ORGANOANTIMONY(V) CYANOXIMATE COMPOUNDS AND METHODS OF PRODUCTION AND USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS/INCORPORATION BY REFERENCE STATEMENT

[0001] This application claims benefit under 35 USC § 119(e) of US Provisional Application No. 63/152,490, filed February 23, 2021. The entire contents of the above-referenced application(s) are hereby expressly incorporated herein by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] Not Applicable.

BACKGROUND

[0003] Microbial infections are associated with considerable morbidity and costs. The continuous spread of antimicrobial resistance (AMR) among bacterial and fungal pathogens imperils the usefulness of antibiotics and antifungals, which previously revolutionized and enabled medical interventions. The increasing AMR is manifesting as multidrug resistance (MDR) leading to a global crisis that if not addressed will soon have a huge adversarial impact on human race. The main causes of the rapid increase of AMR include an overuse of antimicrobials, inappropriate prescribing, and the inability of patients to follow the prescribed drug regimen. Extensive use of antibiotics in agricultural settings, as well as wide availability of some antibiotics are also important factors. This situation led to the appearance of antibiotic resistant bacterial pathogens, exemplified by Methicillin-Resistant *Staphylococcus aureus* (MRSA), and Vancomycin-Resistant *Enterococci* (VRE), which are becoming increasingly difficult to treat with the current range of available antibiotics. Fungal pathogens *Cryptococcus neoformans and Candida albicans* are also becoming increasingly resistant to antifungal drugs, and different *Candida* species with natural drug resistance, such as *Candida auris*, are evolving in hospital settings and becoming major pathogens. The MDR phenomenon is one of the greatest global public health challenges that humanity is currently facing. According to the Center for Disease Control (CDC) Prevention's report on antimicrobial resistance conducted in 2019, annually, there are more than 2.8 million antibiotic-resistant bacterial infections, from which more than 35,000 people die in the US.

Although fungal drug-resistant strains are more difficult to track, current estimates indicate that fungal infections lead to more than 1.5 million deaths annually worldwide. Aside from that, the economic impacts due to treating these infections as well as from the loss of productivity is astronomical – about $4.6 billion annually for bacterial infections and about $7.2 billion annually for fungal infections.

[0004] With modern medicine's reliance on antimicrobials in treating diseases such as pneumonia, tuberculosis, sexually transmitted diseases, and bloodstream infections, the problem with AMR is that soon no current treatments will be effective. Therefore, the development of new antimicrobial agents is imperative. The critically important progress in this field requires the development of new non-antibiotic chemical compounds that would inhibit bacterial and fungal growth, but at the same time, would not be toxic to human tissues. Pure organic compounds cannot meet these criteria, as they are rather quickly metabolized by microorganisms, while simple inorganic salts and Werner-type complexes are toxic or not soluble.

[0005] The successful application of metal complexes and organometallic compounds in treatments of numerous human diseases is a vigorously expanding area in both biomedical and bioinorganic chemistry research. Remarkably, the group V elements in the Periodic Table – pnictogens – contains all biologically active elements. Nitrogen (N) and phosphorous (P) are crucial for life on the planet, while arsenic (As), antimony (Sb), and bismuth (Bi) possess very useful properties and biomedical applications. Paul Ehrlich, widely known as the father of antimicrobial chemotherapy and bioinorganic chemistry, found the "magic bullet" (compound 606) capable of treating the highly infectious bacterial pathogen *Treponema pallidum* without hurting the host. This compound is a heterocyclic arsenic-based small molecule ($C_{12}H_{13}As_2ClN_2O_2$) that was used to successfully treat syphilis and was marketed as *Salvarsan* ("Lifesaving"). Despite the well-known toxicity of arsenic, this drug saved >560,000 lives within fifty years. Bismuth, contrary to arsenic, is not toxic and is widely used as subsalicylate ($C_7H_5BiO_4$) in the well-known drug Pepto-Bismol. In turn, antimony has been used since early Egyptian's civilization. For example, $NaSbO_3$ was commonly used as an emetic compound until the late 1700's. The other successful application of Sb is the treatment of leishmaniasis, caused by a protozoan parasite *Leishmania* transmitted through the bite of infected sandflies in South and Central America, Bangladesh, southern Europe, and North Africa. The active compounds against the disease were found to be several Sb(V) carbohydrates, such as sodium antimony gluconate (Pentostam) and meglumine antimonate

(Glucantime). These have been in use for more than six decades to treat leishmaniasis. Moreover, several organoantimony compounds have been studied and shown to possess antimicrobial, antifungal, and antitumor activities (Gasser et al., *J Med Chem* (2011) 54(1):3-25; Frezard et al., *Molecules* (2009) 14(7):2317-36); Agrawal et al., *J Coord Chem* (2011) 64(3):554-563; Khan et al., *Middle East Journal of Scientific Research* (2013) 17:705-711; Oliveira et al., *Molecules* (2011) 16(12):10314-23; Sharma et al., *Bioinorg Chem Appl* (2006) 16895; and Singh et al., *Main Group Metal Chemistry* (2010) 33).

[0006] Recently, it was discovered that Sb(V) cyanoximates are thermally and chemically stable both in solid state and solutions (Domasevitch et al., *Inorg Chem* (2000) 39(6):1227-1237). In addition, no intrinsic *in vitro* cytotoxicity was detected for free cyanoximes, organic ligands used to synthesize organoantimony(V) (Eddings et al., *Inorg Chem* (2004) 43(13):3894-909; Gerasimchuk et al., *Inorg Chem* (2007) 46(18):7268-84); and Mann et al., *Inorganica Chimica Acta* (2016) 440:118-128).

[0007] Therefore, there is a need in the art for new and improved antimicrobial organometallic compounds and compositions that efficiently inhibit pathogenic bacteria and fungi. It is to such new and improved compounds and compositions, as well as methods of producing and using same, that the present disclosure is directed.

NON-ANTIBIOTIC ANTIMICROBIAL COMPOUNDS AND METHODS OF PRODUCTION AND USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS/INCORPORATION BY REFERENCE STATEMENT

[0001] Not Applicable.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] Not Applicable.

DETAILED DESCRIPTION

[0003] Before explaining at least one embodiment of the inventive concept(s) in detail by way of exemplary language and results, it is to be understood that the inventive concept(s) is not limited in its application to the details of construction and the arrangement of the components set forth in the following description. The inventive concept(s) is capable of other embodiments or of being practiced or carried out in various ways. As such, the language used herein is intended to be given the broadest possible scope and meaning; and the embodiments are meant to be exemplary - not exhaustive. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

[0004] Unless otherwise defined herein, scientific and technical terms used in connection with the presently disclosed inventive concept(s) shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. The foregoing techniques and procedures are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. The nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques are used for chemical syntheses and chemical analyses.

[0005] All patents, published patent applications, and non-patent publications mentioned in the specification are indicative of the level of skill of those skilled in the art to which this presently

disclosed inventive concept(s) pertains. All patents, published patent applications, and non-patent publications referenced in any portion of this application are herein expressly incorporated by reference in their entirety to the same extent as if each individual patent or publication was specifically and individually indicated to be incorporated by reference.

[0006] All of the compositions and/or methods disclosed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of the inventive concept(s) have been described in terms of particular embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the methods described herein without departing from the concept, spirit, and scope of the inventive concept(s). All such similar substitutions and modifications apparent to those skilled in the art are deemed to be within the spirit, scope, and concept of the inventive concept(s) as defined by the appended claims.

[0007] As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

[0008] The use of the term “a” or “an” when used in conjunction with the term “comprising” in the claims and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.” As such, the terms “a,” “an,” and “the” include plural referents unless the context clearly indicates otherwise. Thus, for example, reference to “a compound” may refer to one or more compounds, two or more compounds, three or more compounds, four or more compounds, or greater numbers of compounds. The term “plurality” refers to “two or more.”

[0009] The use of the term “at least one” will be understood to include one as well as any quantity more than one, including but not limited to, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, 100, etc. The term “at least one” may extend up to 100 or 1000 or more, depending on the term to which it is attached; in addition, the quantities of 100/1000 are not to be considered limiting, as higher limits may also produce satisfactory results. In addition, the use of the term “at least one of X, Y, and Z” will be understood to include X alone, Y alone, and Z alone, as well as any combination of X, Y, and Z. The use of ordinal number terminology (i.e., “first,” “second,” “third,” “fourth,” etc.) is solely for the purpose of differentiating between two or more items and is not meant to imply any sequence or order or importance to one item over another or any order of addition, for example.

[0010] The use of the term "or" in the claims is used to mean an inclusive "and/or" unless explicitly indicated to refer to alternatives only or unless the alternatives are mutually exclusive. For example, a condition "A or B" is satisfied by any of the following: A is true (or present) and B is false (or not present), A is false (or not present) and B is true (or present), and both A and B are true (or present).

[0011] As used herein, any reference to "one embodiment," "an embodiment," "some embodiments," "one example," "for example," or "an example" means that a particular element, feature, structure, or characteristic described in connection with the embodiment is included in at least one embodiment. The appearance of the phrase "in some embodiments" or "one example" in various places in the specification is not necessarily all referring to the same embodiment, for example. Further, all references to one or more embodiments or examples are to be construed as non-limiting to the claims.

[0012] Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for a composition/apparatus/ device, the method being employed to determine the value, or the variation that exists among the study subjects. For example, but not by way of limitation, when the term "about" is utilized, the designated value may vary by plus or minus twenty percent, or fifteen percent, or twelve percent, or eleven percent, or ten percent, or nine percent, or eight percent, or seven percent, or six percent, or five percent, or four percent, or three percent, or two percent, or one percent from the specified value, as such variations are appropriate to perform the disclosed methods and as understood by persons having ordinary skill in the art.

[0013] As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include"), or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

[0014] The term "or combinations thereof" as used herein refers to all permutations and combinations of the listed items preceding the term. For example, "A, B, C, or combinations thereof" is intended to include at least one of: A, B, C, AB, AC, BC, or ABC, and if order is important in a particular context, also BA, CA, CB, CBA, BCA, ACB, BAC, or CAB. Continuing with this example, expressly included are combinations that contain repeats of one or more item or term, such as BB, AAA, AAB, BBC, AAABCCCC, CBBAAA, CABABB, and so forth. The skilled artisan will understand that

typically there is no limit on the number of items or terms in any combination, unless otherwise apparent from the context.

[0015] As used herein, the term "substantially" means that the subsequently described event or circumstance completely occurs or that the subsequently described event or circumstance occurs to a great extent or degree. For example, when associated with a particular event or circumstance, the term "substantially" means that the subsequently described event or circumstance occurs at least 80% of the time, or at least 85% of the time, or at least 90% of the time, or at least 95% of the time. For example, the term "substantially adjacent" may mean that two items are 100% adjacent to one another, or that the two items are within close proximity to one another but not 100% adjacent to one another, or that a portion of one of the two items is not 100% adjacent to the other item but is within close proximity to the other item.

[0016] The terms “analog,” “derivative,” or “variant” as used herein will be understood to refer to a variation of the normal or standard form or the wild-type form of molecules. For polypeptides, an analog may be a variant (polymorphism), a mutant, and/or a naturally or artificially chemically modified version of the wild-type polypeptide (including combinations of the above). Such analogs may have higher, full, intermediate, or lower activity than the normal form of the molecule, or no activity at all. Alternatively, and/or in addition thereto, for a chemical, an analog may be any structure that has the desired functionalities (including alterations or substitutions in the core moiety), even if comprised of different atoms or isomeric arrangements.

[0017] As used herein, the phrases "associated with" and “coupled to” include both direct association/binding of two moieties to one another as well as indirect association/binding of two moieties to one another. Non-limiting examples of associations/couplings include covalent binding of one moiety to another moiety either by a direct bond or through a spacer group, non-covalent binding of one moiety to another moiety either directly or by means of specific binding pair members bound to the moieties, incorporation of one moiety into another moiety such as by dissolving one moiety in another moiety or by synthesis, and coating one moiety on another moiety, for example.

[0018] As used herein, "substantially pure" means an object species is the predominant species present (i.e., on a molar basis it is more abundant than any other individual species in the composition), and a substantially purified fraction is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present.

Generally, a substantially pure composition will comprise more than about 80 percent of all macromolecular species present in the composition, such as (but not limited to) more than about 85%, 90%, 95%, and 99%. In a particular (but non-limiting) embodiment, the object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods), wherein the composition consists essentially of a single macromolecular species.

[0019] The term "pharmaceutically acceptable" refers to compounds and compositions which are suitable for administration to humans and/or animals without undue adverse side effects such as (but not limited to) toxicity, irritation, and/or allergic response commensurate with a reasonable benefit/risk ratio.

[0020] The term “pharmaceutically-acceptable excipient” refers to any carrier, vehicle, and/or diluent known in the art or otherwise contemplated herein that may improve solubility, deliverability, dispersion, stability, and/or conformational integrity of the compositions disclosed herein.

[0021] The term "patient" as used herein includes human and veterinary subjects. "Mammal" for purposes of treatment refers to any animal classified as a mammal, including (but not limited to) humans, domestic and farm animals, nonhuman primates, and any other animal that has mammary tissue.

[0022] The term "treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include, but are not limited to, individuals already having a particular condition/disease/infection as well as individuals who are at risk of acquiring a particular condition/disease/infection (e.g., those needing prophylactic/preventative measures). The term "treating" refers to administering an agent/element/method to a patient for therapeutic and/or prophylactic/preventative purposes.

[0023] A "therapeutic composition" or "pharmaceutical composition" refers to an agent that may be administered *in vivo* to bring about a therapeutic and/or prophylactic/preventative effect.

[0024] Administering a therapeutically effective amount or prophylactically effective amount is intended to provide a therapeutic benefit in the treatment, prevention, and/or management of a disease, condition, and/or infection. The specific amount that is therapeutically effective can be readily determined by the ordinary medical practitioner, and can vary depending on factors known

in the art, such as (but not limited to) the type of condition/disease/infection, the patient's history and age, the stage of the condition/disease/infection, and the co-administration of other agents.

[0025] The term "effective amount" refers to an amount of a biologically active molecule or conjugate or derivative thereof, or an amount of a treatment protocol (i.e., an alternating electric field), sufficient to exhibit a detectable therapeutic effect without undue adverse side effects (such as (but not limited to) toxicity, irritation, and allergic response) commensurate with a reasonable benefit/risk ratio when used in the manner of the inventive concept(s). The therapeutic effect may include, for example but not by way of limitation, preventing, inhibiting, or reducing the occurrence of at least one tumor and/or cancer. The effective amount for a subject will depend upon the type of subject, the subject's size and health, the nature and severity of the condition/disease/infection to be treated, the method of administration, the duration of treatment, the nature of concurrent therapy (if any), the specific formulations employed, and the like. Thus, it is not possible to specify an exact effective amount in advance. However, the effective amount for a given situation can be determined by one of ordinary skill in the art using routine experimentation based on the information provided herein.

[0026] As used herein, the term "concurrent therapy" is used interchangeably with the terms "combination therapy" and "adjunct therapy," and will be understood to mean that the patient in need of treatment is treated or given another drug for the condition/disease/infection in conjunction with the treatments of the present disclosure. This concurrent therapy can be sequential therapy, where the patient is treated first with one treatment protocol/pharmaceutical composition and then the other treatment protocol/pharmaceutical composition, or the two treatment protocols/pharmaceutical compositions are given simultaneously.

[0027] The terms "administration" and "administering," as used herein, will be understood to include all routes of administration known in the art, including but not limited to, oral, topical, transdermal, parenteral, subcutaneous, intranasal, mucosal, intramuscular, intraperitoneal, intravitreal, and intravenous routes, and including both local and systemic applications. In addition, the compositions of the present disclosure (and/or the methods of administration of same) may be designed to provide delayed, controlled, or sustained release using formulation techniques which are well known in the art.

[0028] Turning now to the inventive concept(s), certain non-limiting embodiments of the present disclosure are directed to compositions that comprise at least one non-antibiotic

antimicrobial compound as described or otherwise contemplated herein. Particular non-limiting examples of non-antibiotic antimicrobial compounds include those listed in the exemplary claims section below.

[0029] In certain particular (but non-limiting) embodiments, the non-antibiotic antimicrobial compound is an organoantimony(V) compound.

[0030] Certain non-limiting embodiments of the present disclosure are directed to a method of synthesizing any of the compounds as described or otherwise contemplated herein. The individual method steps may be performed as described in any of the attached Appendices, which are expressly incorporated herein by reference.

[0031] Certain non-limiting embodiments of the present disclosure are directed to a method of synthesizing an organoantimony(V) compound. The individual method steps may be performed as described in any of the attached Appendices, which are expressly incorporated herein by reference. In addition, while the Appendices may describe synthesis reactions for one or more particular compounds, it will be understood that these same synthesis reactions will be applicable to the synthesis of other related compounds.

[0032] Each of the methods described or otherwise contemplated herein may produce the non-antibiotic antimicrobial compounds with any level of yield. For example (but not by way of limitation), the non-antibiotic antimicrobial compounds can be synthesized with a yield of at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, and at least about 99%. In addition, the scope of the presently disclosure also includes the production of the compounds at any percent yield that falls within any range formed from the combination of two values listed above (for example, a range of from about 10% to about 99%, a range of from about 30% to about 98%, a range of from about 50% to about 97%, a range of from about 60% to about 96%, a range of from about 70% to about 95%, etc.).

[0033] Certain non-limiting embodiments of the present disclosure are directed to a non-

antibiotic antimicrobial compound produced by any of the methods described or otherwise contemplated herein. Particular non-limiting examples of compounds include those listed in the exemplary claims section below.

[0034] Certain non-limiting embodiments of the present disclosure are directed to methods of using one or more of the non-antibiotic antimicrobial compounds, as described or otherwise contemplated herein.

EXAMPLES

[0035] Examples are provided hereinbelow. However, the present disclosure is to be understood to not be limited in its application to the specific experimentation, results, and laboratory procedures disclosed herein. Rather, the Examples are simply provided as one of various embodiments and are meant to be exemplary, not exhaustive.

[0036] In addition to the disclosure provided above, attached hereto are Appendices A, B, C, and D related to the inventive concept(s) of the present disclosure. Said documents provide exemplary drawings, experimentation, results, and laboratory procedures in accordance with the present disclosure. Such information is to be understood to be explicitly included within this Specification. However, it is to be understood that the information contained therein is provided for the purpose of description, and the present disclosure is not limited to such exemplary information contained therein. The inventive concept(s) is capable of other embodiments or of being practiced or carried out in various ways.

[0037] Thus, in accordance with the present disclosure, there have been provided compounds, as well as methods of producing and using same, which fully satisfy the objectives and advantages set forth hereinabove. Although the present disclosure has been described in conjunction with the specific drawings, experimentation, results, and language set forth hereinabove, it is evident that many alternatives, modifications, and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications, and variations that fall within the spirit and broad scope of the present disclosure.

EXEMPLARY CLAIMS

Exemplary, non-limiting claims are provided herein below. However, the scope of the present inventive concept(s) is to be understood to not be limited in any manner by the exemplary claims presented below.

1. A composition, comprising:

at least one of any of the compounds disclosed or otherwise contemplated herein.

2. The composition of claim 1, wherein the at least one compound is an organoantimony(V) compound.

3. The composition of claim 1, wherein the organoantimony(V) compound is an organoantimony(V) cyanoximate.

4. The composition of claim 3, wherein the compound is an organoantimony(V) derivative of the formula:

$$SbR_{4-n}L_y,$$

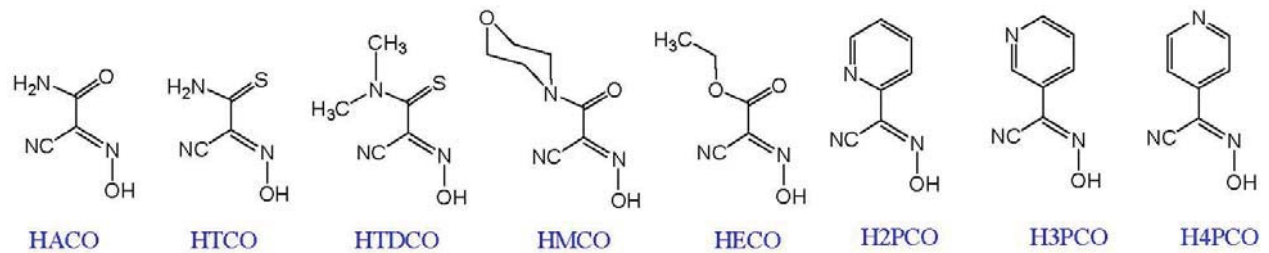
wherein:

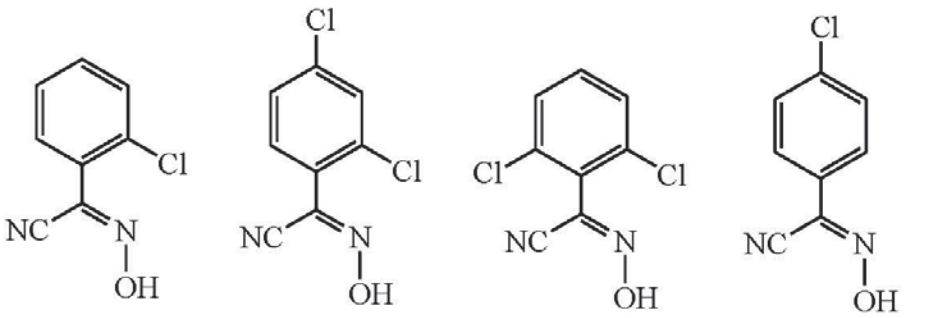
R is C_6H_5, n is 0, and y is 1; or

R is CH_3, n is 1 or 2, and y is 2 or 3; and

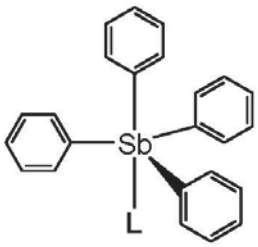
wherein L is a cyanoxime molecule.

5. The composition of claim 4, wherein the cyanoxime molecule is selected from the following structures:



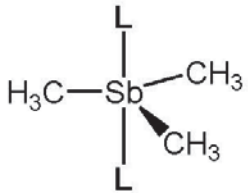


6. The composition of claim 3, wherein the compound is further defined as having the structure:



wherein L is 2PCO, 3PCO, 4PCO, ECO, ACO, MCO, TCO, or TDCO.

7. The composition of claim 3, wherein the compound is further defined as having the structure:



wherein L is ACO, MCO, ECO, TCO, TDCO, or a chlorinated arylcyanoxime.

8. The composition of claim 1, further defined as an antimicrobial composition.

9. The composition of claim 8, further defined as being effective at reducing, inhibiting, and/or substantially preventing growth of at least one bacterium selected from the group consisting of

Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and the like, as well as any other bacteria disclosed or otherwise contemplated herein.

10. The composition of claim 9, wherein the at least one bacterium is a Methicillin-resistant strain.

11. The composition of claim 8, further defined as being effective at reducing, inhibiting, and/or substantially preventing growth of at least one fungus selected from the group consisting of *Cryptococcus neoformans, Candida albicans*, and the like, as well as any other fungus disclosed or otherwise contemplated herein.

12. The composition of claim 1, further defined as a therapeutic drug composition.

13. The composition of claim 1, further defined as a glue or adhesive.

14. The composition of claim 1, further defined as comprising at least two of any of the compounds disclosed or otherwise contemplated herein.

15. A method of synthesizing the composition of any one of claims 1-14, wherein the method comprises one or more of any of the steps disclosed or otherwise contemplated herein.

16. The method of claim 15, wherein the method comprises a metathesis reaction.

17. An assembly, comprising:

a device (wherein the device is any of the devices disclosed or otherwise contemplated herein); and

the composition of any of claims 1-14 incorporated within the device and/or applied to at least a portion of at least one surface of the device.

18. A method of reducing, inhibiting, and/or substantially preventing microbial growth on a surface and/or in a composition/device, the method comprising the step of:

applying the composition of any of claims 1-14 to at least a portion of the surface and/or incorporating the composition of any of claims 1-14 within the composition/device.

19. A pharmaceutical composition, comprising:
at least one of the compositions of any of claims 1-14; and
a pharmaceutically-acceptable excipient or carrier.

20. A method, comprising the step of:
administering an effective amount of the pharmaceutical composition of claim 19 to a patient.

APPENDIX A

NEW CLASS OF NON-ANTIBIOTIC ANTIMICROBIAL COMPOUNDS.

Abstract:

A series of new organoantimony(V) compounds has been synthesized and characterized using a variety of conventional chemical and physical methods (crystallographic, spectroscopic, thermal analysis). Compounds represent air/moisture and thermally stable to 120o C small molecules of non-antibiotic in nature. All obtained compounds were tested in vitro against diverse group of pathogenic bacteria and fungi and showed considerable activity that allows them to be further developed as antimicrobials for different applications.

Keywords:

Organoantimony(V)

Oxime-based small molecules

Non-antibiotic Covalent, molecular compounds

Technical Description:

The requirement of new antimicrobial treatments has become an urgent field in the last two decades. Multi-drug resistant (MDR) pathogenic bacteria and fungi are now resistant to most available antibiotics, including new generation, ceftazidime and daptomycin. Our previous work with cyanoximes allowed identification of several biologically active compounds, some of which were selected for this investigation (Figure 1).

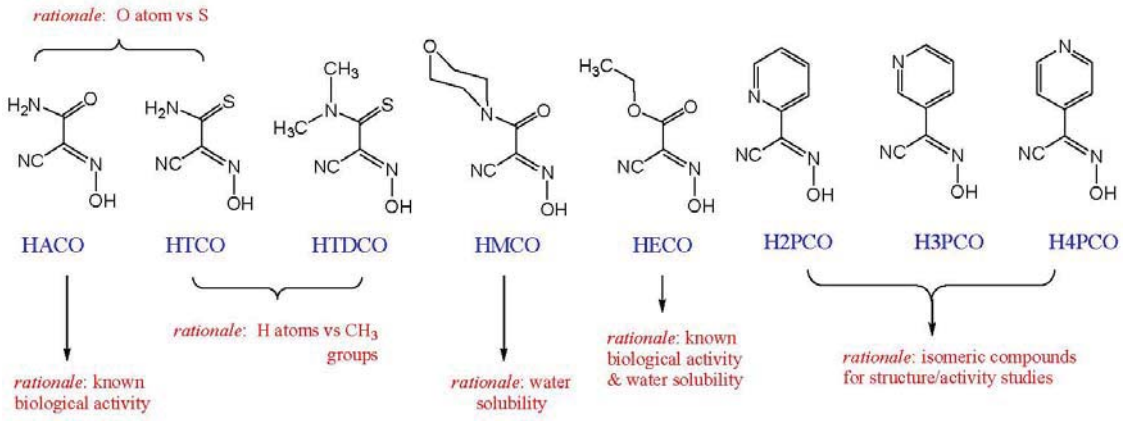


Figure 1. Some Cyanoximes Used in our Studies

To antagonize diverse bacteria and fungi, eight novel organoantimony(V) cyanoximates were: 1) synthesized using the metathesis reaction in dry acetonitrile between tetraphenylantimony(V) bromide or triemethylantimony(V) dibromide and Ag(I) or Tl(I) salts of several cyanoximes, 2) *in vitro* studied using several antibiotic resistant pathogenic microbes.

Synthesis. Desired organoantimony(V) cyanoximates obtained in the metathesis reaction were left in solution while AgBr or TlBr were separated off using centrifugation (Figure 2). Removal of solvent in desiccator led to crystalline molecular compounds of $SbPh_4L$ or $SbMe_3L_2$ composition (L = selected for investigations biologically active cyanoximes). Identity and structures of compounds was confirmed by elemental analysis on C, H, N, and S content, thermal analysis, IR-, $^{13}C\{^1H\}$ NMR, some with UV-visible spectroscopy, and single crystal X-ray analysis. It was found that in all organoantimony(V) cyanoximates the cyanoxime moiety is bound to the central atom via oxygen atom and there is a formation of lightly distorted trigonal bipyramid polyhedronof the Sb(V) atom.

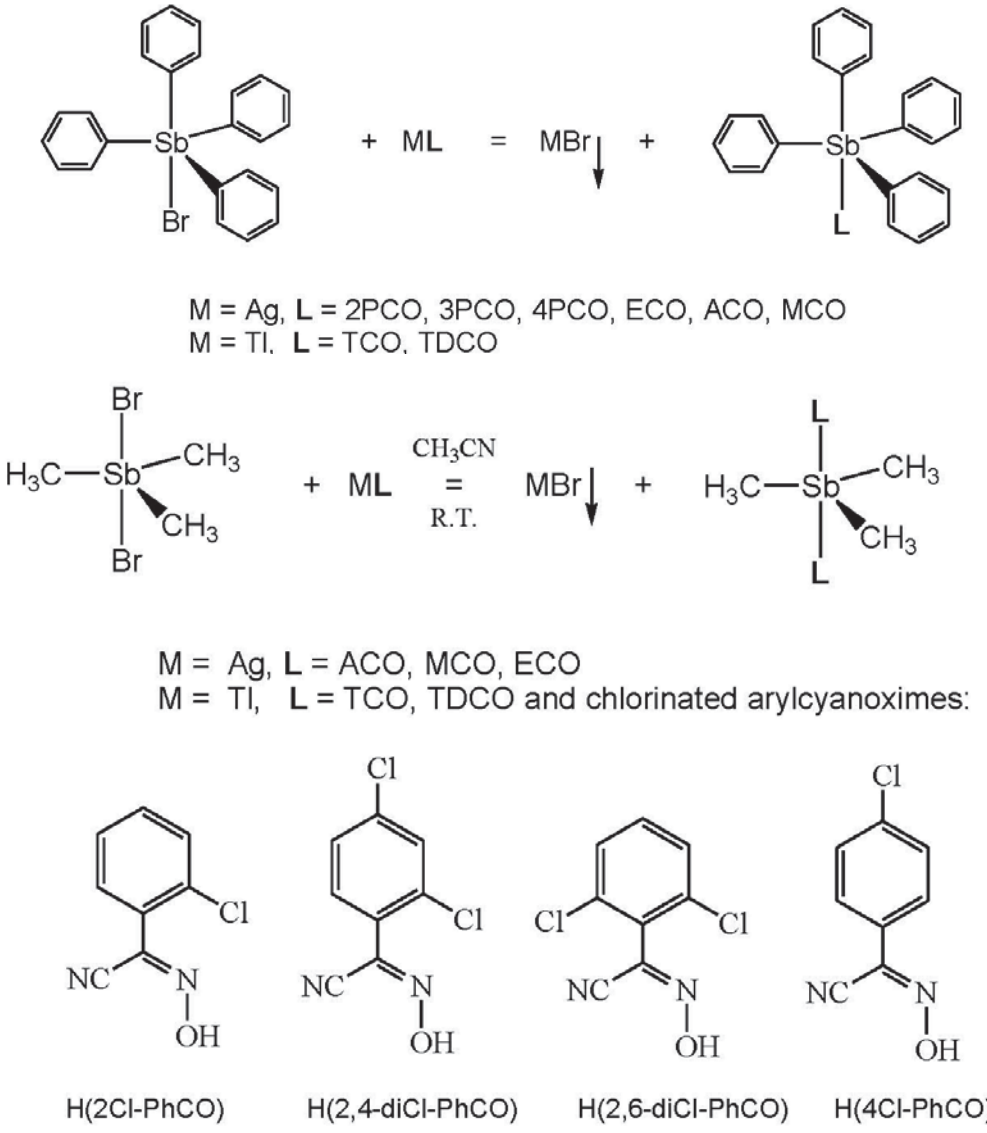


Figure 2. Synthetic Routes to Desired Organoantimony(V) Cyanoximates

Antimicrobial studies involved: 1) paper disk studies during growth on solid media; 2) determining MIC during growth in liquid media. It was found that that $Sb(Ph)_4(ACO)$ and $Sb(Ph)_4(ECO)$ had significant antimicrobial effect against all three selected for investigation strains: two Gram-negative *Escherichia coli* strain S17 and *Pseudomonas aeruginosa* strain PAO1, and Gram-positive Methicillin-resistant *Staphylococcus aureus* strain NRS70. Compounds $Sb(Ph)_4(TCO)$ and $Sb(Ph)_4(TDCO)$ had significant effects on the Gram- positive Methicillin-resistant *Staphylococcus aureus* strain NRS70, but essentially no antimicrobial activity for Gram-

negative strains used. Antifungal disk assays concluded that $Sb(Ph)_4(ECO)$ was effective against *Cryptococcus neoformans* and *Candida albicans*. $Sb(Ph)_4(TCO)$ followed in antifungal activity against both strains. $Sb(Ph)_4(ACO)$ and $Sb(Ph)_4(TDCO)$ were only effective at inhibition of *Cryptococcus neoformans*. For MIC assays, $Sb(Ph)_4(MCO)$ as the only Sb(V) cyanoximate along with the free cyanoximes H(MCO), H(ECO), and $Na[H(ACO)_2]$ were tested so far. As expected, free cyanoximes have no antimicrobial effect.

Specific modifications of the organic components of the compounds may be made to enhance their antimicrobial effect (for instance, make them better water soluble, or increase the amount of oxime groups attached to antimony atoms).

There are several applications of these new compounds.

1) Development of new non-antibiotic antimicrobials drugs.
2) Mucus-type antimicrobial hydrophobic additives to adhesives working in aqueous environment.
3) Antimicrobial wearable sweat resistant electronic devises for electrodes, contact pads.
4) Antimicrobial polymer glue that sticks to wet surfaces for under water applications.

Organoantimony-cyanoximates exhibit appreciable and valuable antimicrobial activity. Can modify the periphery of these newly obtained compounds to make them more water soluble.

CLAIMS: a series of new non-antibiotic antimicrobial compounds that include organoantimony(V) derivatives of $SbR_{4-n}L_y$, where R = C_6H_5, n=0, y=1; R = CH_3, n=1 or 2, y=2 or 3; L= cyanoxime molecules shown above in Figures 1 and 2.

Amendment of the disclosure of the latest IP form from Missouri State University showing the role of scientists involved in proposed co-patent.

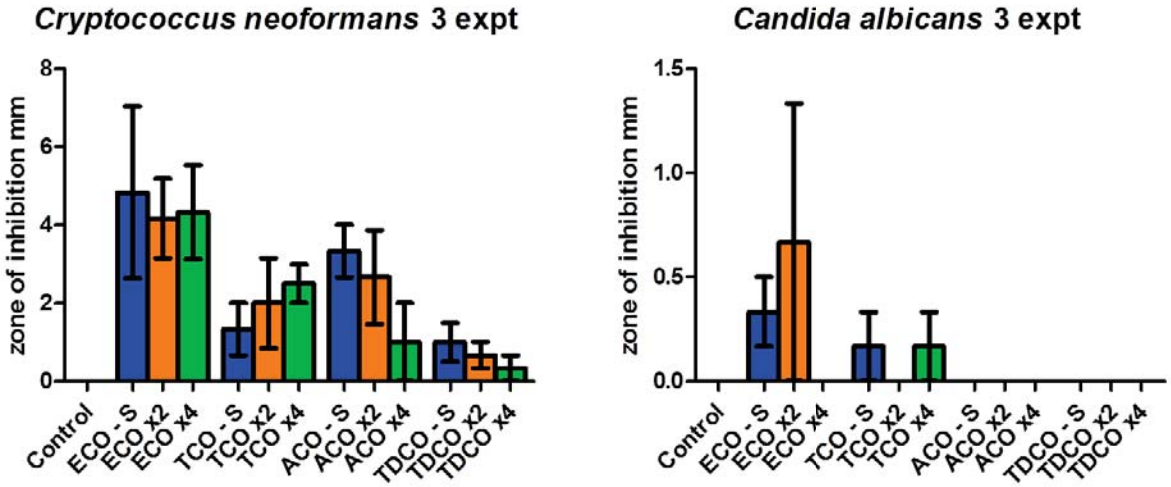
This is a compilation of results provided by our collaborator – Oklahoma State University – to my research group on a subject of studies of antimicrobial activity of a series of new organoantimony(V) compounds. Two directions were taken with this research: antimicrobial testing against notorious human pathogens and antifungal testing. In both studies there were investigated solid powders of our compounds and their solutions.

Unsolicited by me antifungal studies suggested and carried out by Professor Karen Wozniak:

1) Solid state compounds testing as deposited on paper disks powders:

Disk assays

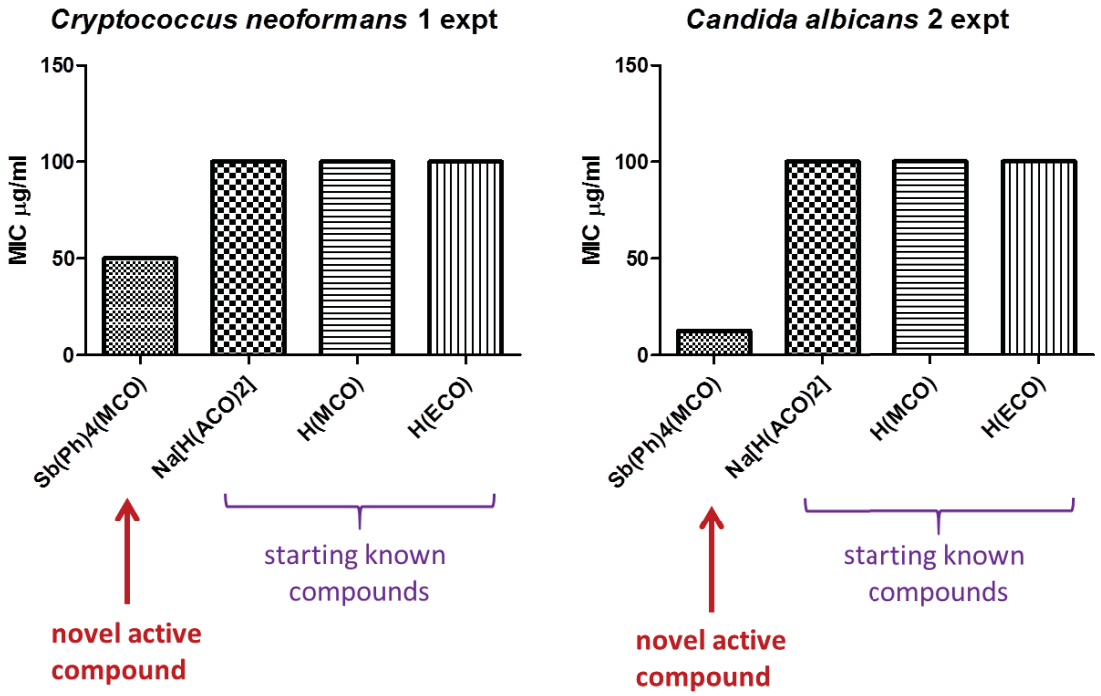
- Triplicate experiments with each compound at each concentration of deposited solid compound
- Control was a blank paper (100% cotton) disk



2) Compounds were dissolved and their Minimal Inhibitory Concentration (MIC) was determined.

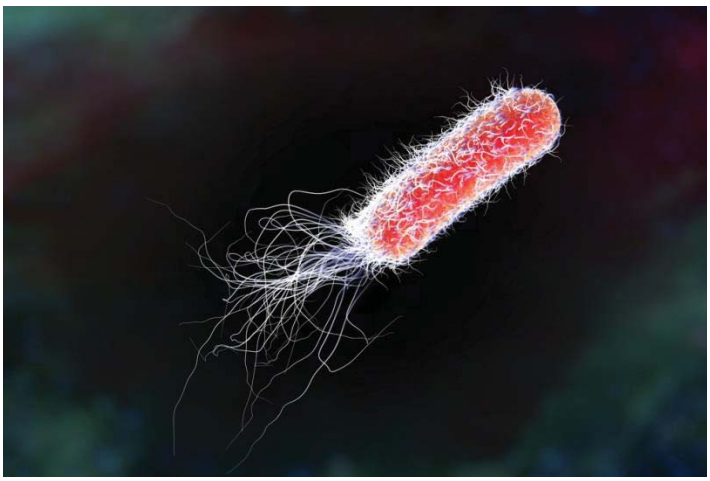
MIC assays – solutions, dissolved powders

- Each solubilized in DMSO to 5ug/ml
- Starting concentration is 100ug/ml (then diluted 1:2)
  - No effect of drugs if MIC is 100ug/ml
- Antifungal drug (Amphotericin B) MIC concentration 0.5ug/ml

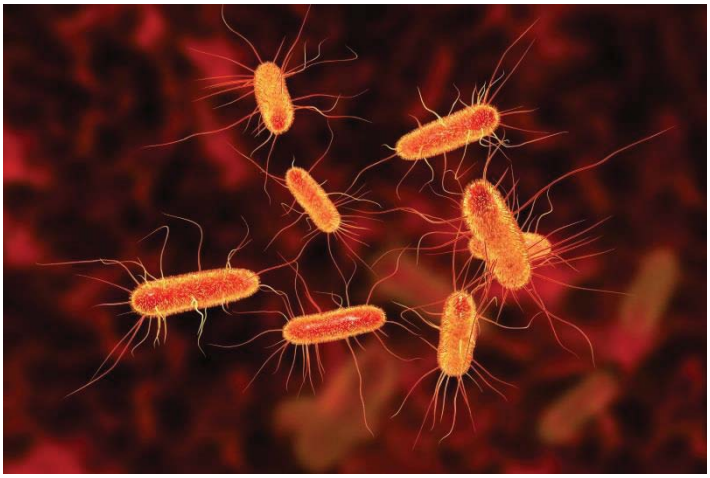


All submitted and studied thus far new organoantimony compounds are active and exhibit potential for further improvement of their antifungal activity. That will be done in our laboratory at Missouri State University by designing and making of new compounds based on results of already carried out work.

Detailed antimicrobial testing carried out by Professor Marianna Patrauchan. Three pathogens were considered for studies: *Pseudomonas Aeruginosa*, *Escherichia Coli*, and *Staphylococcus Aureus*. All considered a significant public health hazard. The first one – *P. Aeruginosa* – has been considered in the last years as the most important enemy of personnel by the US Army.



Pseudomonas Aeruginosa



Escherichia Coli

Staphylococcus Aureus

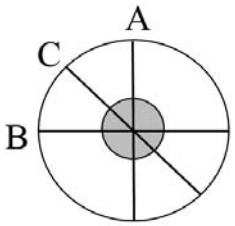
Procedure

Precultures
- 3ml of LB medium was inoculated with culture, incubated at 37◦C, 200 rpm for 12 hours

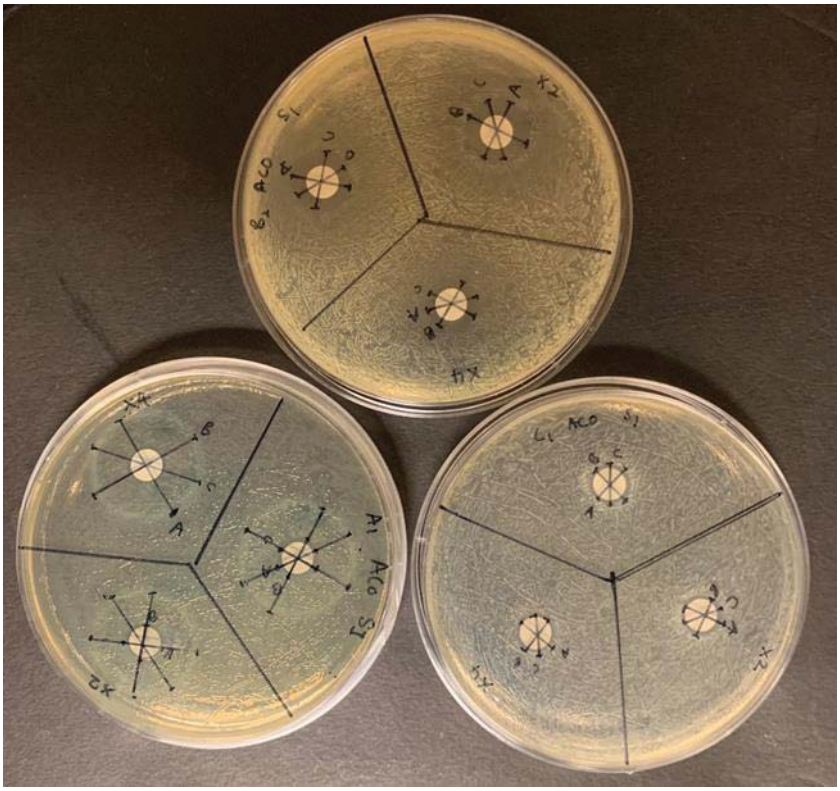
Spread on LB plates
- The OD600 was measured and normalized to 0.1, 100 ul of normalized culture was spread on LB plates (15ml LB in each plate)

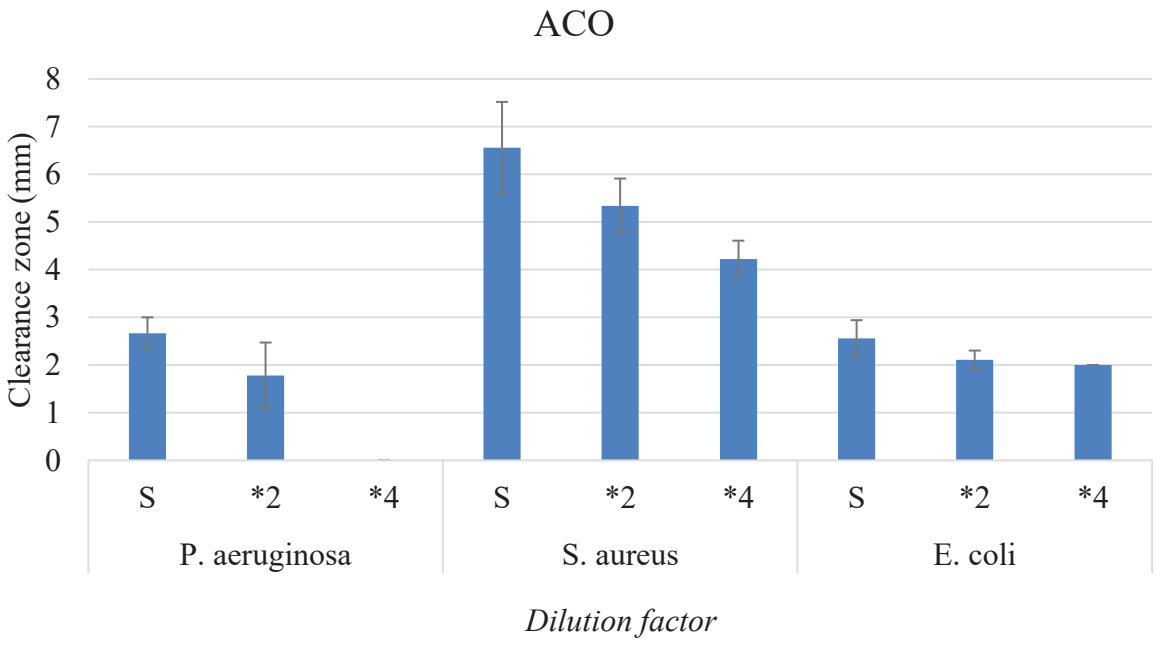
Susceptibility testing
- 10 ul of PBS was applied on the plate and disc was placed on it
- Plates were incubated at 37◦C for 12-24 hours
- Clearance zones were measured: diameter of the zone minus diameter of the disc

how measurements were conducted:



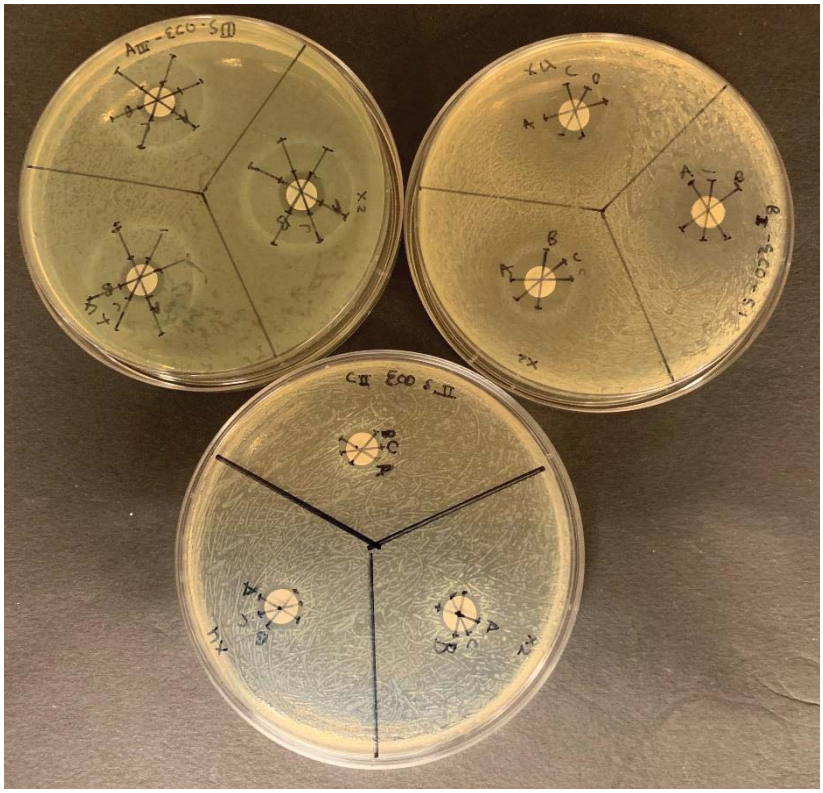
Results for solid samples (as deposited in paper disks powders) for the $SbPh_4(ACO)$ compound:

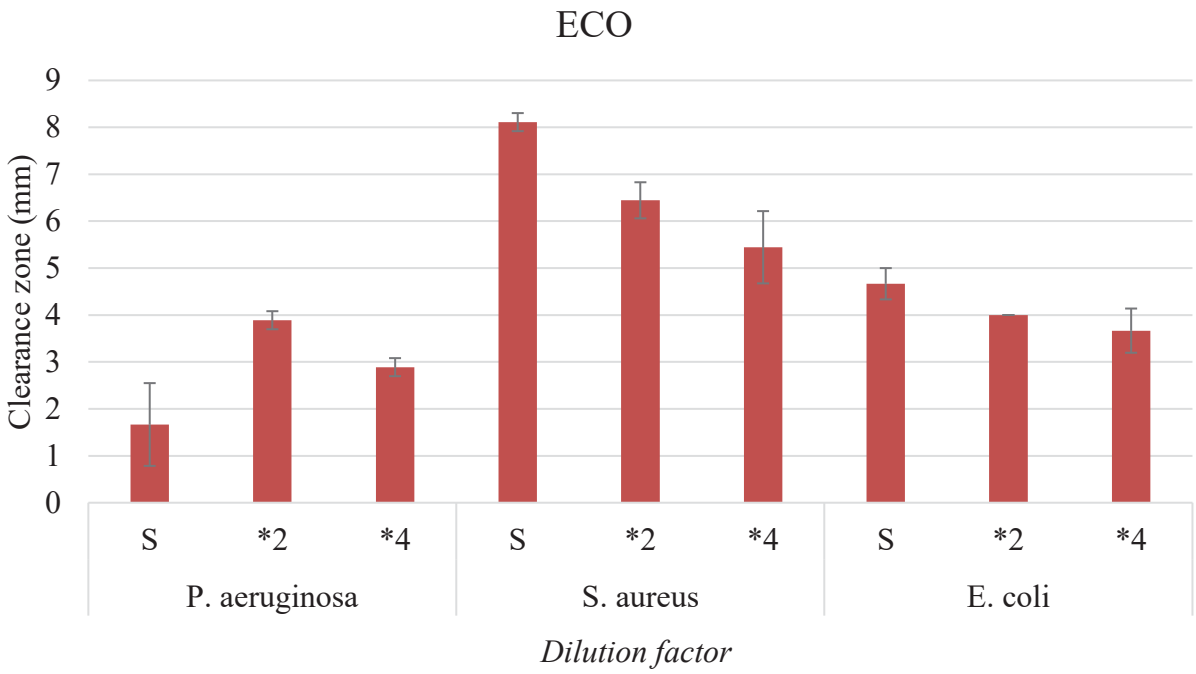




The cyanoxime molecule labeled as ACO used here is: 2-cyano-2-oximino-acetamide anion.

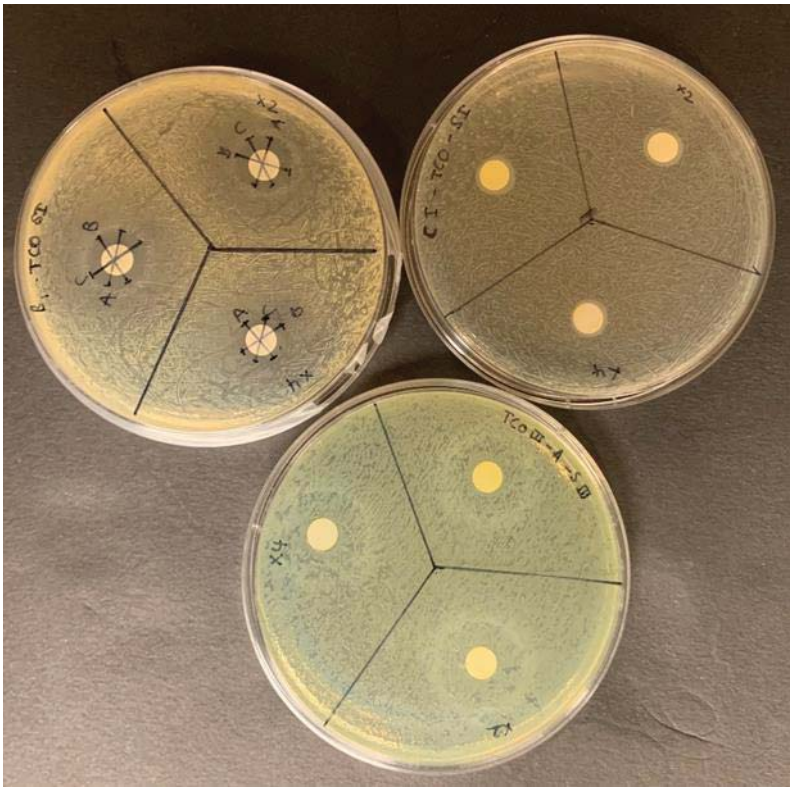
Results for solid samples (as deposited in paper disks powders) for the $SbPh_4$(ECO) compound:

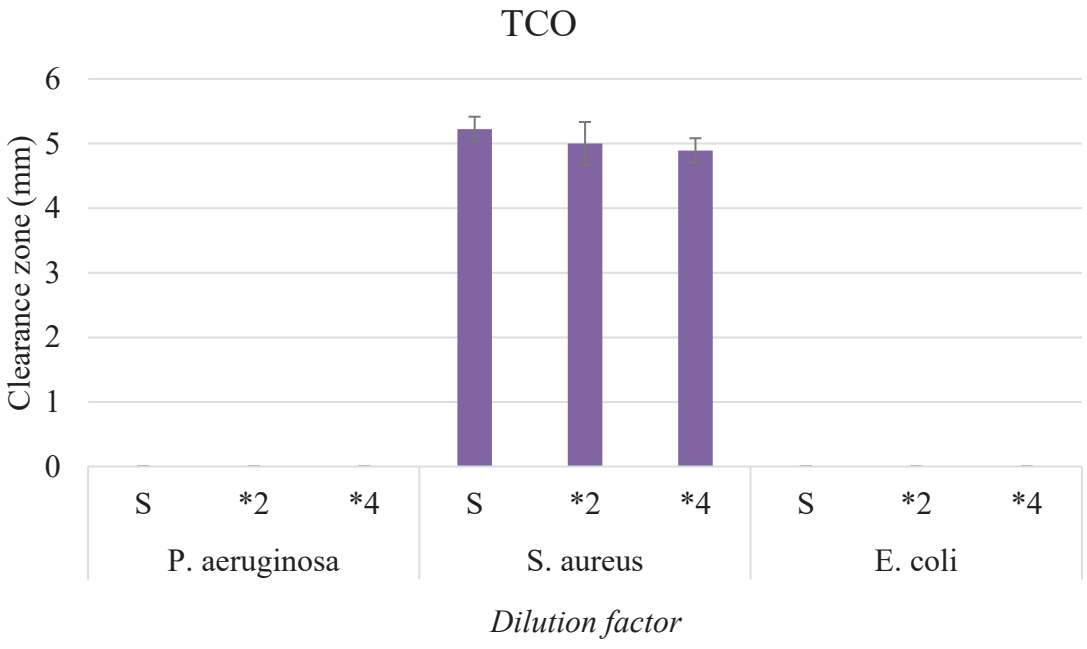




The cyanoxime molecule labeled as ECO used here is: 2-cyano-2-oximino-ethylacetate anion.

Results for solid samples (as deposited in paper disks powders) for the $SbPh_4$(TCO) compound:





The cyanoxime molecule labeled as TCO used here is: 2-cyano-2-oximino-thioacetamide anion.

Studies of our compounds in solutions to determine Minimal Inhibitory Concentration (MIC):

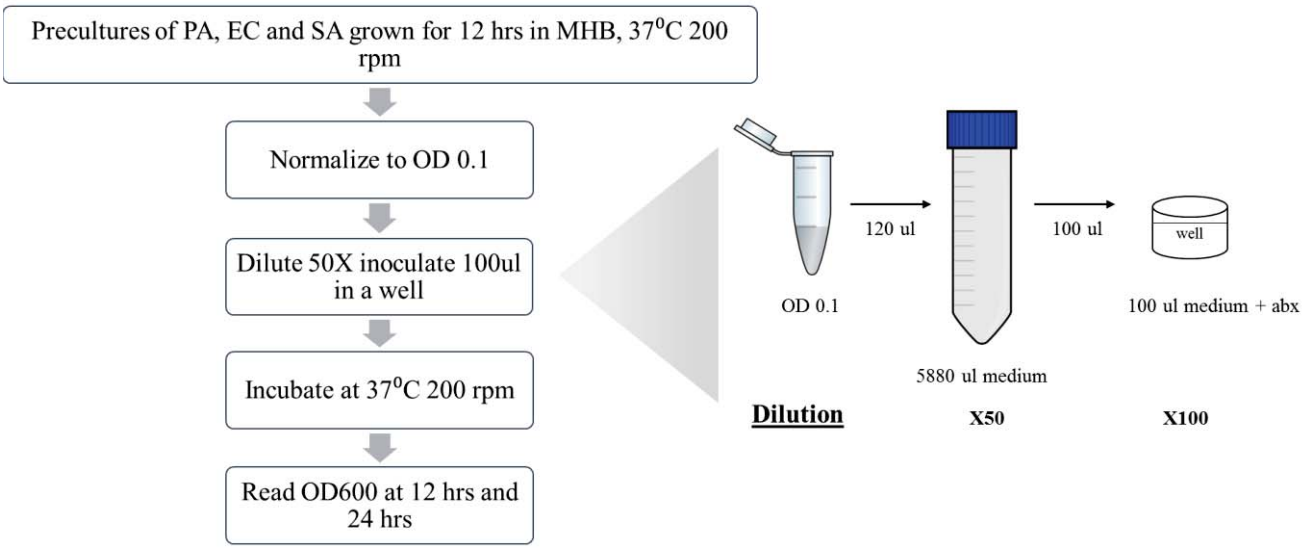
Summary of broth dilution MIC tests

Organisms	Compounds
P. aeruginosa (PA) *E. coli (EC)* *S. aureus (SA*	H(MCO) H(ECO) $Sb(Ph_4)MCO$ $Na[CH(ACO)_2]$

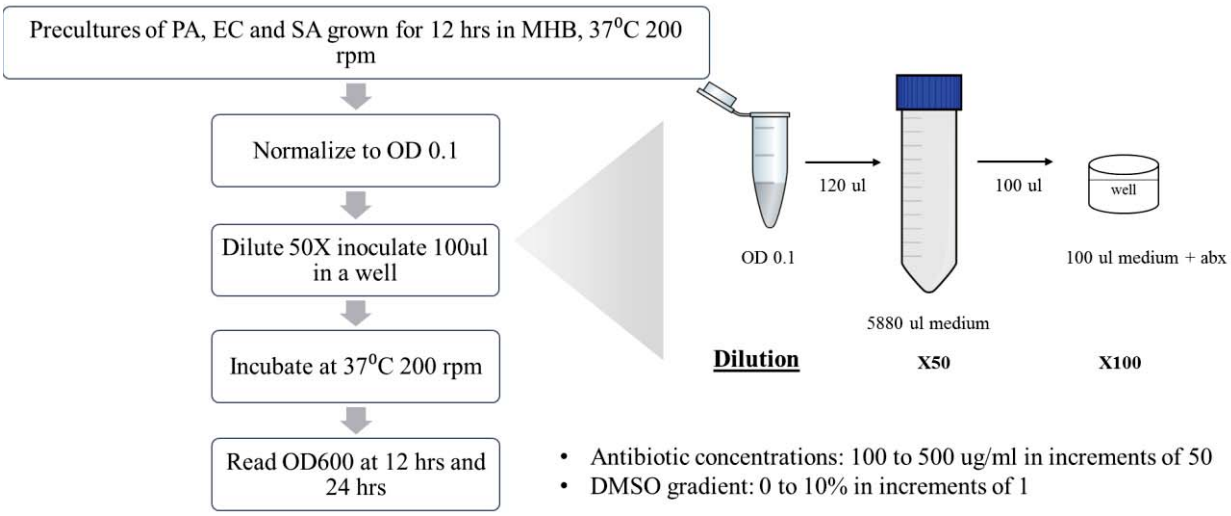
- Concentration of stock solutions in DMSO: 5 mg/ml

Methods designed for experiments:

MIC determination for H(MCO), H(ECO), $Sb(Ph_4)MCO$ and $Na[CH(ACO)_2]$ in *P. aeruginosa, E. coli* and *S. aureus* cultures

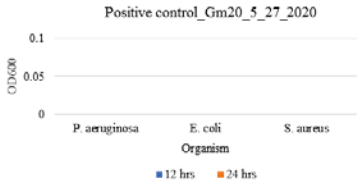


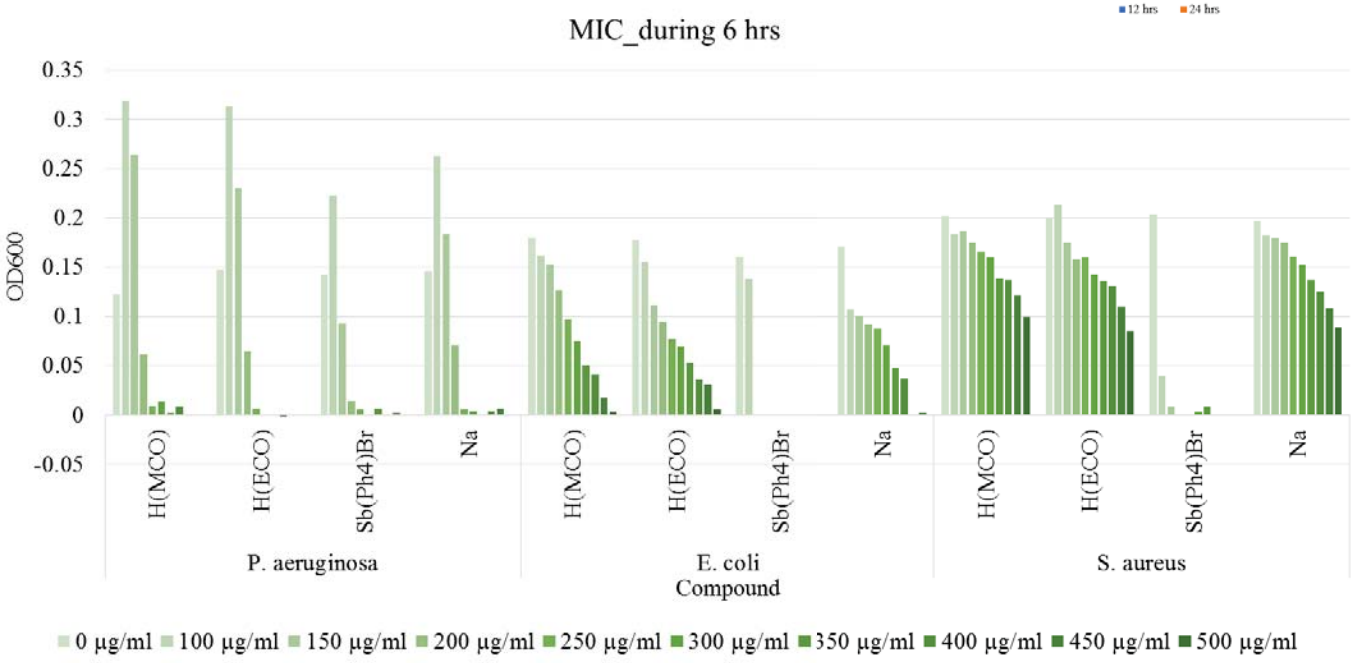
MIC of H(MCO), H(ECO), Sb(Ph$_4$)MCO and Na[CH(ACO)$_2$] in *P. aeruginosa, E. coli* and *S. aureus* cultures

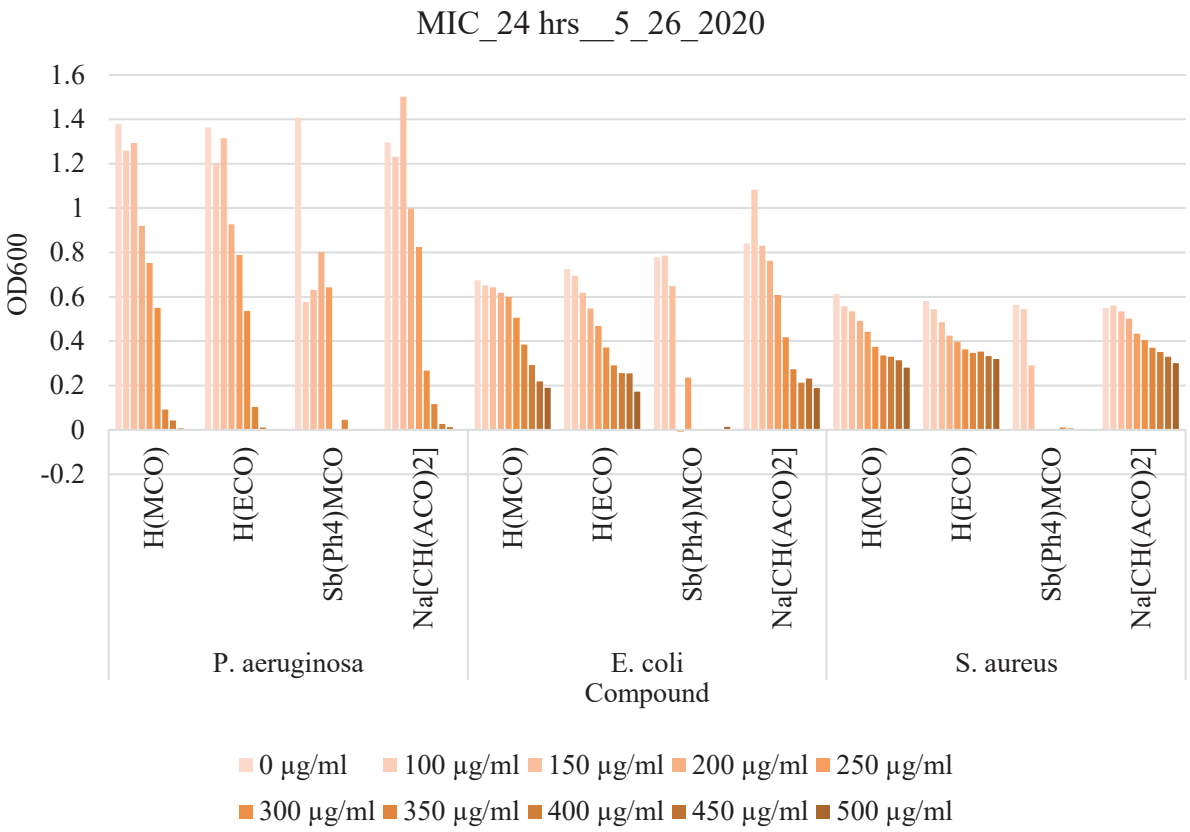


- Antibiotic concentrations: 100 to 500 ug/ml in increments of 50
- DMSO gradient: 0 to 10% in increments of 1

- 2 readings: 6 hrs, 24 hrs
- PA still shows higher growth than the other EC and SA







Results of this solutions study evidenced that the starting compounds do not inhibit growth of microorganisms, and there is pronounced efficacy of our new series of organoantimony(V) compounds prepared at MSU.

APPENDIX C

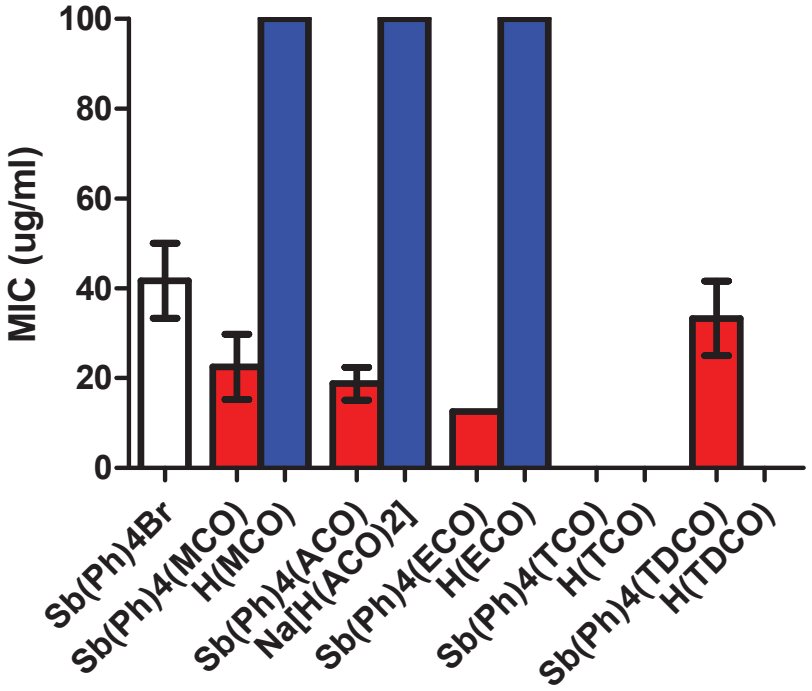
ANTIFUNGAL ACTIVITY OF COMPOUNDS

Experiment information

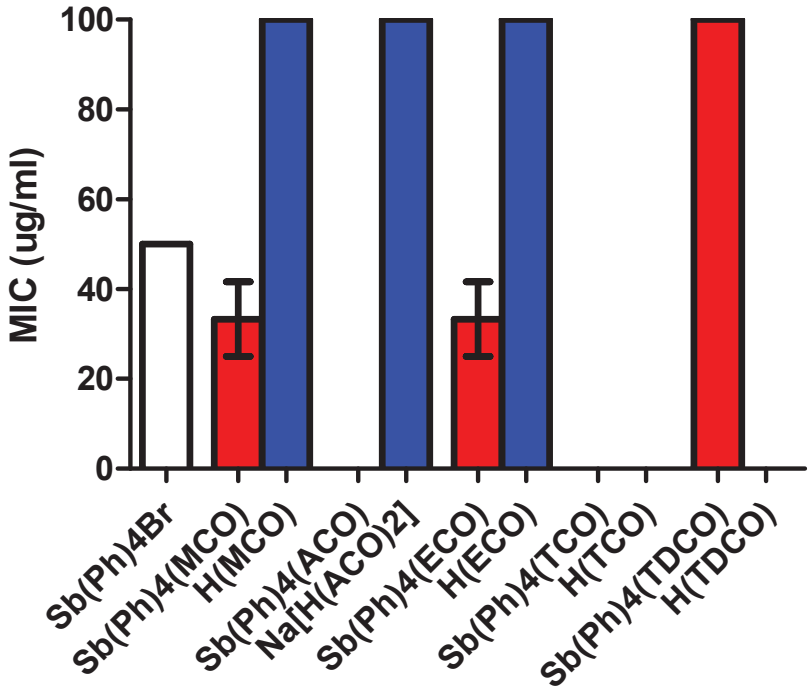
- Disk diffusion assays:
  - No inhibition of Cryptococcus neoformans or Candida albicans for the following compounds at any concentration (S, x2, x4): H(ACO), H(ECO), H(MCO)
  - Still several disk compounds remaining to be tested

- MIC assays:
  - Each solubilized in DMSO at 5mg/ml, starting concentration is 100µg/ml
  - Several compounds tested had antifungal activity (inhibited growth) – see attached graphs
    - Each compound was tested with each organism in three independent experiments. Error bars represent standard error of the mean (if none, the values were identical in each experiment).
      - Untested compounds have no bar
      - Bars reaching 100µg/ml were not considered to have antifungal activity
    - One compound - Sb(Ph)4(MCO) - had **fungicidal activity** (killed *C. neoformans*) at a concentration of 100µg/ml
      - MIC for this compound with *C. neoformans* was 22.5 µg/ml

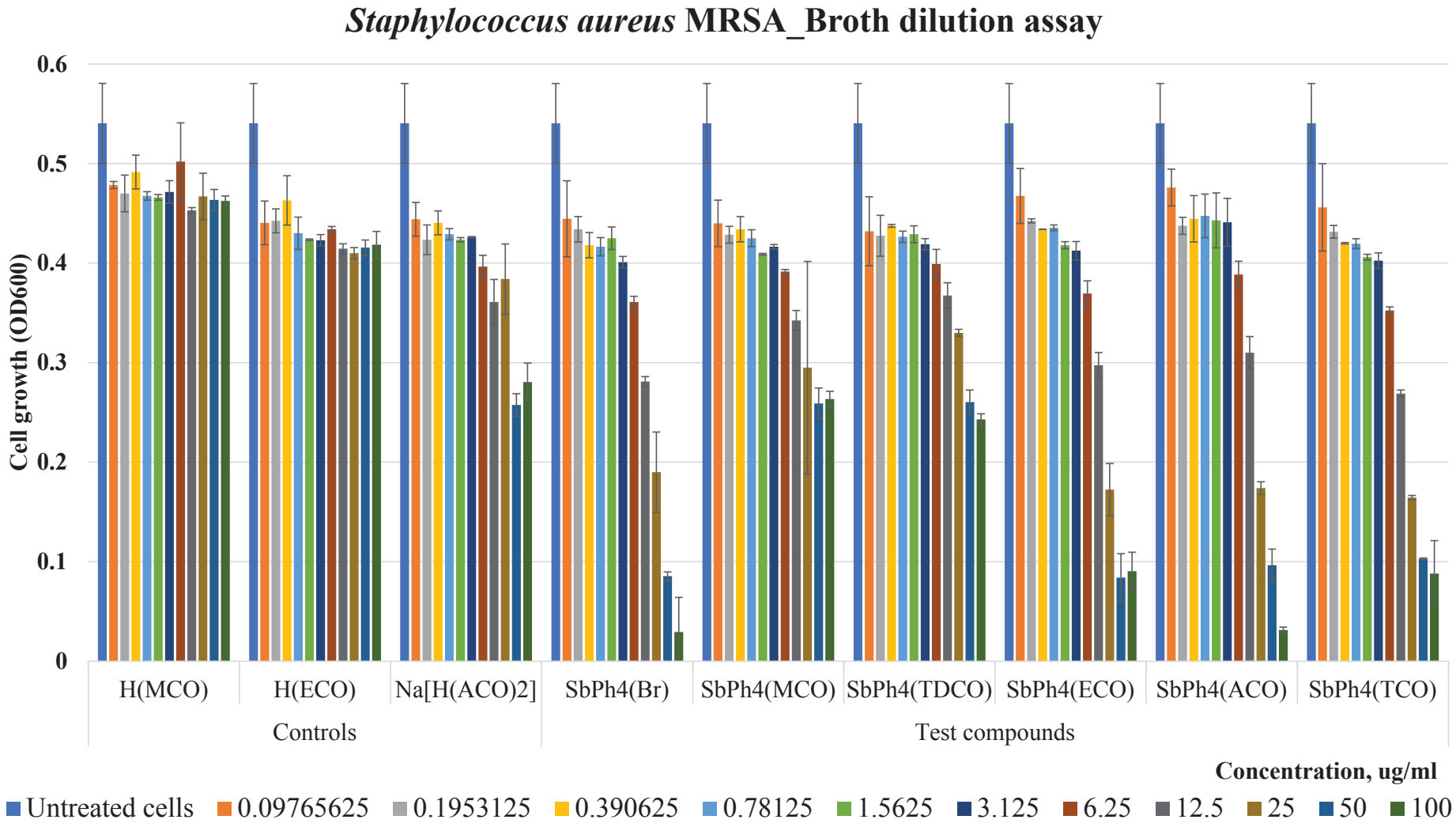
Cryptococcus neoformans

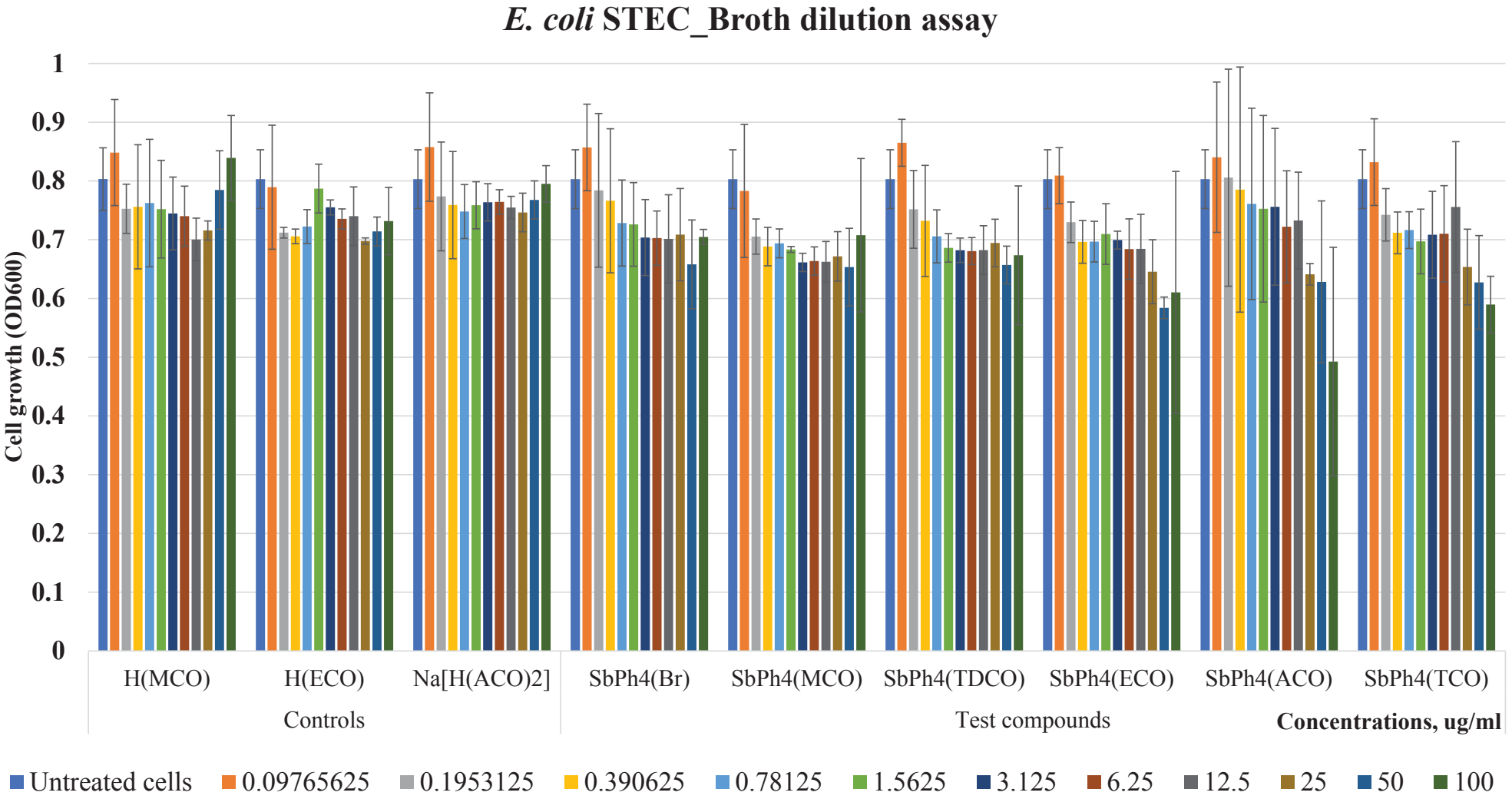


Candida albicans



APPENDIX D


Staphylococcus aureus MRSA_Broth dilution assay
Cell growth (OD600)
0.6
0.5
0.4
0.3
0.2
0.1
0
H(MCO)
H(ECO)
Na[H(ACO)2]
SbPh4(Br)
SbPh4(MCO)
SbPh4(TDCO)
SbPh4(ECO)
SbPh4(ACO)
SbPh4(TCO)
Controls
Test compounds
Concentration, ug/ml
Untreated cells
0.09765625
0.1953125
0.390625
0.78125
1.5625
3.125
6.25
12.5
25
50
100


E. coli STEC_Broth dilution assay
Cell growth (OD600)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
H(MCO)
H(ECO)
Na[H(ACO)2]
SbPh4(Br)
SbPh4(MCO)
SbPh4(TDCO)
SbPh4(ECO)
SbPh4(ACO)
SbPh4(TCO)
Controls
Test compounds
Concentrations, ug/ml
Untreated cells
0.09765625
0.1953125
0.390625
0.78125
1.5625
3.125
6.25
12.5
25
50
100

Conclusions:

- Compounds containing Sb display higher bactericidal activity against Gram positive *Staphylococcus aureus* then Gram negative *E.coli*

- Sb(Ph4)Br, Sb(Ph4)ECO, Sb(Ph4)ACO, and Sb(Ph4)TCO show significant bactericidal activity against multi-drug resistant strain of Gram positive *S. aureus* with Sb(Ph4)Br and Sb(Ph4)ACO showing MIC (minimum inhibitory concentrations) of 100 μg/ml

- 25-40% reduction of growth was also observed for Sb(Ph4)Br, Sb(Ph4)ECO, Sb(Ph4)ACO, and Sb(Ph4)TCO against Gram negative *E.coli*