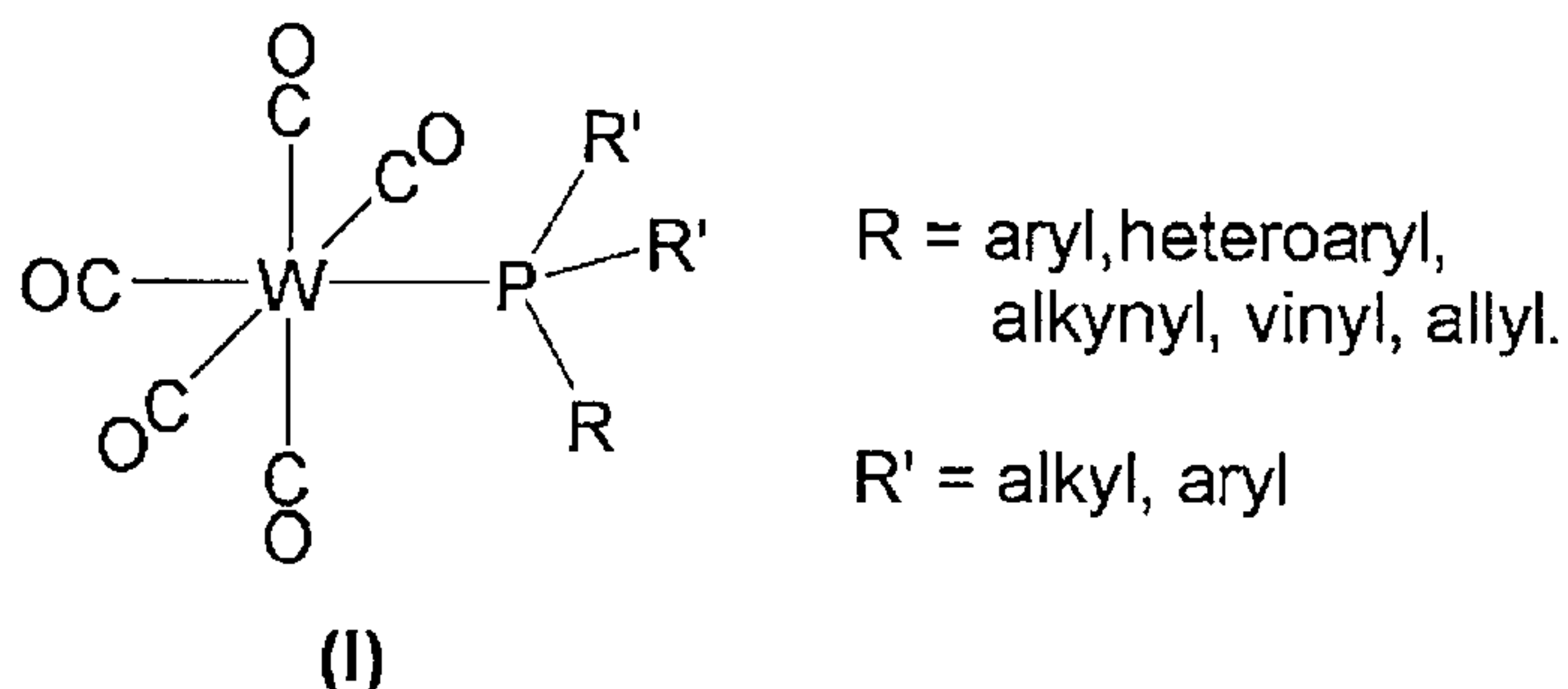
The acronymously named "ESKAPE" pathogens represent a group of bacteria that continue to pose a serious threat to human health, not only due to their propensity for repeated emergence, but also due to their ability to "escape" antibiotic treatment^{1,2}.

The evolution of multi-drug resistance in these pathogens alone has greatly outpaced the development of new therapeutics, necessitating an alternative strategy for antibiotic development that considers the evolutionary mechanisms driving antibiotic resistance. As described herein, we synthesized a novel inorganic antibiotic, phosphopyricin, which has antibiotic activity against the Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus*

faecium (VRE). As a synthetic compound that does not occur naturally, phosphopyricin would be evolutionarily foreign to microbes, thereby slowing the evolution of resistance. In addition, it degrades in light, and will break down in the general environment where strong selective pressures imposed by antibiotic residuals are known to accelerate antibiotic resistance. Phosphopyricin represents an innovation in bactericidals, having a synthetic core, and a photosensitive chemical architecture that would reduce accumulation in the environment.

Synthetic organic antibiotics based on conserved cores have expanded the pool of effective antibiotics in the short term³¹, yet their inherent common structure has enabled microbes to acquire new resistance mechanisms that target the common backbone, or to evolve resistance through incremental mutations of existing genetic determinants^{32,33}. Here we demonstrate that organophosphorus compounds, a group that has not been evaluated for antimicrobial activity in any systematic fashion, have the capacity to yield new architectures for clinically relevant pathogens. In addition, non-phosphate organophosphorus compounds have the incredible advantage over existing antibiotics in that these compounds do not occur naturally in the environment, such that resistance to these foreign compounds would not have already evolved. Although microbes have been shown to possess specific strategies for utilizing, metabolizing, or simply contending with phosphate compounds in the environment³⁴, the evolution of resistance to these synthetic organophosphorus compounds would be slowed considerably, since microbes are unlikely able to metabolize these xenobiotics^{35,36}. The exploration of synthetic organophosphorus compounds has the potential to make a significant impact for antimicrobial discovery, and to move the field in a new direction that considers not only molecular mechanisms of resistance, but also the evolutionary pressures that drive it.

According to an aspect of the invention, there is provided a compound comprising the structure of Formula (I):



wherein:

"W" is tungsten

"C" is carbon

5 "O" is oxygen

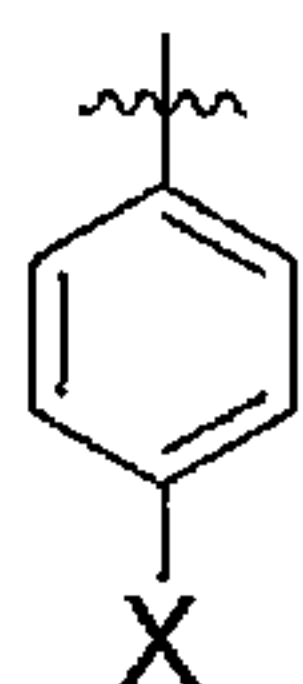
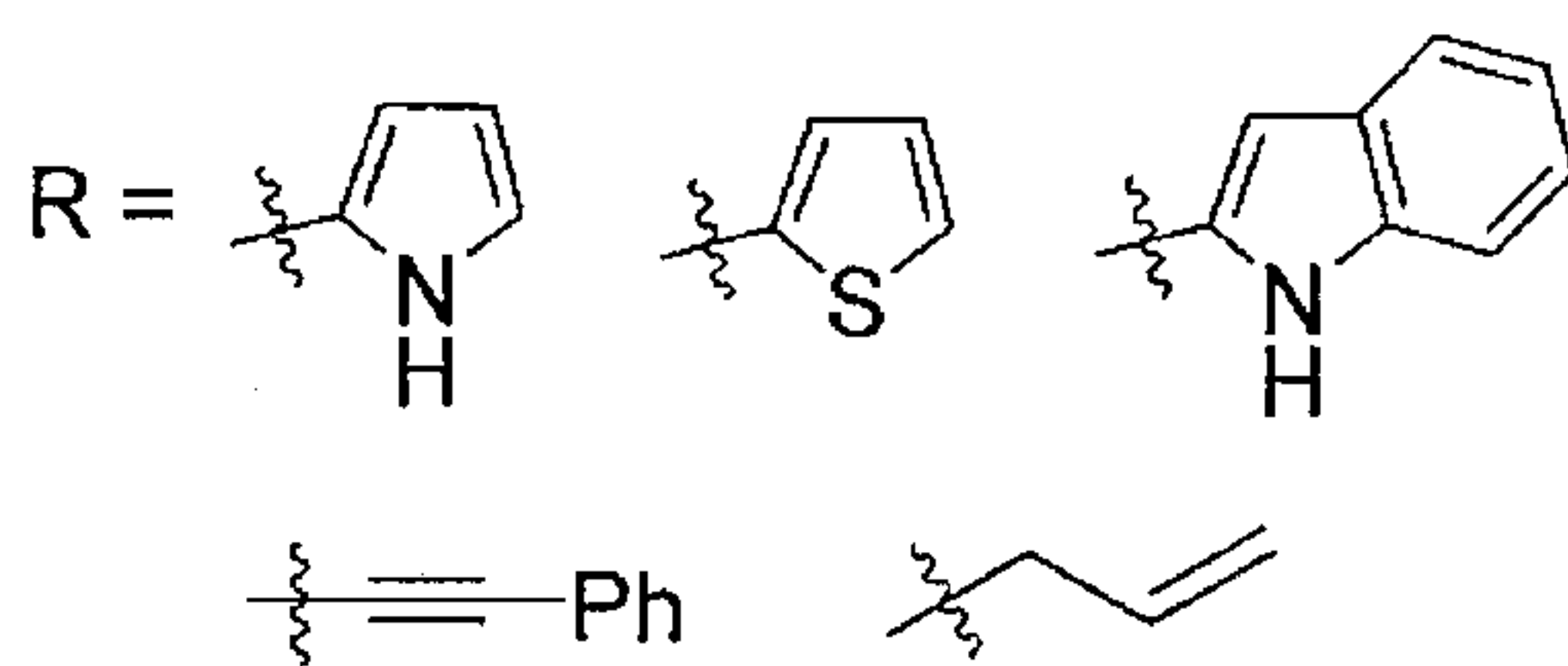
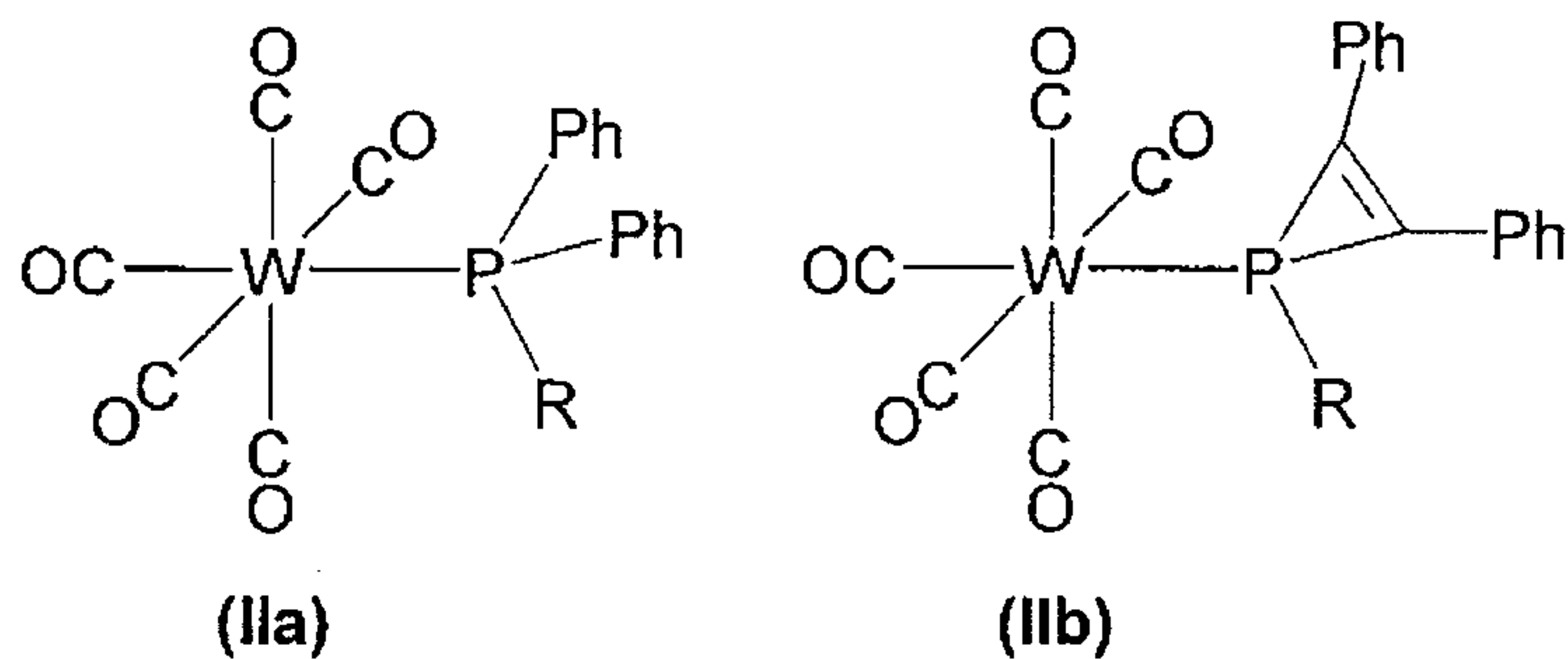
"P" is phosphorus

R is selected from substituted aryl (including, but not limited to anisyl, anisyl, tolyl, naphthyl), heteroaryl (including, but not limited to pyrrol, thienyl, indolyl, pyrazolyl, imidazolyl), alkynyl (C2-C6, phenylalkynyl), vinyl, cyclic vinyl and substituted vinyl (C2-C6), allyl, cyclic allyl and substituted allyl (C3-C7); and R' is alkyl or aryl (Ph, C1-C6 alkyl, C3-C6 cyclic alkyl).

As discussed herein, the R, P, and W(CO)₅ moieties are essential to the function of the compound. For example, variation of R changes the spectrum of antibacterial activity (ie different R groups result in antimicrobial activity against different groups of microbes) and increases or decreases MIC against specific microbes, while removal or replacement of W(CO)₅ eliminates antimicrobial activity.

R' is less essential to function, and variation of R' can be used to alter properties of the compound, for example, solubility and membrane permeability, as discussed below.

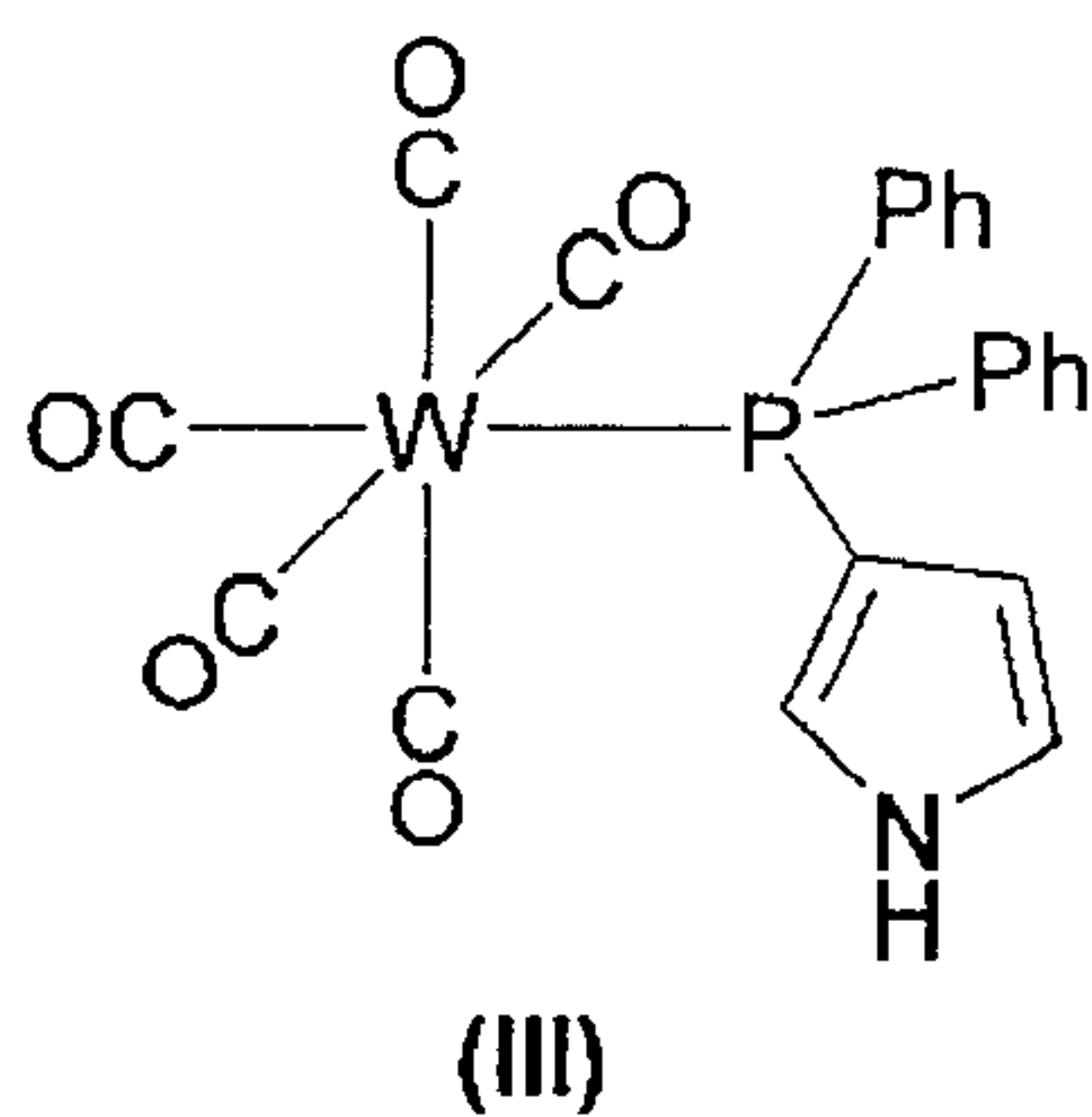
20 According to another aspect of the invention, there is provided a compound comprising the structure of Formula (IIa) or (IIb):



X = H, OMe,
NEt₂, CH₃,
Ph

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According to another aspect of the invention, there is provided a compound comprising the structure of Formula (III):

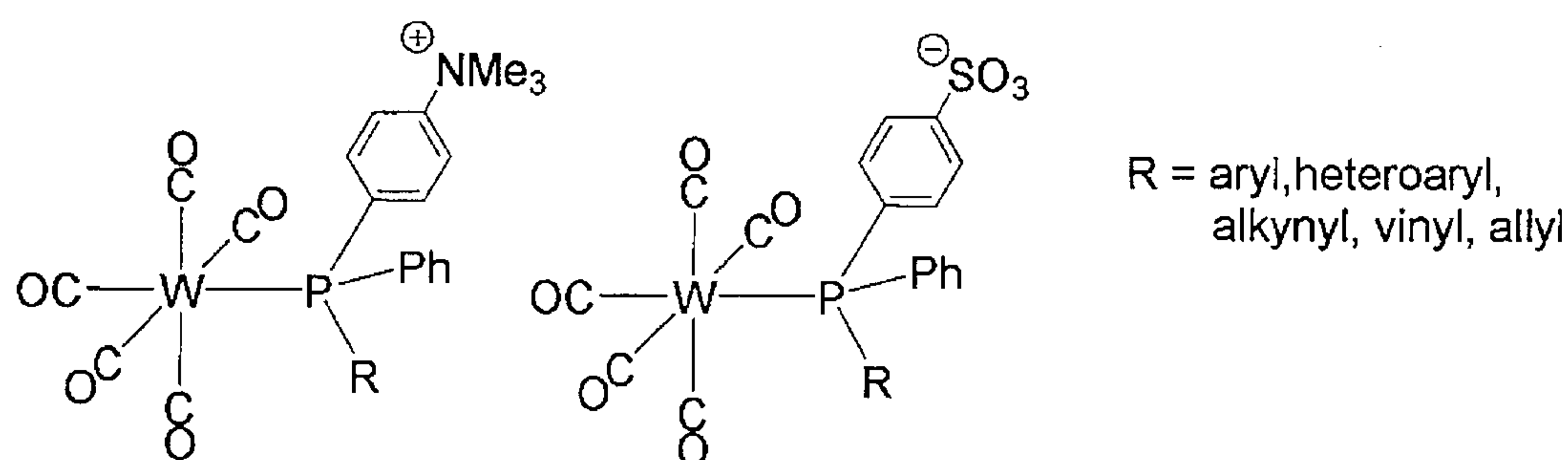


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As will be appreciated by one of skill in the art, many modifications can be made to a compound according to Formula (I), Formula (IIa), Formula (IIb) or Formula (III) which will alter and/or improve a beneficial property thereof. For example, water solubility of phosphopyricin can be improved by introducing ionic groups such as sulfonate (SO₃⁻) or quaternary ammonium (NR₃⁺) groups onto the functionally non-

10

essential auxiliary phenyl rings.



As discussed above, for example, the R' group can be modified. As such,
 5 many additional modifications to the compounds of Formula (I), Formula (IIa),
 Formula (IIb) and Formula (III), for example, to improve stability, improve solubility,
 improve permeability and the like, will be readily apparent to one of skill in the art.

As discussed herein, the compounds of Formula (I), Formula (IIa), Formula
 (IIb) and Formula (III) are light-labile. Specifically, the tungsten complex is susceptible
 10 to light-induced ligand dissociation, leading to a coordinatively unsaturated complex
 that is susceptible to oxidation, leading to decomposition and loss of antibacterial
 activity. In that absence of light, the complex remains coordinatively saturated,
 rendering it less susceptible to oxidation and decomposition, meaning that it will
 persist in biological systems long enough to be an effective antimicrobial. As will be
 15 appreciated by one of skill in the art, the presence of pharmaceutical compounds in
 the environment, particularly in water, is an on-going concern. Furthermore, when
 these pharmaceutical compounds are antibiotics, their presence in the environment
 can lead to development of bacterial resistance. However, because the compounds of
 the invention are light-labile, while they are effective when in the body in the patient,
 20 they quickly decompose within the environment, as discussed herein.

As discussed herein, the compounds of the invention will break down under
 direct light in between about 8-16 hours. As discussed herein, that is sufficient time for
 the compounds of the invention to exert their effects on bacteria. Also, if it's
 maintained in the dark during the disinfection process, it maintains its activity.

25 While not wishing to be bound to a particular theory or hypothesis, the

inventors believe that the mode of action of phosphopyricin involves competitive binding of molybdopterin, which normally binds molybdenum to form an integral protein cofactor, molybdenum cofactor. By taking the place of molybdenum, phosphopyricin may be interfering with key metabolic processes at multiple levels. In this scenario, bacteria that utilize molybdenum as a cofactor would be susceptible to phosphopyricin, unlike those bacteria and members of the Archaea that normally utilize tungsten (most often extremophiles, such as *Desulfovibrio*, *Thermococcus*, *Methanobacterium*, *Pyrococcus*). Because of the universality of its target, phosphopyricin is a general antibacterial that is active against a broad range of bacteria. Consistent with this, biological activity was demonstrated for several Gram-positive bacteria within the class *Bacilli* including species of *Staphylococcus* (order *Bacillales*), and both *Streptococcus* and *Enterococcus* (order *Lactobacillales*), suggesting that there would be biological activity against other clinically relevant pathogens that are also within these orders such as *Bacillus* (anthrax) and *Listeria*. Similarly, the demonstration that *Salmonella* can be made susceptible in the presence of EDTA is strongly suggestive that the target is present in Gram-negatives and that the antibiotic would also be effective against a broad range of other pathogens such as *E. coli*, *Klebsiella* and *Enterobacter*, which exhibit extended-spectrum resistance against several major classes of antibiotics.

According to another aspect of the invention, there is provided a method of treating a bacterial infection in an individual in need of such treatment comprising administering to an individual who has a bacterial infection or who is suspected of having a bacterial infection an effective amount of a compound as set forth in Formula (I), Formula (IIa), Formula (IIb) or Formula (III).

As will be appreciated by one of skill in the art, an individual in need of such treatment is a person who is known to be or is suspected of suffering from a bacterial infection. As will be appreciated by one of skill in the art, symptoms associated with bacterial infections vary greatly but are well known to those of skill in the art.

As discussed above, while not wishing to be bound to a particular theory or hypothesis, the inventors believe that the mode of action of phosphopyricin involves

competitive binding of molybdopterin, which normally binds molybdenum to form an integral protein cofactor, molybdenum cofactor. By taking the place of molybdenum, phosphopyricin may be interfering with key metabolic processes at multiple levels. In this scenario, bacteria that utilize molybdenum as a cofactor would be susceptible to phosphopyricin.

Accordingly, the bacterial infection treated by a compound as set forth in Formula (I), Formula (IIa), Formula (IIb) or Formula (III) may be caused by any bacteria, as discussed herein.

For example, the bacteria may be Gram-positive bacteria selected from for example but by no means limited to the class *Bacilli* including species of *Staphylococcus* (order *Bacillales*), *Streptococcus* and *Enterococcus* (order *Lactobacillales*), *Bacillus* (anthrax) and *Listeria*.

Alternatively, the bacteria may be Gram-negative bacteria, for example, but by no means limited to *Salmonella*, *E. coli*, *Klebsiella* and *Enterobacter*.

As discussed above, the compounds described herein have wide applicability and have been demonstrated to have effectiveness against a wide variety of bacteria. As such, it is to be understood that specific bacterial strains identified herein are for illustrative purposes.

The individual may be an animal, for example a mammal, for example, a human, although as will be appreciated by one of skill in the art, uses such as for example veterinary uses are also contemplated and are within the scope of the invention.

Furthermore, as discussed herein, given the wide range of bacterial targets sensitive to the compounds of the invention, the compounds may also be used for the treatment of bacterial infections of plants, that is, for the treatment or elimination of plant pathogens.

According to another aspect of the invention, there is provided an antibacterial composition comprising a compound as described above.

According to another aspect of the invention, there is provided the use of a compound as described herein in an antibacterial composition.

According to another aspect of the invention, there is provided a method of preparing an antibacterial composition comprising admixing a compound as described herein with a suitable excipient.

As will be appreciated by one of skill in the art, the antibacterial composition
5 may be a pharmaceutical composition or a medicament for treatment of an animal, for example, a mammal, for example a human.

Alternatively, the antibacterial composition may be used for the treatment of a non-human animal, for example but by no means limited to a livestock animal, a domestic animal or a wild animal.

10 The antibacterial composition may be formulated for agricultural use, that is, as an anti-bacterial agent against plant pathogens. As will be appreciated by one of skill in the art, in these embodiments, the antibacterial composition may be formulated for administration as a spray, for example as a hand-held spray or pump spray for administration to individual plants or as a spray from a tank, tanker, sprayer or similar
15 agricultural equipment.

The antibacterial composition may be a disinfectant, for example but by no means limited to formulated as a spray, a solution, a gel or a powder for use in the environment, that is, ex vivo, for example, on surfaces that are to be cleaned, for example, kitchen or food preparation surfaces, floors, exterior body surfaces such as
20 hands and the like, that is, anything that may need or benefit from disinfection prior to human contact.

The antibacterial composition may be used as a general disinfectant for surfaces, for example, on medical devices and/or medical instruments prior to contact with or insertion into patients.

25 As used herein, an effective amount refers to an amount of the compound that is sufficient to reduce at least one of the symptoms associated with the bacterial infection, for example, reducing colony forming units per ml or reducing severity of symptoms associated with the bacterial infection. For example, the daily adult dose for treating Gram-positive infections using approved antibiotics having a MIC < 16
30 ug/mL range between 200-2500 mg. It is further noted that an effective amount can

be determined through routine experimentation and is within the scope of the invention.

For example, an effective amount may be an amount that is sufficient to reduce the severity of at least one symptom associated with a bacterial infection, for example, reducing fever, reducing colony forming units per ml, reducing swelling, reducing pain, reducing redness, reducing shaking, improving low blood pressure, reducing severity of conjunctivitis, reducing irritation, reducing nausea, reducing general feeling of discomfort and reducing congestion.

The invention will now be further elucidated by way of examples; however, the invention is not necessarily limited by or to the examples.

We used metal-mediated electrophilic substitution²⁰⁻²² to rapidly generate a diverse library of synthetic organophosphorus compounds containing carbon-phosphorus bonds. One compound in the library, 9aWC2, a tungsten phosphine complex (Figure 1), showed inhibitory activity against *Staphylococcus aureus* K1-7 at a minimum inhibitory concentration (MIC) of 4 – 8 µg/mL. This promising candidate antimicrobial was modified to eliminate the synthetically challenging phosphirene ring, yielding compound 9bWC2, which exhibited similar potency towards *S. aureus* (MIC of 4 – 8 µg/mL) (Figure 1). To identify features of 9bWC2 that were essential for antimicrobial activity, we systematically varied chemical side groups, beginning with substitution of the C2-bound pyrrole with C2-bound thiophene, which resulted in loss of antimicrobial activity against *S. aureus* (11bW, MIC > 1024 µg/mL) (Figure 1). Substitution of pyrrole with indole resulted in slightly reduced potency against *S. aureus* (10bW, MIC of 8 – 16 µg/mL), while substitution of the pyrrole with a phenyl group, significantly reduced potency against *S. aureus* (12bW, 256 – 512 µg/mL), but increased the spectrum of activity to include Gram-negative, *Klebsiella* sp. and *Salmonella* sp. (MIC of 256 – 512 µg/mL) (Figure 1). We then replaced the C2-bound pyrrole ring with a C3-bound pyrrole (9bWC3), resulting in an increased potency against *S. aureus*, from a MIC of 4 – 8 µg/ml to 2 – 4 µg/ml (Figure 2A). An evaluation of the potency of this compound against the Gram-positive *Enterococcus faecium* K02G0810 and *S. mutans* revealed that both were also inhibited at MICs of 2 – 4

µg/mL, and spot testing on bacterial overlay plates containing all three Gram-positive bacteria revealed no spontaneously resistant mutants. This compound, 9bWC3, was named phosphopyricin (Figure 1). Substitution of the tungsten pentacarbonyl group of phosphopyricin with molybdenum pentacarbonyl (9bMoC3) or oxygen (9bOC3) abolished detectable antibiotic activity (below 1024 µg/mL), suggesting an important role for the W(CO)₅ fragment, and the P-bound N-heterocycle.

We observed two important properties of 9bWC2 and phosphopyricin (9bWC3). Firstly, we noted that potency of 9bWC2 decreased significantly upon exposure to light. After 4 hours of fluorescent light exposure at 92.7 µmol/s .m², the MIC of 9bWC2 for *S. aureus* K1-7 increased from 4 – 8 µg/mL to 8 – 16 µg/mL, suggesting photolytic degradation. Like its parent compound, phosphopyricin activity was also markedly reduced after 24 hours in continuous fluorescent light at the same intensity (Figure 2B), suggesting that phosphopyricin would photodegrade gradually in the general environment. Secondly, we determined that both 9bWC2 and phosphopyricin were bactericidal. Bacteriostatic agents are able to arrest bacterial growth, while bactericidal agents compromise bacterial cell viability and eradicate >99.9% of a given inoculum ²³. Approximately 5 x 10⁵ cfu of *S. aureus* K1-7 was exposed to either 50 µg/mL phosphopyricin or 9bWC2 for 24 hours in 1 mL 10 mM MgSO₄ buffer, and the viability of the bacteria subsequently assessed. Relative to the antibiotic-free control, viable cell number was reduced by 99.9993% for 9bWC2, and 100% for phosphopyricin (Figure 2C). Given this potency, we assessed its activity against the Gram-negative *Pseudomonas aeruginosa*, *S. enterica* subsp. Typhimurium 14028, *Cronobacter* sp. 12202, *Enterobacter* sp. TX1, *Klebsiella* sp. B011499, and *Acinetobacter baumannii* ATCC17978, and all were shown to be resistant (>1024 µg/mL). The outer membrane of Gram-negative strains can act as a barrier to foreign agents, including antimicrobials, but can be destabilized with compounds like the chelating agent, ethylenediaminetetraacetic acid (EDTA) ^{24,25}. For example, the Gram-negative *S. enterica* subsp. Typhimurium 14028 becomes sensitive to the Gram-positive-specific antibiotic, nisin, in the presence of EDTA, which destabilizes the outer membrane, thereby overcoming physical exclusion of the

antibiotic from the cell ²⁴. We incubated *S. enterica* subsp. Typhimurium 14028 for 24 hours at 37°C in the presence of 32 µg/mL phosphopyricin, with and without 1.5 mM EDTA. Bacterial titres were reduced significantly when EDTA was combined with phosphopyricin, as compared to cultures containing only phosphopyricin (Tukey's
 5 HSD, $p < 2 \times 10^{-12}$) (Figure 2D). This suggests that the target(s) of phosphopyricin are also present in Gram-negative bacteria, and that the spectrum of activity is determined by the ability of the compound to cross the bacterial cell wall. Thus, the spectrum of activity of phosphopyricin can be expanded with additives that increase bacterial cell wall permeability. Phosphopyricin contains a transition metal, tungsten,
 10 which could bind competitively to essential proteins where other co-factors, like molybdenum cofactor, would normally bind ²⁶.

Methods

Bacterial isolates and culturing conditions

15 *Staphylococcus aureus* K1-7, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecium* K02G0810, *Cronobacter* sp. 12202, *Enterobacter* sp. TX1, *Klebsiella* sp. B011499, *Klebsiella* sp. G4061350, *Acinetobacter baumannii* ATCC17978, and *Streptococcus mutans* UAIS9:wt were cultured in lysogeny broth (LB, BD Biosciences) at 37 °C, except for *E. faecium* K02G0810, which was cultured
 20 in brain-heart infusion (BHI, BD Biosciences) medium at 37 °C.

Chemical synthesis and derivatization

Compounds 9aWC2, 9bWC2, 9bWC3, 10bW, and 11bW were synthesized using published procedures ²⁰⁻²². To synthesize O=PPh₂(C₄H₃NH) (9bO), compound
 25 [W(CO)₅{PPh₂(C₄H₃NH))}] (9bW, 60 mg, 0.104 mmol, 2 isomers) and dppe (43.6 mg, 0.110 mmol) were dissolved in THF (2 mL) and irradiated with UV for 2 h, resulting in a color change from colorless to yellow. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography (silica gel, 50/50 v/v diethyl ether/petroleum ether). After purification, the free phosphine oxide
 30 {O=PPh₂(C₄H₃NH))} (9bO, 2 isomers) was obtained as a white powder. Yield: 17 mg,

62%. $^{31}\text{P}\{^1\text{H}\}$ NMR (CDCl_3): δ 16.7 [$\text{O}=\text{PPh}_2(2\text{-C}_4\text{H}_3\text{NH})$] and 18.2 [$\text{O}=\text{PPh}_2(3\text{-C}_4\text{H}_3\text{NH})$].

The synthesis of $[\text{W}(\text{CO})_5\{\text{PPh}_3\}]$ (12bW) was carried out by first adding $(\text{CH}_3)_3\text{NO}\cdot 2\text{H}_2\text{O}$ (66.3 mg, 0.597 mmol) to a solution of $[\text{W}(\text{CO})_6]$ (200 mg, 0.568 mmol) in acetonitrile (15 mL), in small portions, over 5 min. The resulting yellow solution was stirred for 40 min, and the solvent was removed under reduced pressure. The residue was dissolved in toluene (2 mL) and the solvent was again removed under reduced pressure. The crude $[\text{W}(\text{CO})_5\{\text{CH}_3\text{CN}\}]$ was dissolved in THF (15 mL), PPh_3 (164 mg, 0.625 mmol) was added, and the mixture was heated at 50 °C for 16 h. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography (silica gel, 10/90 diethyl ether/petroleum ether). The white product was crystallized by cooling a saturated hexane/diethylether solution to -20 °C. Yield: 35%. IR (ν_{CO} , CH_2Cl_2 , cm^{-1}): 2071(w), 1944(vs). $^{31}\text{P}\{^1\text{H}\}$ NMR (CDCl_3): δ 21.6 ($^1J_{\text{PW}} = 244$ Hz).

To synthesize $[\text{Mo}(\text{CO})_5\{\text{PPh}_2(\text{C}_4\text{H}_3\text{NH})\}]$ (9bMo), a solution of $\text{Mo}(\text{CO})_6$ (2.00 g, 7.58 mmol, MW= 264.01 g/mol) and PPh_2Cl (1.36 mL, 9.09 mmol) in dry toluene (50 mL) was heated under reflux for 90 minutes resulting in a colour change from yellow to amber colour. The volume was reduced under vacuum to ~10 mL, and 10 mL of Petroleum ether was added. The solution was filtered and anhydrous alumina (1 g) was added, the mixture was shaken and then filtered. The filtrate was evacuated to yield $[\text{Mo}(\text{CO})_5(\text{PPh}_2\text{Cl})]$ as a pale yellow powder. Yield: 2.25 g (65%). $^{31}\text{P}\{^1\text{H}\}$ NMR = δ 123, IR (ν_{CO} , CH_2Cl_2 , cm^{-1}): 2081, 1969, 2020 cm^{-1} . Silver trifluoromethanesulfonate (90 mg, 0.350 mmol) was added to a solution of $[\text{Mo}(\text{CO})_5(\text{PPh}_2\text{Cl})]$ (80 mg, 0.175 mmol) in CH_2Cl_2 (2 mL). The solution was stirred for 3 hours, and then filtered to remove AgCl . Pyrrole (24 μL , 0.350 mmol) was added 24 μL , the solution was stirred for 5 minutes and then the solvent was evaporated under reduced pressure. The residue was purified using column chromatography (silica gel, 10:90 v/v diethyl ether/petroleum ether) to yield 30 mg of a white powder, which was shown to be $[\text{Mo}(\text{CO})_5(\text{PPh}_2(\text{C}_4\text{H}_3\text{NH}))]$ (2 isomers). Yield: 37%. $^{31}\text{P}\{^1\text{H}\}$ NMR (CDCl_3): δ 15.8, and 15. Yield = 37%. $^{31}\text{P}\{^1\text{H}\}$ NMR = δ 15.8; δ 15. For bioassays, powdered

organophosphorus compounds were resuspended in either 100% isopropanol (9aWC2, 9bWC2, 9bWC3, 9bOC3, 10bW), dimethylsulfoxide (11bW) or a 50:50 isopropanol-tetrahydrofuran (v/v) solution (9bMoC3).

Minimum inhibitory concentration and bactericidal assays

5 The minimum inhibitory concentrations (MIC) of each compound was assessed in 96-well plates by serial dilution of a 10 mg/mL stock solution of the organophosphorus compound in LB or BHI medium from 1 µg/mL to 1024 µg/mL (one per column). A no-compound (solvent-only) control was used in the final column of the 12-column plate. Approximately 1×10^5 cfu of bacteria was added to each well to a
10 final volume of 200 µL, the microplate covered with sterile, breathable rayon film (VWR International), and incubated at 30°C with shaking at 220 RPM for 24 hours.

To evaluate bacteriostatic or bactericidal activity, an overnight culture of *S. aureus* K1-7 in LB medium was diluted to yield 1 mL suspensions of 5×10^5 cfu in 10 mM MgSO₄. Each suspension was treated with a final concentration of 50 µg/mL of
15 either 9bWC2 or 9bWC3 (in isopropanol). Control treatments were supplemented with an equal volume of isopropanol. Cultures were shaken at 220 RPM for 24 hours at 21°C, and bacterial survivorship assessed by colony enumeration of serial dilutions plated on LB.

EDTA Assay

20 To evaluate the effects of phosphopyricin on Gram-negative bacteria, we adapted the protocol of Stevens et al.²⁴. *Salmonella typhimurium* 14028 was cultured in BHI broth at 37 °C overnight, and 1 mL of bacterial culture (OD_{600nm} = ~1.25) was transferred to a 1.5 mL microfuge tube and bacteria pelleted by centrifugation (1 minute at $12.0 \times 1000 \text{ min}^{-1} \times g$). The supernatant was removed, and cells were re-
25 suspended in 1 mL 10 mM MgSO₄. Using a 96-well plate, 1×10^4 cfu were incubated in BHI broth containing 1.5 mM EDTA solution, 32 µg/mL phosphopyricin, or both at 37 °C for 24 hours. *S. typhimurium* cells grown in BHI broth alone served as a no-treatment control.

Photolysis assay

Glass vials containing compound stock solution of 9bWC3 in isopropanol at a concentration of 10 mg/mL were placed in a light chamber at 21°C, and subjected to either 8, 16 or 24 hours of 92.7 $\mu\text{mol/s}\cdot\text{m}^2$ continuous fluorescent light generated by three, 60 cm Philips F20T12/CW, 20-watt bulbs. The amount of light was measured with a Li-cor Quantum / Radiometer / Photometer (LI-189). Control vials were wrapped in aluminum foil and placed in the same conditions. Following treatment, compounds were spotted on overlay media plates containing *S. aureus* K1-7, as prepared previously ³⁷.

Statistical analysis

For the EDTA assay, SPSS Statistics was used to perform a one-way ANOVA, which identified a statistically significant difference between groups ($F(3,14) = 374.102$, $p = 1.3 \times 10^{-13}$). A Tukey post hoc test revealed statistically significant differences in growth between the phosphopyricin with EDTA treatment ($\text{OD}_{600\text{nm}} = 0.407 \pm 0.077$) and the other three treatment groups: only EDTA ($\text{OD}_{600\text{nm}} = 1.236 \pm 0.021$), only phosphopyricin ($\text{OD}_{600\text{nm}} = 1.4420 \pm 0.053$), and no treatment ($\text{OD}_{600\text{nm}} = 1.260 \pm 0.037$); $p < 1.3 \times 10^{-11}$. Levene's test of homogeneity showed no statistically significant differences between variances across treatments (1.421, $p = 0.279$).

Statistical evaluation of bactericidal activity of 9bWC2 and 9bWC3 (phosphopyricin) was carried out with SPSS Statistics using the Mann-Whitney test, which indicated statistically significant differences between the control group (supplemented with isopropanol) and both 9bWC2 ($U = 0$, $p < 2 \times 10^{-6}$) and phosphopyricin ($U = 0$, $p < 3 \times 10^{-7}$).

The scope of the claims should not be limited by the preferred embodiments set forth in the examples but should be given the broadest interpretation consistent with the description as a whole.

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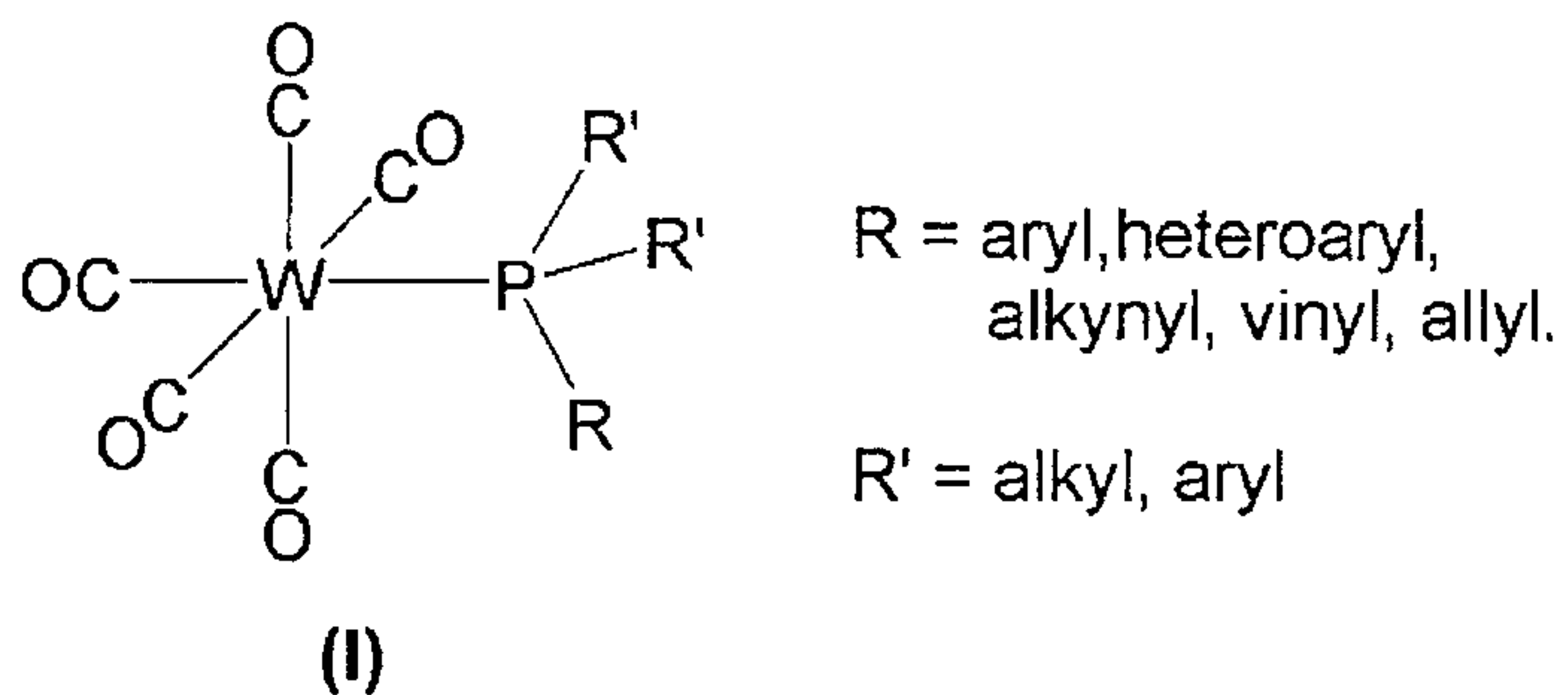
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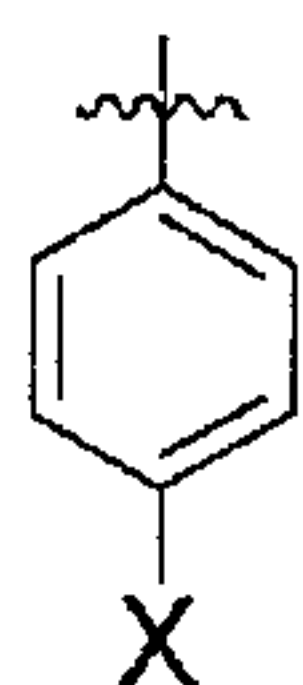
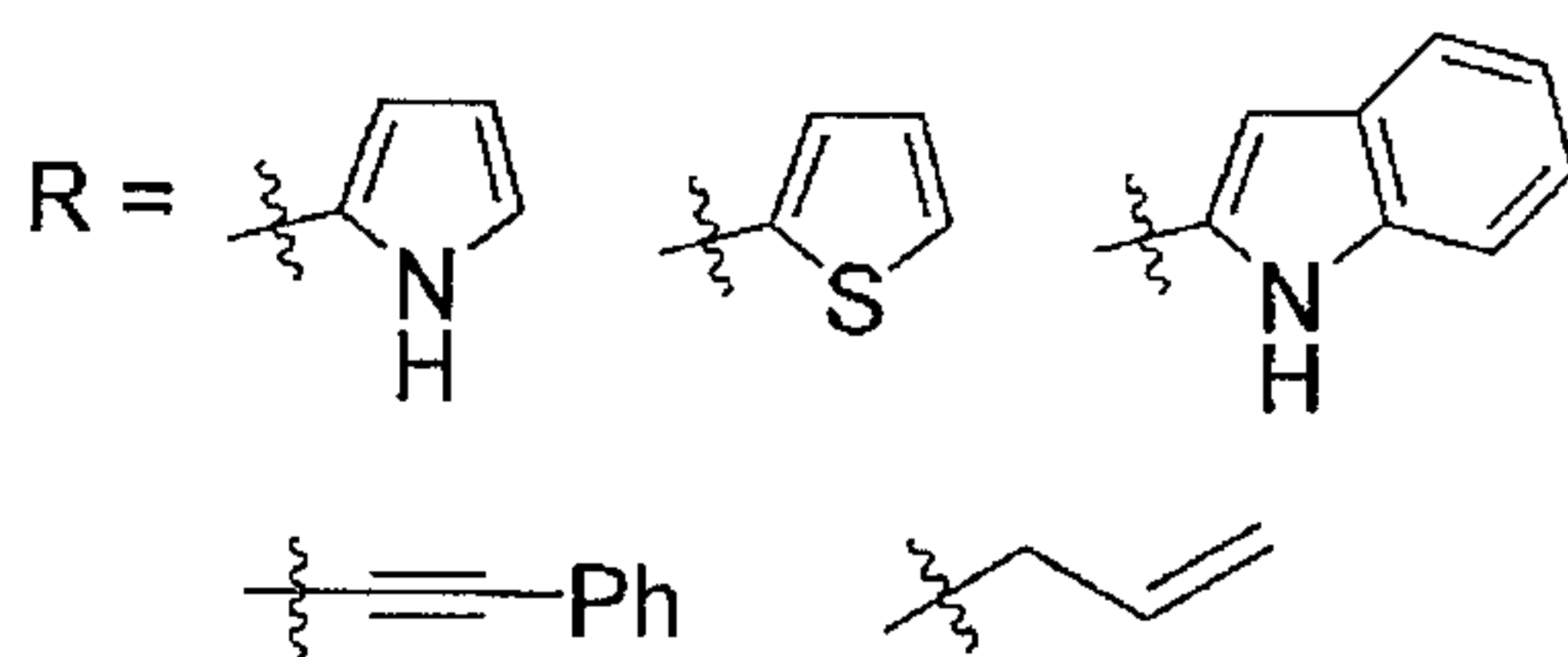
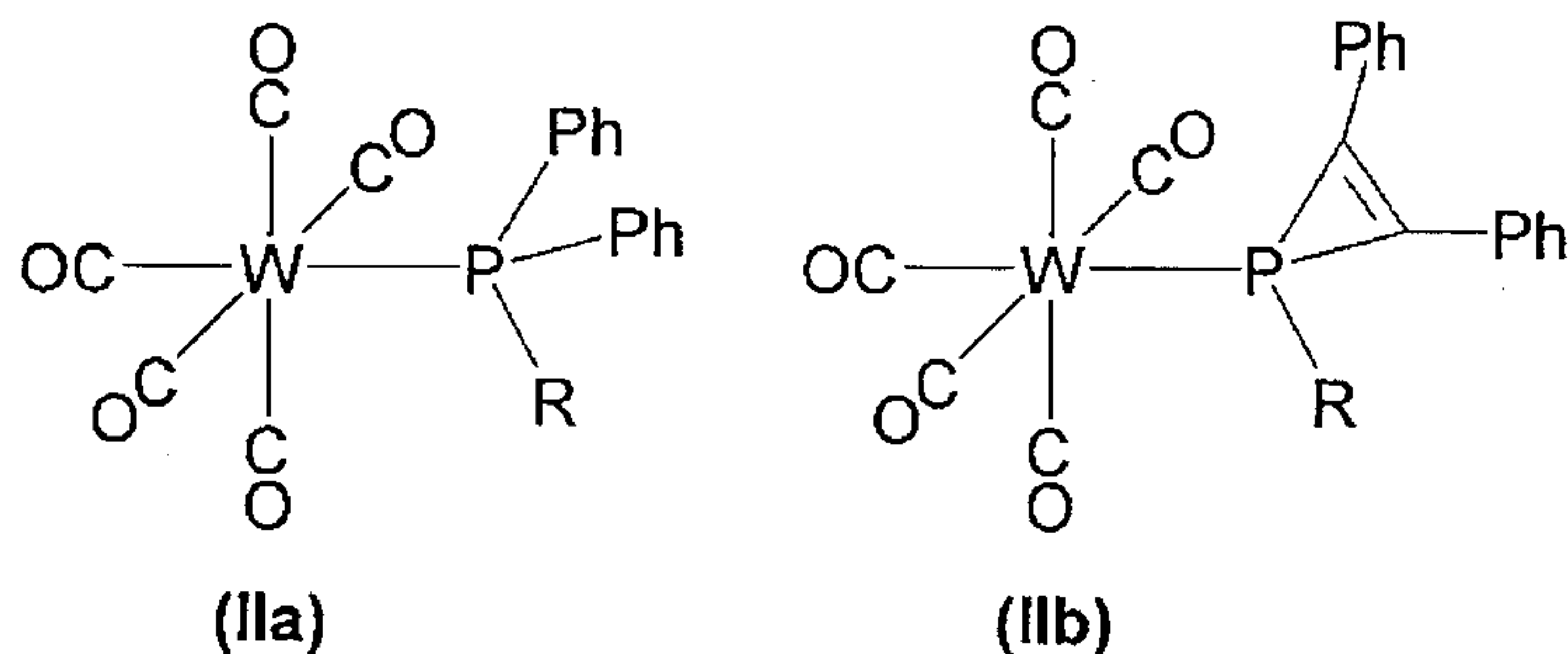
CLAIMS

1. A compound comprising the structure of Formula (I):



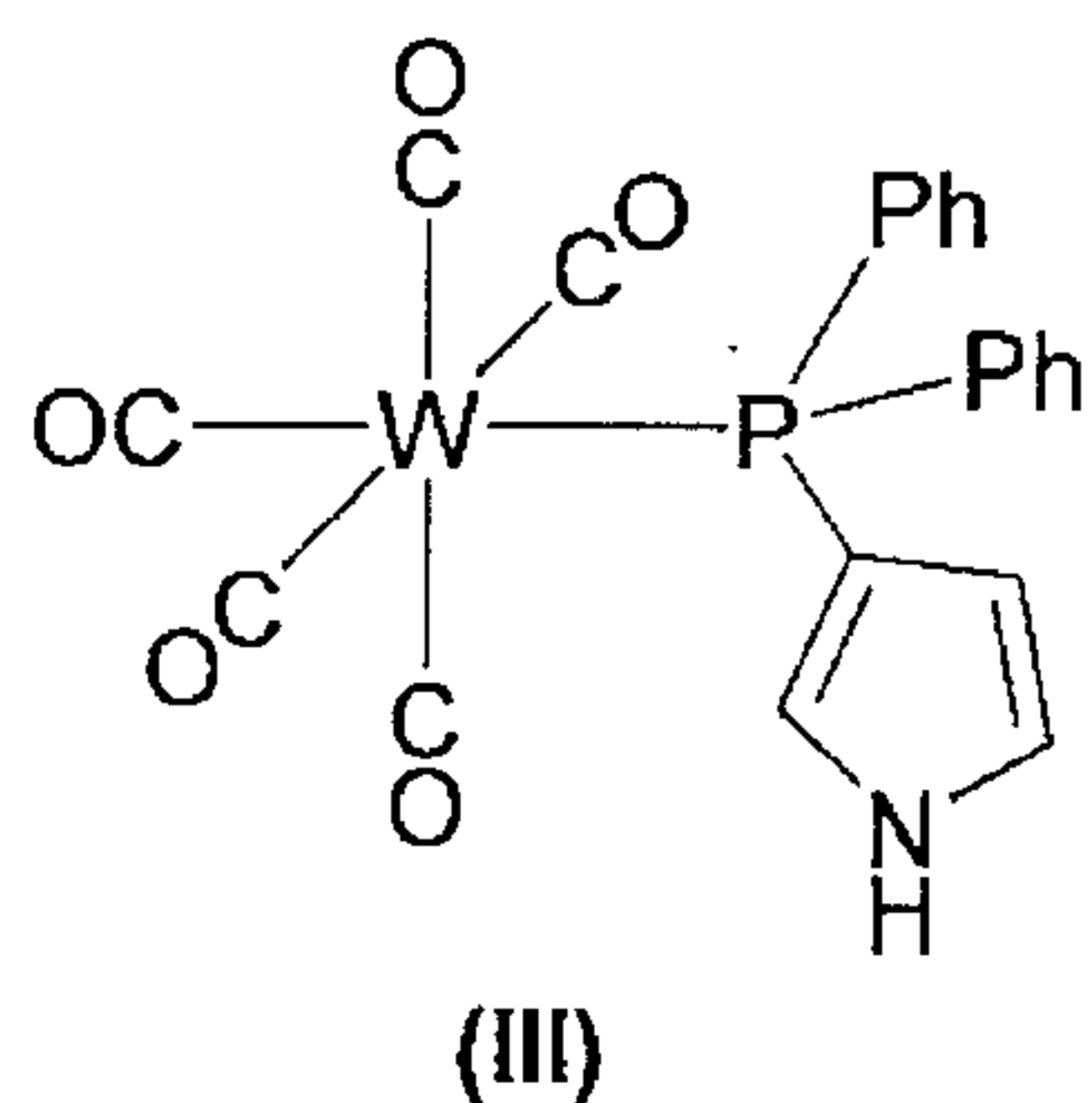
wherein:

- 5 "W" is tungsten;
 "C" is carbon;
 "O" is oxygen;
 "P" is phosphorus;
 R is selected from substituted aryl, alkynyl, vinyl, cyclic vinyl, substituted vinyl, allyl,
 10 cyclic allyl and substituted allyl; and
 R' is alkyl or aryl.
2. The compound according to claim 1 wherein the compound comprises the structure of Formula (IIa) or (IIb):



X = H, OMe,
NEt₂, CH₃,
Ph

3. The compound according to claim 1 wherein the compound comprises the structure of Formula (III):



- 5 4. Use of a compound according to any one of claims 1 to 3 for treating a bacterial infection.
5. The use according to claim 4 wherein the bacterial infection is of an animal.
6. The use according to claim 5 wherein the bacterial infection is of
- 10 a human.
7. The use according to claim 4 wherein the bacterial infection is of

a plant.

8. A method of treating a bacterial infection comprising administering to an individual in need of such treatment an effective amount of a compound according to any one of claims 1-3.

5 9. A method of preparing a medicament for treating a bacterial infection comprising admixing a compound according to any one of claims 1 to 3 with a suitable excipient.

10. A compound according to any one of claims 1 to 3 for treating a bacterial infection.

10 11. An antibacterial composition comprising a compound according to any one of claims 1 to 3.

12. Use of a compound according to any one of claims 1 to 3 in an antibacterial composition.

15 13. A method of preparing an antibacterial composition comprising admixing a compound according to any one of claims 1 to 3 with a suitable excipient.

14. The method according to claim 13 wherein the excipient is an excipient suitable for agricultural use.

15. The method according to claim 13 wherein the excipient is suitable for formulation of a spray.

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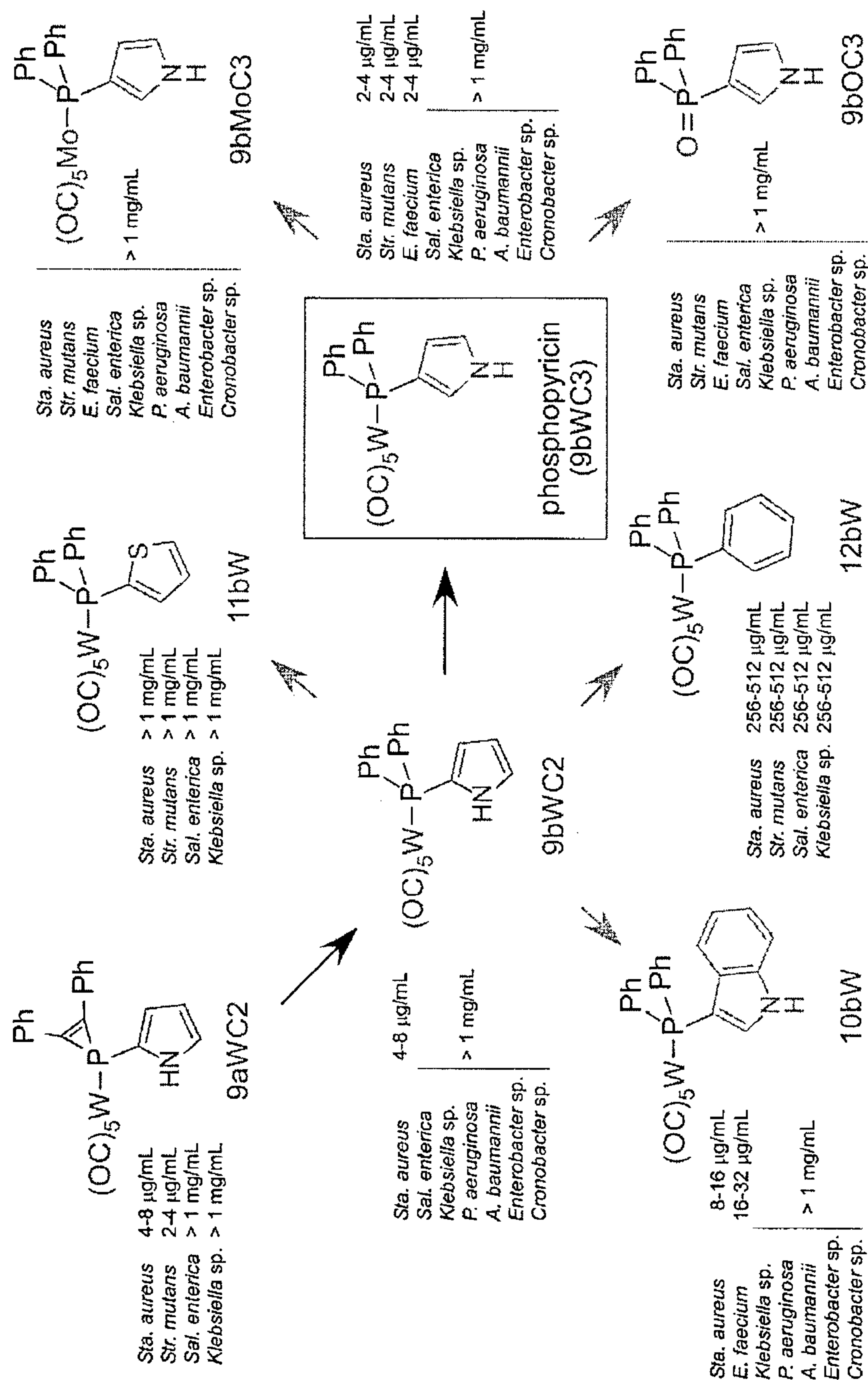


Figure 1

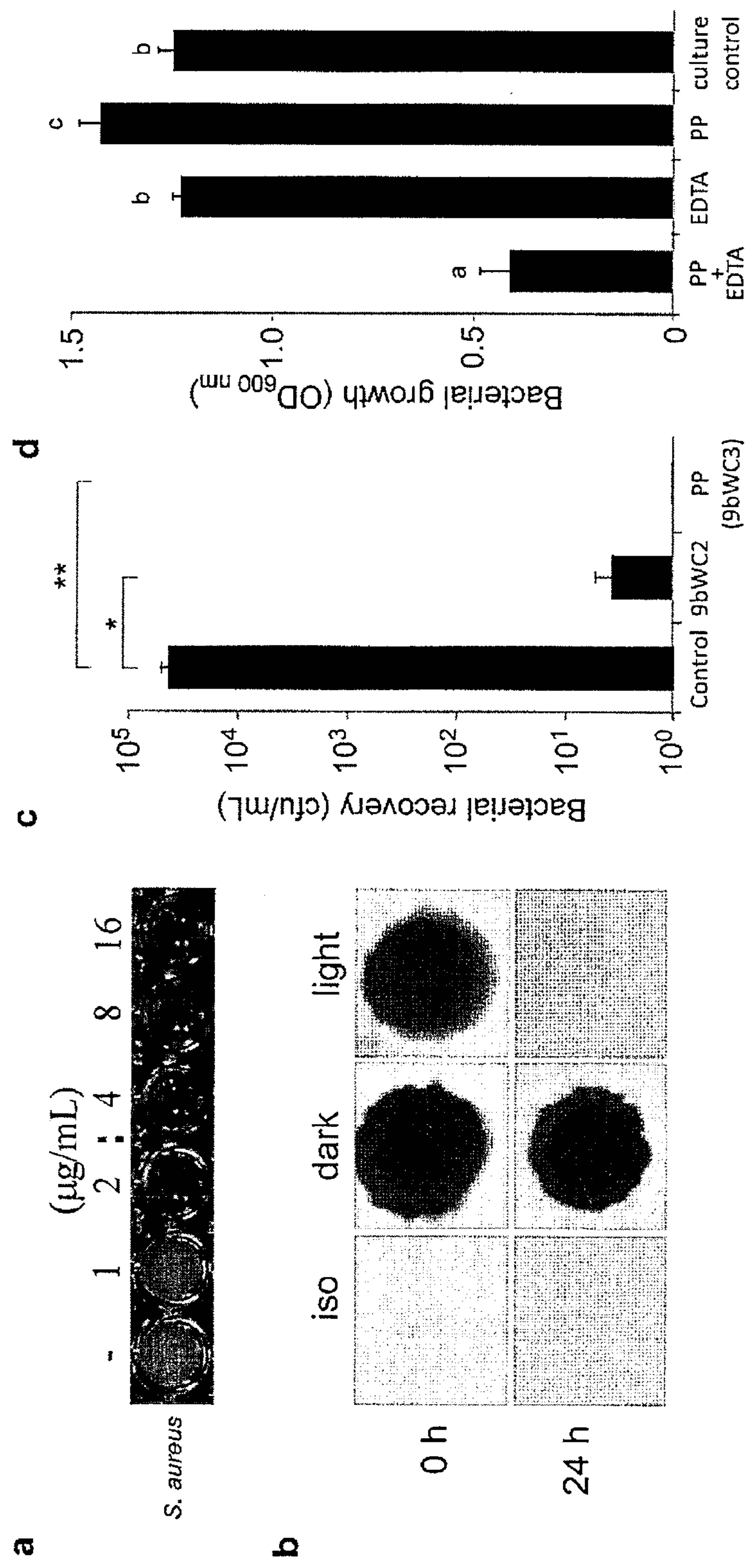
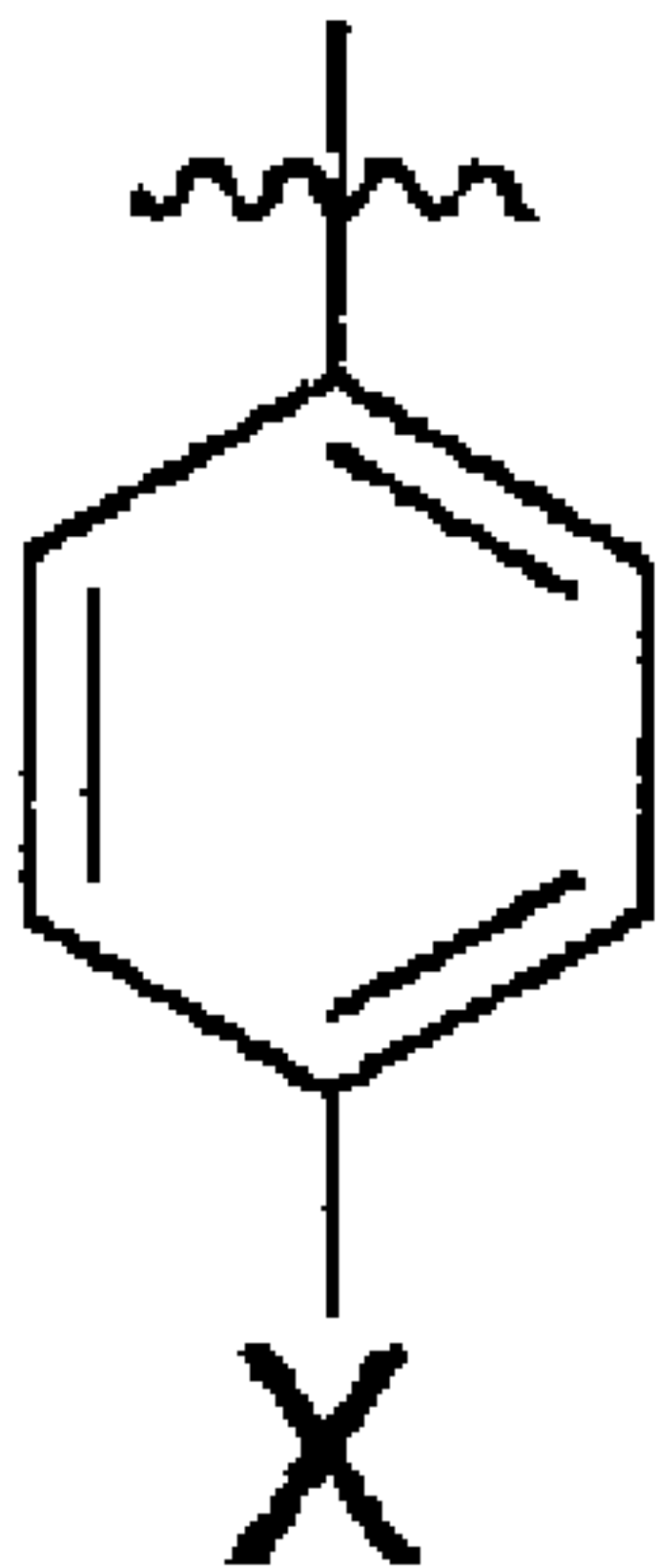


Figure 2.



$X = \text{H, OMe,}$
 $\text{NEt}_2, \text{CH}_3,$
 Ph