



Article

Exploring Titanium(IV) Complexes as Potential Antimicrobial Compounds

Israel Rodríguez , Lauren Fernández-Vega , Andrea N. Maser-Figueroa, Branlee Sang, Patricia González-Pagán and Arthur D. Tinoco *

Department of Chemistry, University of Puerto Rico–Río Piedras Campus, San Juan 00925, Puerto Rico; israel.rodriguez6@upr.edu (I.R.); lauren.fernandez@upr.edu (L.F.-V.); andrea.maser@upr.edu (A.N.M.-F.); branlee.sang@upr.edu (B.S.); patricia.gonzalez6@upr.edu (P.G.-P.)

* Correspondence: arthur.tinoco@upr.edu; Tel.: +1-939-319-9701

Abstract: Due to the rapid mutation of pathogenic microorganisms, drug-resistant superbugs have evolved. Antimicrobial-resistant germs may share their resistance genes with other germs, making them untreatable. The search for more combative antibiotic compounds has led researchers to explore metal-based strategies centered on perturbing the bioavailability of essential metals in microbes and examining the therapeutic potential of metal complexes. Given the limited knowledge on the application of titanium(IV), in this work, eight Ti(IV) complexes and some of their corresponding ligands were screened by the Community for Open Antimicrobial Drug Discovery for antimicrobial activity. The compounds were selected for evaluation because of their low cytotoxic/antiproliferative behavior against a human non-cancer cell line. At pH 7.4, these compounds vary in terms of their solution stability and ligand exchange lability; therefore, an assessment of their solution behavior provides some insight regarding the importance of the identity of the metal compound to the antimicrobial therapeutic potential. Only one compound, Ti(deferasirox)₂, exhibited promising inhibitory activity against the Gram-positive bacteria methicillin-resistant *Staphylococcus aureus* and minimal toxicity against human cells. The ability of this compound to undergo transmetalation with labile Fe(III) sources and, as a consequence, inhibit Fe bioavailability and ribonucleotide reductase is evaluated as a possible mechanism for its antibiotic effect.

Keywords: transmetalation; titanium(IV) antimicrobial compounds; iron chelation



Citation: Rodríguez, I.; Fernández-Vega, L.; Maser-Figueroa, A.N.; Sang, B.; González-Pagán, P.; Tinoco, A.D. Exploring Titanium(IV) Complexes as Potential Antimicrobial Compounds. *Antibiotics* **2022**, *11*, 158. <https://doi.org/10.3390/antibiotics11020158>

Academic Editor:
Enrique Domínguez-Álvarez

Received: 1 January 2022
Accepted: 20 January 2022
Published: 26 January 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The urgent need for new classes of antimicrobial drugs to fight resistance and the evolution of superbugs has forced researchers to explore different strategies. The classical approach has been the synthesis of derivatives of already known compounds that can specifically attack microbes rather than human cells, the majority of which are purely organic compounds. This approach is quickly reaching the limit of its possibilities. Given the central role that essential metals can play in microbial growth and the distinctive chemistry that can be facilitated by metals coupled with the varied structures and properties that they can adopt from ligand complexation, metal-centered antimicrobial strategies are an exciting new research avenue. One approach is developing compounds that can uniquely perturb the essential metal homeostasis within pathogenic microbes and possibly even attenuate their bioavailability [1–4]. Another approach is assessing the therapeutic potential of metals in ion or complex form. Bismuth(III) and silver(I) are currently the only metals used in the treatment of certain infections [5–9]. Ag(I) has been tested in combination with different classes of antibiotic agents and exhibits excellent synergism [10]. The Ag(I) complex of penicillin-G expands the spectrum of the effect of the antibiotic so that it might be useful against Gram-negative bacteria [11]. A variety of organometallic derivatives of known antibacterial drugs have been explored [12]. In this regard, the ferrocenyl moiety

holds tremendous potential as a multipurpose antimicrobial agent [13]. A recent review highlights some of the most important antibiotic metal complexes developed over the last decade [14].

Recently Blaskovich et al. undertook a very ambitious effort to screen the results of over 295,000 compounds that were submitted to the Community for Open Antimicrobial Drug Discovery (CO-ADD) from laboratories throughout the world with the intent of identifying promising antimicrobial metal-based compounds with little to no toxicity against human cells [9]. The CO-ADD provides a free service to test compounds at a one dose concentration (32 µg/mL) against four Gram-negative bacteria (*Escherichia coli*, *E. coli*; multidrug-resistant *Klebsiella pneumoniae*, *K. pneumoniae* (MDR); *Acinetobacter baumannii*, *A. baumannii*; and *Pseudomonas aeruginosa*; *P. aeruginosa*), the Gram-positive bacteria (methicillin-resistant *Staphylococcus aureus*, methicillin resistant *S. aureus* (MRSA)), and the fungi (*Candida albicans*, *C. albicans* and *Cryptococcus neoformans*, *C. neoformans*) [15]. Depending on the potency demonstrated, the compounds are then evaluated in a dose-response screening (32–0.25 µg/mL) against the microbes and are also tested for cytotoxicity against the human embryonic kidney cell line HEK293 and hemolysis against human red blood cells. Blaskovich et al. found that 906 metal-based compounds had been screened, of which 88 met the criteria of displaying a minimum inhibitory concentration (MIC) of ≤ 16 µg/mL against at least one microbe and no toxicity against the human cells [9]. They made a valiant call to the academic community to continue the effort to discover new antimicrobial metal-based compounds and contribute compounds from their own labs to the CO-ADD. To our surprise, not one of the 906 metal-based compounds in the study of Blaskovich et al. was a Ti(IV) compound [9]. In this work, we respond to this call by contributing a series of titanium(IV) compounds.

Titanium, the ninth most abundant element on the earth's crust, is generally non-toxic to the human body and is commonly used in the medical field in prosthetics [16–18]. Depending on how Ti(IV) is coordinated by ligands in coordination compounds, the metal ion can be transformed into a cytotoxic species, which is why it has been explored for anticancer applications [17,19–21]. Two Ti(IV) compounds, titanocene dichloride and budotitane, were tested in clinical trials as anticancer drug candidates, although they did not advance to the drug market [22,23]. Ti(IV) has been far less investigated for its antimicrobial properties. Due to their semiconductor properties, TiO₂ nanoparticles (TiO₂ NPs) in the anatase and rutile forms have been extensively studied for their UV-induced photocatalytic ability to generate reactive oxygen species, which can effectively kill microbes [24]. TiO₂ NPs have been shown to kill Gram-positive and Gram-negative bacteria [25–27] and can be useful in the disinfection of water contaminated with *E. coli* [28]. TiO₂ NPs also demonstrate some antifungal capacity against a variety of fungi [29–31]. In *C. albicans*, the NPs cause morphological changes that indicate that their direct contact with the cells is the main mechanism of cell death [30]. Much less is known about the microbial properties of soluble Ti(IV) compounds. The German physician Julius Pick observed that hydrolyzed Ti(IV) sulfate and Ti(IV) mono- and disalicylates could inhibit putrefaction of protein-rich materials and served as an effective topical and oral treatment of tuberculosis [27]. These compounds were likely some form of titanyl (TiO²⁺) species in solution [17]. McCue et al. reported that titanyl sulfate could inhibit the growth of *E. coli*, *S. typhimurium*, and *P. aeruginosa*, possibly by a general inhibition of the bacterial serine proteases [32]. The bacteria exhibited loss of motility and compromised metabolism. Motility is linked to bacterial virulence. Titanyl sulfate was also able to inhibit *E. coli* in soil, but this ability was decreased in the presence of increasing amounts of oxalate, possibly owed to the formation of an inactive titanyl oxalate species [33,34]. In related studies, white mice fed titanyl oxalate supplied in bread exhibited higher weight in the form of lean mass compared to control mice [33,35], which might have been correlated with a 50% decrease in the total intestinal bacterial count [35]. The bacteriostatic/bactericidal activity of titanyl oxalate in these mice is probably attributable to the acidification and dissociation of the compound during digestion, liberating the titanyl moiety [34].

With the context of what is known about the antimicrobial behavior of soluble Ti(IV) compounds, we selected eight Ti(IV) compounds that are commonly utilized/studied in our laboratory to be screened by the CO-ADD (Figure 1). These compounds were selected based on our own in-house assessment of their low antiproliferative activity/cytotoxicity against the noncancerous lung cell line MRC-5 and because they range in their aqueous solution stability and ligand exchange lability. The results reveal little to no activity against the screened microbes with the exception of one compound (titanium(IV) bis(deferasirox) against MRSA. Insight into the inhibitory mechanism of this compound is explored with regard to its iron-binding capability.

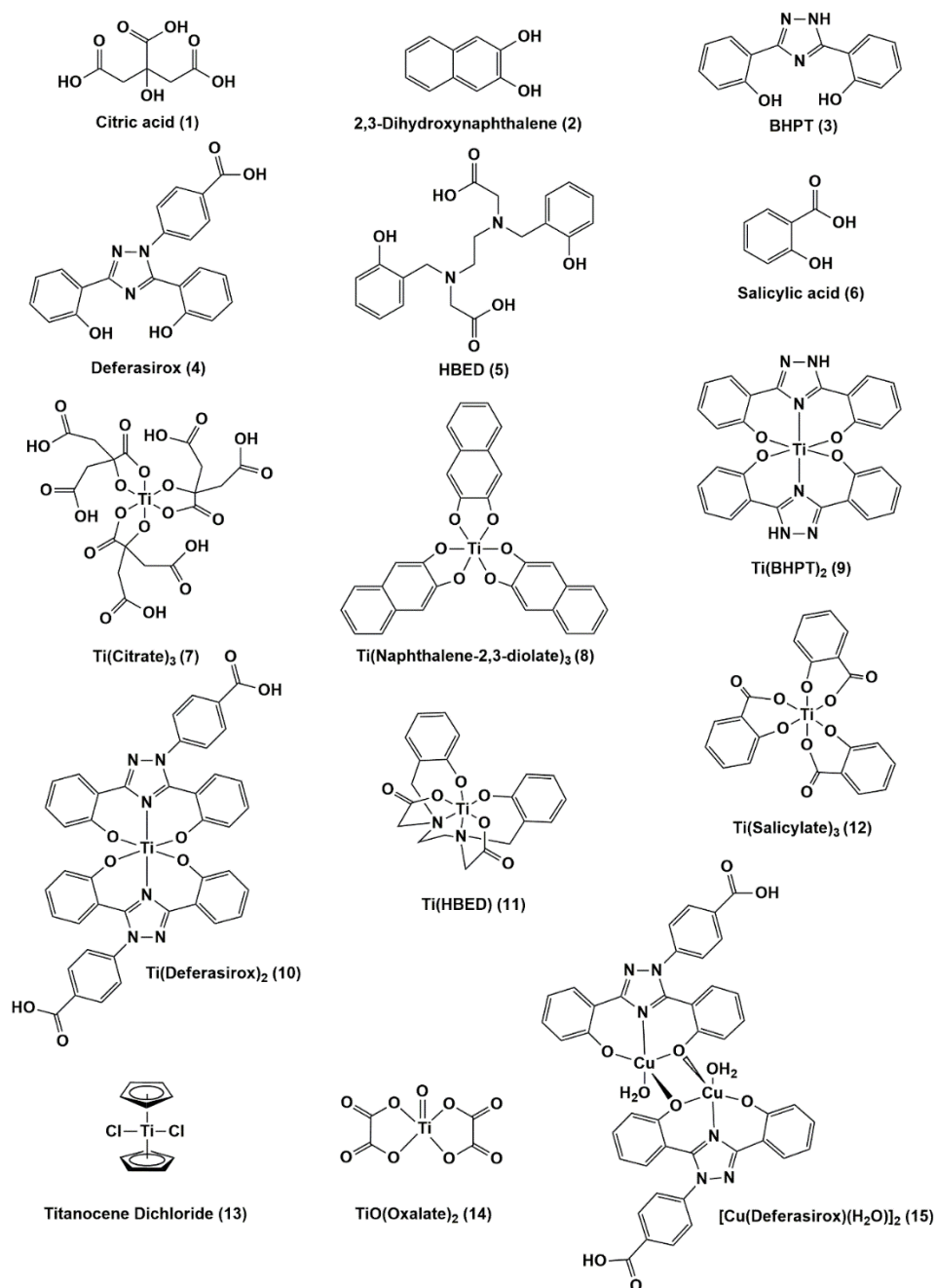


Figure 1. The known/proposed structures of eight Ti(IV) complexes and some of their corresponding ligands and the compound [Cu(Deferasirox)(H₂O)]₂ that were tested for antimicrobial activity.

2. Materials and Methods

2.1. Chemicals

N,N'-di(o-hydroxybenzyl)ethylenediamine-*N,N'*-diacetic acid monohydrochloride hydrate (HBED·HCl·H₂O) was purchased from Strem Chemicals, Inc. (Newburyport, MA, USA). 4-[(3Z,5E)-3,5-Bis(6-oxocyclohexa-2,4-dien-1-ylidene)-1,2,4-triazolidin-1-yl] benzoic acid (deferiasirox) was purchased from Focus Synthesis LCC (San Diego, CA, USA). 2-[3-(2-Hydroxyphenyl)-1H-1,2,4-triazol-5-yl]phenol (BHPT) was purchased from Princeton Biomolecular Research, Inc. (Sonesta ES Suites South Brunswick-Princeton, NJ, USA). Trisodium citrate (Na₃Citrate) was purchased from Thermo Fisher Scientific (Waltham, MA, USA). 2,3-dihydroxynaphthalene, salicylic acid, titanium(IV) tetrachloride (TiCl₄), sodium chloride, sodium hydroxide pellets, hydrochloric acid (37%), and ethanol (99.5%, 200 proof) were obtained from Fisher Scientific (Nazareth, PA, USA). Cisplatin was purchased from Abcam (Cambridge, UK). *N,N*-dimethylformamide (DMF, 99.9%, HPLC grade) and dimethyl sulfoxide (DMSO, 99.9%) were purchased from Sigma-Aldrich (St. Louis, MN, USA). Compressed Argon (Ar, UN1006) was obtained from Messer (Puerto Rico, USA). MRC-5 human lung normal cells were obtained from ATCC (CCL-185 and CCL-171, respectively) (Manassas, VA, USA). Mycoplasma removal agent was used in the preparation of cell stocks (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The cell line was cultured in phenol red Dulbecco's Modified Eagle's Medium (DMEM, D6429) Millipore Sigma, Burlington, MA, USA) supplemented with 10% Fetal Bovine Serum (FBS; HyClone, Thermo Fisher Scientific, Waltham, MA, USA) and 1% of penicillin-streptomycin (Prepared from the solid obtained from Sigma-Aldrich (St. Louis, MO, USA) and prepared as follows: Streptomycin 11 mg/mL and Penicillin 7 mg/mL) at 37 °C in a humidified atmosphere of 5% (*v/v*) CO₂. Phenol red-free DMEM (CellGro REF 17-205-CV, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with L-glutamine (Millipore Sigma, Burlington, MA, USA) at 2.4 mM, 10% FBS, and 1% of penicillin-streptomycin was used for cell viability studies. 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Autoclavable 0.2 micron filters were obtained from Thermo Fisher Scientific (Waltham, MA, USA) and were used to sterile filter solutions. Tris (biotechnology grade) from Amresco—VWR International LLC (Solon, OH, USA) was used to prepare 1M Tris buffer (pH 11). Dodecyl sulfate sodium salt (SDS, electrophoresis grade, 98% pure) was obtained from Acros Organics—Thermo Fisher Scientific (Waltham, MA, USA). Trypan blue solution (0.4%) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and was used as a cell staining solution. Ingredients to prepare sterile 10X Phosphate buffer saline (PBS) was obtained from Thermo Fisher Scientific (Waltham, MA, USA) and was diluted tenfold to produce 1X PBS (pH 7.4). A Hausser Scientific hemacytometer (Thermo Fisher Scientific (Waltham, MA, USA)) was used to perform cell counts. All commercially purchased materials were of high purity and used as received. All aqueous solutions were prepared with autoclaved (121 °C and 18 psi) high-quality nanopure water (18.2 MΩ·cm resistivity) at 25 °C, PURELAB flex system (ELGA Corporation, Woodridge, IL, USA).

2.2. Sources of Metal Complexes

The metal compounds submitted to the CO-ADD for screening were either commercially purchased or synthesized and confirmed to be at ≥95% purity. Bis(cyclopentadienyl)titanium(IV) dichloride (aka titanocene dichloride; 248.96 g/mol) was purchased from Sigma Aldrich. Potassium dioxalatoxotitanate(IV) dihydrate (K₂[TiO(Oxalate)₂]·2H₂O; 354.13 g/mol) was obtained from Fisher Scientific. Ti(HBED)·0.5H₂O (441.40 g/mol), Ti(Deferiasirox)₂·H₂O (790.57 g/mol), Ti(BHPT)₂·2H₂O·C₃H₆O (C₃H₆O = acetone) (644.46 g/mol), and K₂[Ti(Naphthalene-2,3-diolate)₃]·3H₂O (654.58 g/mol) were synthesized by following our previously published protocols [36–38]. Na₂[Ti(Salicylate)₃]·4H₂O·0.5C₂H₆O (C₂H₆O = ethanol) (597.26 g/mol) and K₂[Ti(Citrate)₃]·2H₂O (732.41 g/mol) were synthesized using published synthetic routes [39,40]. [Cu(Deferiasirox)H₂O]₂ (905.83 g/mol) was synthesized following our laboratory protocol [41]. The molecular formulas for all synthesized metal compounds submitted to the CO-ADD screening were proposed by using C,H,N elemental analyses performed by Atlantic Microlab,

Inc. (Norcross, GA, USA) and are within 0.4% of the calculated values for the proposed formula and where applicable, these formulas are consistent with our previously published use of the compounds [36–38,42,43]. For simplicity, all compounds in this study will be referred to without their solvates or co-crystals.

2.3. Instrumentation

Absorbance measurements were conducted on the Agilent Technologies Cary Series UV-Vis spectrophotometer Cary 100 UV-Vis model using the UV Cary Scan software version v.20.0.470. All analyses are carried out with a scan of 600–200 nm. pH titrations were conducted with ThermoScientific Orion Star A211 and an Orion 9157BNMD electrode, calibrated at pH 4, 7, and 10 with standard buffer solutions. X-ray diffraction was performed using a Rigaku XtalLAB SuperNova single-micro-focus copper-K α -007 radiation (5-007 = 1.5417 Å) source equipped with a HyPix3000 X-ray detector in 50 kV transmission mode and 1 mA in CrystAllisPRO software ver. 1.171.39.43c. A Nicolet iS50 FTIR Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to collect FTIR absorbance spectra. The EDS was obtained with 20 kV operating voltage in a scanning electron microscope JEOL JSM-5800LV. An Infinite M200 PRO Tecan Microplate Reader was used for cell viability measurements.

2.4. MRC-5 Cell Viability Study of Dose–Response Treatment with Different Compounds by the MTT Assay

A thawed stock of MRC-5 cells was washed with 1X PBS and resuspended in phenol red DMEM media (supplemented with 10% FBS and 1% of penicillin-streptomycin) and then seeded in a 100 mm \times 20 mm (complete, O.D. \times H) petri dish and grown in a 5% (v/v) CO $_2$ humidified atmosphere at 37 °C. At least three passages were performed to ensure the integrity of the cells. After this point, cells were collected, thoroughly washed with 1X PBS, and then resuspended in phenol red-free DMEM (supplemented with 10% FBS, 1% Streptomycin/Penicillin, and 2.4 mM L-glutamine) at a 2.0×10^5 cells/mL concentration. Approximately 100 μ L of the cell solution was seeded into wells of several 96-well plates to begin the MTT assay [44], which was performed in the 5% (v/v) CO $_2$ humidified atmosphere at 37 °C. At least one lane of wells in each plate was supplied media without cells as a control. After the plates were left incubating for one day, cells were treated with 100 μ L of 1 to 100 μ M final concentrations of the following compounds: K $_2$ [Ti(Naphthalene-2,3-diolate) $_3$], titanocene dichloride, K $_2$ [TiO(Oxalate) $_2$], K $_2$ [Ti(Citrate) $_3$], Na $_2$ [Ti(Salicylate) $_3$], [Cu(Deferasirox)(H $_2$ O) $_2$], 2,3-dihydroxynaphthalene, salicylic acid, and Na $_3$ Citrate. Most of the compounds were dissolved in 1X PBS buffer at pH 7.4. Due to poor aqueous solubility, [Cu(Deferasirox)(H $_2$ O) $_2$] was extensively sonicated in the buffer for 30 min at 30 °C until no particulate matter could be observed. Titanocene dichloride was prepared as a fresh stock solution in DMSO and then diluted in 0.1 M NaCl (aq) instead of 1X PBS (to minimize hydrolysis induced precipitation [45]) to the desired concentration while maintaining a constant 1% (v/v) DMSO background (0.5% DMSO after mixing with the well media). A control of cells treated with media containing 1% (v/v) DMSO was included in the study. The titanocene solutions were used within an hour of preparation in the final cell solution. Cisplatin at 40 μ M final concentration after mixing with cells was used as a negative control. The cisplatin stock solution had to be prepared in DMF (and not DMSO [46]), then diluted in buffer and used soon after dilution. At 3 h before a total of 72 h of incubation with the compounds, the cells were treated with 25 μ L of 9 mg/mL MTT prepared in 1X PBS and sterile-filtered. Upon completion of the three hours, 50 μ L of 10% (w/v) SDS (prepared in 0.1 M Tris buffer at pH 11) [47] was added to solubilize the purple formazan product crystals and left overnight in the incubator. Control wells in the plates consisted of: (1) Untreated cells + MTT as the measure of 100% viable cell growth in the media and (2) Media with no cells + MTT as the measure of zero cells in the media. Two sets of compounds could be tested per plate. The plates were read at the wavelength of 570 nm (absorbance of the formazan product at pH 10.0) and 800 nm (background

control) using a Tecan plate reader. The absorbance of all wells was compared to the absorbance of the untreated cells with the MTT set as the 100% viable cell standard and the absorbance of the zero cell control set as the 0% viable cell standard. The 40 μ M cisplatin negative control produced $5.2 \pm 1.4\%$ viable cells in accordance with expectation. Nonlinear regression in Origin 8.5 was utilized to fit the growth curves using the pharmacology dose-response equation to determine the half-maximal inhibitory concentration (IC_{50}) and the corresponding standard deviation. All samples were tested six times.

2.5. Solid-State and pH and Concentration Dependent Characterization of Ti(IV) Salicylate Complexation

The complex $Na_2[Ti(\text{salicylate})_3]$ has been previously characterized by single-crystal X-ray diffraction [39]. In this work, the compound was evaluated by powder X-ray diffraction (PXRD), Fourier transform infrared (FT-IR) spectroscopy, and energy-dispersive X-ray spectroscopy (EDS).

The Ti(IV) and salicylic acid solution complexation at a mole equivalent ratio of 1:3 were studied under pH-dependent conditions by UV-Vis absorbance spectroscopy (250 to 600 nm) at 25.0 °C. Stock solutions of salicylic acid and $TiCl_4$ were prepared in pure ethanol. The $TiCl_4$ stock solution preparation required that the ethanol be deoxygenated by purging with Ar gas and chilled in an ice-water bath prior to syringe addition of $TiCl_4$. Once prepared, the solution could be open to the air. The Ti(IV) concentration of this solution was determined by our previously published 2,3-dihydroxynaphthalene colorimetric assay [48]. The ligand was diluted to 150 μ M to a final background of 50:50 ethanol:water containing 0.1 M NaCl (to maintain an ionic strength (I) of 0.1 M) and then divided into two batches. One batch was pH titrated with aliquots of either 0.1 M or 1.0 M HCl by micropipette to acidify the solution. The other batch was pH titrated with aliquots of either 0.1 M or 1.0 M NaOH to basify the solution. The selection of acid or base concentration was to maintain the total volume of addition low to not alter the concentration of the analyte by greater than 5%. After the addition of each aliquot of acid or base, the solutions were equilibrated for at least 10 min, when the millivoltage reading remained constant. At predefined pH values, 1 mL of the titration solution was removed for analysis by UV-Vis absorbance. The changes in concentration were accounted for. The same pH titration was performed for the ligand (150 μ M) in the presence of Ti(IV) (50 μ M). In total, the pH-metric titrations of the ligand in the presence and absence of Ti(IV) were performed in the pH range 2.0–12.0.

A concentration-dependent study was performed. A stock 10 mM $Na_2[Ti(\text{Salicylate})_3]$ solution was prepared in DMF because it is not soluble in ethanol and has limited solubility in water. The solution was diluted to the concentrations 5, 1, 0.5, 0.25, and 0.15 mM with a fixed background of 50:50 DMF:water, containing 0.1 M NaCl. The solutions were left to equilibrate overnight and were all pH ~ 4.7 without any pH adjustment. UV-Vis absorbance spectra of the solutions were collected.

2.6. Compound Solution Preparation by the CO-ADD

Dry compounds ranging from 1–2 mg were weighed into properly labeled Eppendorf tubes and sealed with parafilm. The compounds were used as received by the CO-ADD. For the screenings, most compounds were dissolved to a stock concentration of 10 mg/mL in DMSO. $K_2[Ti(\text{Citrate})_3]$, $Na_3\text{Citrate}$, and $K_2[TiO(\text{Oxalate})_2]$ were dissolved in warm water. $[Cu(\text{Deferasirox})(H_2O)]_2$ was dissolved in pyridine after its solubility was determined to be excellent in pyridine due to coordination of the solvent to the metal producing $[Cu(\text{Deferasirox})(\text{pyridine})]_2$ in situ [41]. Samples were diluted in water to a final testing concentration of 32 μ g/mL while keeping the final co-solvent concentration to a maximum of 0.5%. Where applicable, compounds were serially diluted 1:2 fold eight times. Each sample concentration was prepared in 384-well plates, non-binding surface plate (NBS; Corning 3640) for each bacterial/fungal strain, tissue-culture treated (TC-treated; Corning 3712/3764), black for mammalian cell types and polypropylene 384-well (PP; Corning 3657)

for hemolysis assays, all in duplicate ($n = 2$), and keeping the final DMSO concentration to a maximum of 0.5%. All sample preparation was conducted using liquid handling robots.

2.7. CO-ADD Antibacterial Assays

For the bacterial assays, four Gram-negative bacteria (*E. coli* (ATCC 25922, Manassas, VA, USA); *K. pneumoniae* (ATCC 700603), *A. baumannii*; (ATCC 19606), and *P. aeruginosa* (ATCC 27853) and the Gram-positive bacteria (*S. aureus* MRSA (ATCC 43300) were used. Each strain was cultured in cation-adjusted Mueller Hinton broth (CAMHB; Bacto Laboratories 212322, Mount Pritchard, Australia) at 37 °C overnight. A sample of each culture was then diluted 40-fold in fresh CAMHB and incubated at 37 °C for 1.5–3 h. The resultant mid-log phase cultures were diluted with CAMHB (CFU/mL measured by OD₆₀₀), then added to each well of the compound-containing plates (384-well non-binding surface (NBS) plates; Corning CLS3640, Millipore Sigma, Burlington, MA, USA), giving a cell density of 5×10^5 CFU/mL and a total volume of 50 µL. Plates were covered and incubated at 37 °C for 18 h without shaking. Inhibition of bacterial growth was determined by measuring absorbance at 600 nm (OD₆₀₀) with a Tecan M1000 Pro monochromator plate reader, using media-only as negative control and bacteria without inhibitors as a positive control. Minimum inhibitory concentration (MIC) values were determined as the lowest concentration at which the growth was inhibited at greater than or equal to 75%. Colistin sulfate (Sigma C4461) and vancomycin HCl (Sigma 861987) were used as internal controls on each plate for Gram-negative and Gram-positive bacteria, respectively. These inhibitors exhibited MIC values (µg/mL) against the microbes within the expected range.

2.8. CO-ADD Antifungal Assays

For the fungal assays, the fungi (*C. albicans* (ATCC 90028) and *C. neoformans*) (ATCC 208821) were used. Both fungi (yeast) strains were cultured for 3 days on Yeast Extract-Peptone Dextrose (YPD; Becton Dickinson 242720, Thermo Fisher Scientific, Waltham, MA, USA) agar at 30 °C. A yeast suspension of 1×10^6 to 5×10^6 CFU/mL (as determined by OD₅₃₀) was prepared from five colonies from the agar plates, subsequently diluted with Yeast Nitrogen Base media (YNB; Becton Dickinson 233520, Thermo Fisher Scientific, Waltham, MA, USA), and added to each well of the compound-containing plates (384-well plates, NBS; Corning CLS3640) giving a final cell density of 2.5×10^3 CFU/mL and a total volume of 50 µL. Plates were covered and incubated at 35 °C for 36 h without shaking. Growth inhibition of *C. albicans* was determined by measuring absorbance at 630 nm (OD₆₃₀), while the growth inhibition of *C. neoformans* was determined by measuring the difference in absorbance between 600 and 570 nm (OD_{600–570}), after the addition of resazurin (0.001% final concentration; Sigma R7017) and incubation at 35 °C for 2 h, using media-only as a negative control and fungi without inhibitors as a positive control. MIC values were determined as the lowest concentration at which the growth was inhibited at greater than or equal to 75%. Fluconazole (Sigma F8929) was used as an internal control on each plate for both strains. These inhibitors exhibited MIC values (µg/mL) against the microbes within the expected range.

2.9. CO-ADD Cytotoxicity Assays

HEK293 ATCC CRL-1573 human embryonic kidney cells were counted manually in a Neubauer hemocytometer and added to compound-containing plates (384-well plates, tissue culture-treated (TC); Corning CLS3712), giving a final density of 5000 cells per well in a total volume of 50 µL, using Dulbecco's Modified Eagle Medium (DMEM; Life Technologies 11995-073) with 10% FBS (GE SH30084.03). The cells were incubated together with the compounds for 20 h at 37 °C in 5% CO₂. Cytotoxicity (or cell viability) was measured by fluorescence, ex: 560/10 nm, em: 590/10 nm (F_{560/590}), after addition of 5 µL of 25 µg/mL resazurin (2.3 µg/mL final concentration; Sigma R7017) and after further incubation for 3 h at 37 °C in 5% CO₂, using media-only as a negative control and cells without inhibitors as a positive control. CC₅₀ (concentration at 50% cytotoxicity) were calculated by curve-fitting

the inhibition values vs. log(concentration) using a sigmoidal dose–response function, with variable fitting values for the bottom, top, and slope. Tamoxifen (Sigma T5648) was used as an internal control on each plate and exhibited a CC_{50} value within the expected range.

2.10. CO-ADD Hemolysis Assays

Human whole blood (Australian Red Cross) was washed three times with three volumes of 0.9% NaCl and resuspended in a concentration of 0.5×10^8 cells per mL, determined by manual cell count in a Neubauer hemocytometer. Washed cells were added to the compound-containing plates (384-well polypropylene plates (PP); Corning 3657) for a final volume of 50 μ L, shaken and incubated for 1 h at 37 °C. After incubation, the plates were centrifuged at $1000 \times g$ for 10 min to pellet cells and debris, 25 μ L of the supernatant was then transferred to reading plates (384 well, polystyrene plated (PS), Corning CLS3680), with hemolysis determined by measuring the supernatant absorbance at 405 nm (OD405), using cells without inhibitors as a negative control and cells with 1% Triton X-100 (Sigma T8787) as a positive control. HC_{10} and HC_{50} (concentration at 10% and 50% hemolysis, respectively) were calculated by curve fitting the inhibition values vs. log(concentration) using a sigmoidal dose–response function with variable fitting values for the top, bottom, and slope. Melittin (Sigma M2272) was used as an internal control on each plate and exhibited HC_{10} and HC_{50} values within the expected range.

3. Results

3.1. MRC-5 Cell Viability Study of Dose–Response Treatment with Different Compounds by the MTT Assay

Ti(IV) compounds (Figure 1) selected for submission to the CO-ADD antimicrobial screening had to meet the criteria of low antiproliferative activity/cytotoxicity against a non-cancer human cell line as measured by the MTT assay at pH 7.4. For this purpose, we selected the MRC-5 lung cell line commonly used in our laboratory as a control in our cancer-related research and opted not to include a cancer cell line given the selectivity toward cancer cells that some of the compounds have demonstrated. We defined low antiproliferative activity/cytotoxicity as either a compound that exhibits an absolute IC_{50} value greater than 20 μ M or inhibits cell proliferation at no greater than 50% within the micromolar concentration range. Absolute IC_{50} values are determined for compounds that inhibit cell proliferation at nearly 100%, whereas relative IC_{50} values are determined for inhibitory activity significantly less than 100%. Some of the IC_{50} values were previously determined, while others were measured in this work (Figure 2 and Table 1). All of the Ti(IV) compounds and the ligands met the criteria for low antiproliferative activity/cytotoxicity, while the compound $[Cu(Deferasirox)(H_2O)]_2$ did not. The data for the compounds that do not show any antiproliferative behavior are not shown with the exception of salicylic acid (monoanionic at pH 7.4), which is included as a representative example (Figure 2). As noted in a previous publication, deferasirox causes a nearly three-fold increase in the proliferation of MRC-5 cells for reasons that are not known [36].

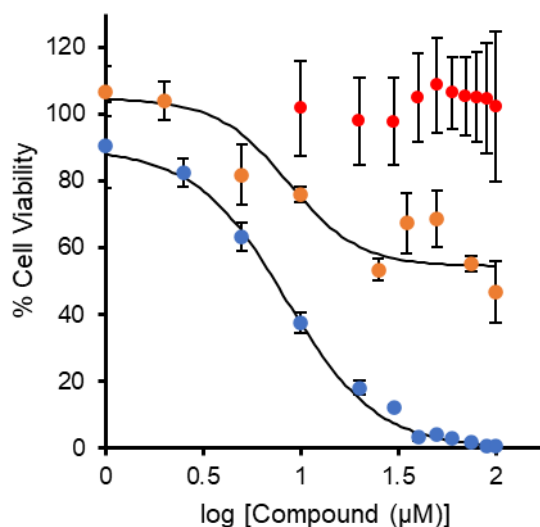


Figure 2. Dose–response treatment ($n = 6$) of MRC-5 cells with $[\text{Ti}(\text{salicylate})_3]^{2-}$ (orange), $[\text{Cu}(\text{Deferasirox})(\text{H}_2\text{O})_2]$ (blue), and salicylic acid (red) at pH 7.4. The relative half-maximal inhibitory concentration was fit by nonlinear regression.

Table 1. The IC_{50} values (\pm standard deviation) for the treatment of metal compounds and some corresponding ligands against MRC-5 cells.

Compound	$\text{IC}_{50} \pm \text{SD} (\mu\text{M})$	$\text{IC}_{50} \pm \text{SD} (\mu\text{g/mL})$	Ref.
$\text{Ti}(\text{Deferasirox})_2$ (10)	25 ± 1	19.8 ± 0.8	[36]
$[\text{Cu}(\text{Def})(\text{H}_2\text{O})_2]$ (15)	8.40 ± 1.62	7.70 ± 1.47	This work
Deferasirox ^a (4)	Proliferative Behavior		[36]
$\text{Ti}(\text{BHPT})_2$ (9)	2.4^b	1.5	[36]
BHPT (3)	$>>100$	$>>25.3$	[36]
$\text{Ti}(\text{HBED})$ (11)	42.0 ± 4.4	18.5 ± 1.9	[49]
HBED ^a (5)	$>>100$	$>>44.3$	[49]
$\text{K}_2[\text{Ti}(\text{naphthalene-2,3-diolate})_3]$ (8)	$>>100$	$>>65.5$	This work
2,3-dihydroxynaphthalene (2)	$>>100$	$>>16.0$	This work
Titanocene Dichloride (13)	$>>100$	$>>24.9$	This work
$\text{K}_2[\text{TiO}(\text{Oxalate})_2]$ (14)	$>>100$	$>>35.4$	This work
$\text{Na}_2[\text{Ti}(\text{Salicylate})_3]$ (12)	8.60 ± 1.54^c	5.14 ± 0.92	This work
Salicylic Acid ^a (6)	$>>100$	$>>13.8$	This work
$\text{K}_2[\text{Ti}(\text{Citrate})_3]$ (7)	$>>100$	$>>73.2$	This work
$\text{Na}_3\text{Citrate}$ (1)	$>>100$	$>>29.4$	This work

^a Note that at pH 7.4, deferasirox will be monoanionic, HBED will be dianionic, and salicylic acid will be monoanionic. ^b An approximated relative IC_{50} value. The compound inhibits cell proliferation at a maximum of 25%. ^c A relative IC_{50} value. The compound inhibits cell proliferation at a maximum of 50%.

3.2. Assessment of the Solution Behavior of the Ti(IV) Compounds

The Ti(IV) compounds in this study range widely in their aqueous solution stability and ligand exchange lability, especially at pH 7.4. Titanocene dichloride is extremely hydrolysis prone and undergoes virtually complete ligand dissociation at pH 7.4 and extensively hydrolyzed Ti(IV) rapidly precipitates from solution in the absence of a suitable chelator [45]. The complex $\text{Ti}(\text{Citrate})_3$ complex is ligand exchange labile [42,43]. In the absence of excess ligand ($\geq 3:1$ ligand:metal ratio), the Ti(IV) citrate speciation consists of a likely mixture of monocitrate, dicitrate, and tricitrate monoTi(IV) species, which nonetheless maintain the Ti(IV) ion in solution [50–53]. In the presence of excess ligand, the species $\text{Ti}(\text{Citrate})_3^{8-}$ dominates [50,51,53]. The $\text{TiO}(\text{Oxalate})_2$ complex is expected to behave similarly to $\text{Ti}(\text{Citrate})_3$ in solution. Monotitanoyl oxalate species are stable in the pH 1.0 to pH 4.0 range [34]. At higher pH values, multimeric oxoTi(IV) oxalate species are

likely present (tetrameric species have been crystallized) [54–56] and are labile to ligand exchange, able to form mixed ligand species [34,52] and serve as precursors for other Ti(IV) complexes [36,57]. The complexes $\text{Ti}(\text{Deferasirox})_2$, $\text{Ti}(\text{BHPT})_2$, $\text{Ti}(\text{naphthalene-2,3-diolate})_3$, and $\text{Ti}(\text{HBED})$ are highly stable in solution as monoTi(IV) species over a broad pH range (at least pH 4 to 8) and ligand exchange inert [36,38,48,49,58]. $\text{Ti}(\text{Deferasirox})_2$, $\text{Ti}(\text{BHPT})_2$, $\text{Ti}(\text{naphthalene-2,3-diolate})_3$ are dianionic at pH 7.4 whereas $\text{Ti}(\text{HBED})$ undergoes hydrolysis-induced partial ligand dissociation forming the anionic titanyl species $\text{TiO}(\text{H}^+-\text{HBED})^-$ [36,38,48,49,58].

Prior to this study, the Ti(IV) salicylate complexation had not been extensively characterized. An orange-yellow $\text{Ti}(\text{salicylate})_3^{2-}$ complex readily forms at pH 4.5 from a 100 mM aqueous solution, which had been studied by Dey et al. via single-crystal X-ray diffraction, displaying the three salicylates coordinating the Ti(IV) in a bidentate manner through the carboxylic and phenolic oxygens in an octahedral arrangement [39]. The carboxylic and phenolic oxygens are coordinated in a facial orientation with respect to each other. A similar structure was obtained from a DMF solution [59], but the carboxylic and phenolic oxygens are coordinated in a meridional orientation with respect to each other. However, from a 1-butanol solution, a Ti(IV) trisalicylate compound was crystallized in which one salicylate was bound through the carboxylate oxygen, and two salicylates were bound through the carbonyl oxygen; all three salicylates retained phenolic oxygen coordination [60]. Given our interest in the aqueous speciation of Ti(IV) salicylate, we repeated the synthesis of Dey et al. [39]. Orange block-like crystals were obtained through slow evaporation from pure ethanol. The crystals were thoroughly rinsed using cold ethanol and cold water and analyzed through PXRD. The experimental diffractogram for the material was compared to the simulated diffractogram from the crystallographic information file (CIF) for Dey et al.'s $\text{Ti}(\text{salicylate})_3$ structure downloaded from the Cambridge Structural Database (CSD) (database identifier: TAQPUX, deposition number: 282262) (Figure S1). The appearance of the same reflections when comparing the diffractograms of one to another suggested that the correct material was produced. EDS and elemental analysis were performed to verify the chemical composition of the compound. Elemental analysis provided a molecular formula of $\text{Na}_2[\text{Ti}(\text{Salicylate})_3] \cdot 4\text{H}_2\text{O} \cdot 0.5\text{C}_2\text{H}_6\text{O}$ with a molecular weight of 597.26 g/mol: found(theoretical) C, 44.24(44.04); H, 3.88(3.91). The EDS spectra of the compound exhibit the characteristic signals of the respective metal (Ti(IV)) and other elements (carbon and oxygen atoms), which are present in the molecular structure of salicylate. The elements identified were Ti, C, O, and Na (Figure S2). FT-IR spectra were collected of $\text{Ti}(\text{salicylate})_3$ and metal-free salicylic acid. Noticeable differences were observed between the two, particularly the reduction in the frequency of the C=O vibrational band of salicylic acid from 1650 cm^{-1} to 1614 cm^{-1} due to Ti(IV) coordination (Figure S3).

The pH-dependent Ti(IV) salicylate complexation was studied by UV-Vis absorbance spectroscopy. Figure 3A demonstrate the spectra of the Ti(IV)-salicylic acid system compared to salicylic acid alone. As can be noted, the spectra are highly similar, and there is no formation of a ligand to metal charge transfer (LMCT) band. Gigant et al. observed an LMCT absorbance between 330–430 nm for their Ti(IV) trisalicylate compound in CCl_4 solution. This absorbance would be expected of an orange-yellow colored compound and its absence in aqueous solution throughout the pH 2 to 12 range at 50 μM Ti(IV) and 150 μM salicylate indicates a high water exchange labile Ti(IV) complexation by salicylate at micromolar concentration, which appears to provide sufficient Ti(IV) binding to prevent hydrolysis induced precipitation. This water exchange lability is comparable to what is observed for Ti(IV) complexation by citrate [50–53]. $\text{Na}_2[\text{Ti}(\text{Salicylate})_3]$ demonstrated an effect on MRC-5 cell viability distinct from free salicylate and even the highly unstable titanocene dichloride supports that some form of Ti(IV) binding is present. This solution behavior is very different from what is exhibited by the catecholate chelation of Ti(IV) [17]. In aqueous solution at pH 7.4 and micromolar concentration, a pronounced LMCT is observed for the Ti(IV) chelation by the catecholate moiety ($\epsilon_{365\text{nm}} = 40,000\text{ M}^{-1}\text{cm}^{-1}$ for $[\text{Ti}(\text{naphthalene-2,3-diolate})_3]^{2-}$; $\epsilon_{370\text{nm}} = 9300\text{ M}^{-1}\text{cm}^{-1}$ for $[\text{Ti}(\text{catecholate})_3]^{2-}$) [38,61].

Thus the wavelength shift observed in the titration from low pH to high pH in the 50% (*w/w*) ethanol/water solution is owed to the deprotonation of the carboxylic acid moiety of the salicylic acid (Figure 3A). The pKa of the carboxylic acid in 50% (*w/w*) ethanol/water is pKa 3.47 [62], and of the phenolic group is 13.61 in water [63]. A concentration-dependent study of solubilized $\text{Na}_2[\text{Ti}(\text{Salicylate})_3]$ (50:50 DMF:water at pH 4.65 and $I = 0.1 \text{ M}$) was monitored by UV-Vis absorbance spectroscopy to identify a concentration at which the putative Ti(IV) trisalicylate solution species is stable (Figure 3B). A clearly defined LMCT shoulder at 360 nm could be identified at 1 mM concentration with an extinction coefficient of $1900 \text{ M}^{-1}\text{cm}^{-1}$. Dissolving $\text{Na}_2[\text{Ti}(\text{Salicylate})_3]$ directly into pH 7.4 buffer produces a colorless solution, which suggests that although the compound is far more water soluble at this pH, the salicylate is far more ligand exchange labile.

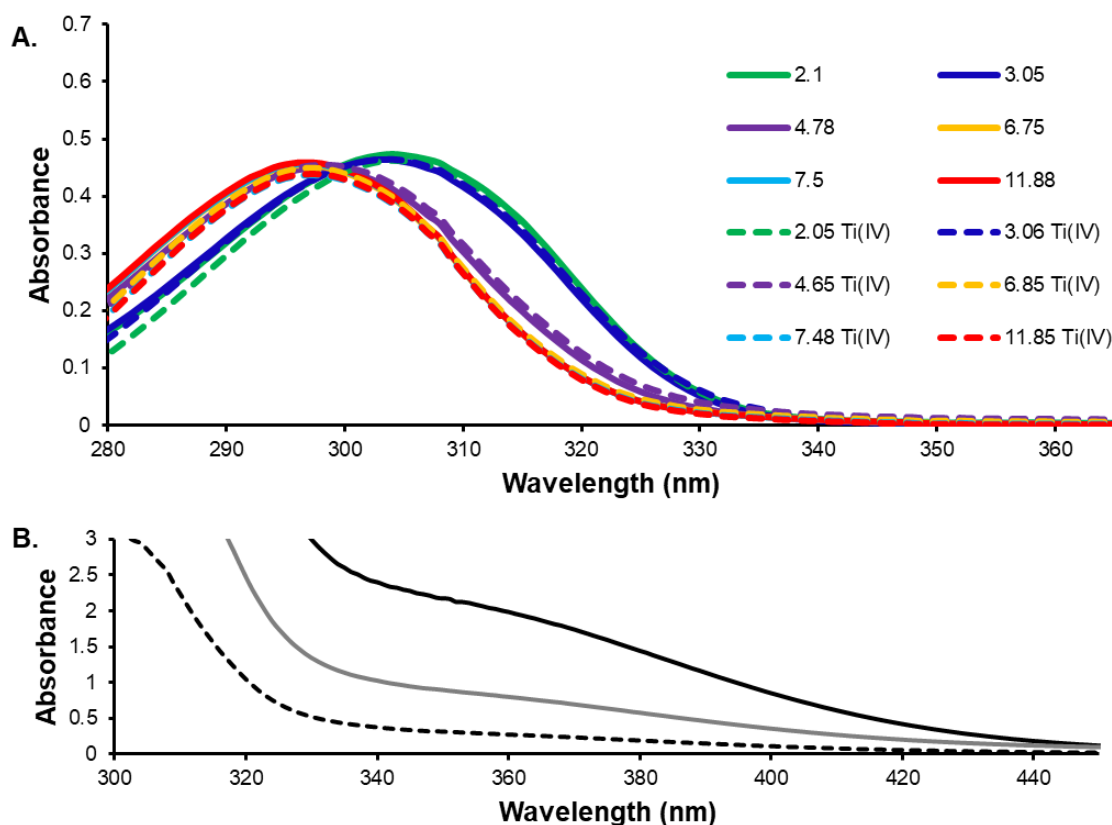


Figure 3. UV-Vis absorbance spectra. (A). pH titration of salicylic acid (solid lines) and Ti(IV)-salicylic acid system (dash lines) recorded at different pH values: [ligand] = 150 μM , metal ligand ratio 1:3; $t = 25.0^\circ\text{C}$, $I = 0.10 \text{ M}$ (NaCl) in 50% (*w/w*) ethanol/water]. (B). Concentration dependent study of solutions of $\text{Na}_2[\text{Ti}(\text{Salicylate})_3]$ at 250 μM (dashed line), 500 μM (gray line), and 1000 μM (black line) (50:50 DMF:water at pH 4.65 and $I = 0.1 \text{ M}$).

3.3. CO-ADD Screening Results

The CO-ADD performs a first antimicrobial screening of compounds at a one-dose concentration of 32 $\mu\text{g/mL}$ to assess general potency. Compounds that demonstrate inhibition of the growth of two or more of the microbes between 50% to 75% or of at least one microbe at greater than 75% are typically then advanced to a dose-response screening (0.25 to 32 $\mu\text{g/mL}$) and are also tested for human cell toxicity to evaluate their therapeutic index (selectivity against microbes versus human cells). Table 2 provide a summary of these two screenings, which lists the compounds numerically as specified in Figure 1. The compounds $\text{Ti}(\text{Deferasirox})_2$ (10), $\text{Ti}(\text{HBED})$ (11), $\text{K}_2[\text{Ti}(\text{naphthalene-2,3-diolate})_3]$ (5), $\text{K}_2[\text{TiO}(\text{Oxalate})_2]$ (14), and HBED (8) met the criteria to advance to the second screening. Although deferasirox (4) and $[\text{Cu}(\text{Deferasirox})(\text{H}_2\text{O})_2]$ (15) did not meet the criteria, they were also included in the second screening.

Table 2. Predicted or measured MIC ($\mu\text{g/mL}$) values for the compounds against the Gram-negative (G−ve) bacteria, Gram-positive (G+ve) bacteria, and the fungi. The maximum % inhibition (D_{max}) exhibited by the compounds against each organism is included. MIC values that could be measured are highlighted in yellow. D_{max} values of $\geq 50\%$ are highlighted in gray. ND = Not determined.

	G+ve		G−ve						Fungi					
	<i>S. aureus</i> (MRSA)		<i>A. baumannii</i>		<i>E. coli</i>		<i>K. pneumoniae</i>		<i>P. aeruginosa</i>		<i>C. albicans</i>		<i>C. neoformans</i>	
	MIC	D_{max}	MIC	D_{max}	MIC	D_{max}	MIC	D_{max}	MIC	D_{max}	MIC	D_{max}	MIC	D_{max}
1	>32	10.59	>32	18	>32	6.14	>32	14.22	>32	10.27	>32	0.28	>32	−8.24
2	>32	12.55	>32	31.32	>32	6.86	>32	26.77	>32	14.61	>32	16.32	>32	−9.97
3	>32	−2.34	>32	−1.93	>32	−9.07	>32	4.11	>32	10.03	>32	6.23	>32	−7.23
4	>32	44.31	>32	18.95	>32	7.72	>32	20.2	>32	5.82	>32	4.31	>32	−25.58
5	>32	24.28	>32	68.29	>32	51.65	>32	52.86	>32	69.01	>32	3.5	>32	−17.49
6	>32	13.86	>32	17.87	>32	−0.01	>32	17.1	>32	9.76	>32	30.95	>32	−10.66
7	>32	38.01	>32	17.94	>32	10.41	>32	15.43	>32	7.99	>32	44.53	>32	−7.88
8	>32	52.5	>32	24.76	>32	2.67	>32	22.94	>32	13.43	>32	57.57	>32	−1.45
9	>32	7.82	>32	1.55	>32	−0.56	>32	6.35	>32	10.27	>32	4.27	>32	−1.7
10	16	98.2	>32	16.28	>32	20.44	>32	34.61	>32	7.57	>32	5.74	>32	−5.72
11	>32	61.46	>32	68.73	>32	48.02	>32	56.3	>32	72.48	>32	10.43	>32	−18.86
12	>32	3.66	>32	9.4	>32	2.6	>32	12.46	>32	9.57	>32	49.3	>32	−5.2
13	>32	51.55	>32	15.78	>32	23.76	>32	16.65	>32	16.01	>32	16.15	>32	6.5
14	>32	40.5	>32	8.2	>32	11.0	>32	17.2	>32	7.5	32	77.3	>32	ND
15	>32	26.9	>32	7.8	>32	−9.5	>32	7.7	>32	19.2	>32	34.0	>32	ND

Only two compounds demonstrated any note-worthy antimicrobial activity. $\text{K}_2[\text{TiO}(\text{Oxalate})_2]$ (14) exhibits a MIC of $32 \mu\text{g/mL}$ and maximum % inhibition (D_{max}) of 77.3% against *C. albicans*. $\text{Ti}(\text{Deferasirox})_2$ (10) exhibits an MIC of $16 \mu\text{g/mL}$ and maximum % inhibition (D_{max}) of 98.2% against *S. aureus* (MRSA). At all concentrations examined, both compounds showed insignificant antiproliferative effect against the HEK293 and red blood cell lines, and thus CC_{50} , HC_{10} , and HC_{50} values could not be measured. The other five compounds included in the second screening were inactive toward inducing hemolysis. $\text{Ti}(\text{HBED})$, $\text{K}_2[\text{Ti}(\text{naphthalene-2,3-diolate})_3]$ (5), and deferasirox (4) were also inactive in inhibiting HEK293 proliferation. However, HBED (8) and $[\text{Cu}(\text{Deferasirox})(\text{H}_2\text{O})]_2$ (15) inhibited the proliferation of the cells at nearly 50% at the maximum concentration tested.

4. Discussion

Although the CO-ADD screening did not produce many positive hits, assessing the results has been very insightful about the importance of the Ti(IV) compound identity to the antimicrobial behavior observed or lack thereof. Previous studies centered on soluble Ti(IV) compounds have pointed to a potent antibiotic behavior of the titanyl species with and without bound sulfate at low pH that could be owed to bacterial serine protease inhibition [27,32,33,35]. Our library of Ti(IV) compounds was screened at pH ~ 7.4 , and at this pH, they vary in terms of their solution stability and ligand exchange lability. The highly unstable titanocene dichloride, which rapidly dissociates and precipitates hydrolyzed Ti(IV), demonstrated virtually no promising antimicrobial behavior. The ligand labile compounds $\text{K}_2[\text{Ti}(\text{Citrate})_3]$ and $\text{Na}_2[\text{Ti}(\text{Salicylate})_3]$ were also not promising whereas $\text{K}_2[\text{TiO}(\text{Oxalate})_2]$ demonstrated modest behavior. It could inhibit *C. albicans* proliferation at greater than 75% and exhibited no toxic behavior against the three human cell lines (MRC-5, HEK293, and red blood cells) tested, thus indicating that it displays a significant antifungal therapeutic index. When considering its *C. albicans* MIC value in units of molarity ($90.4 \mu\text{M}$)

(Table S1), this concentration is relatively high. The absence of antibiotic activity of this compound is consistent with the observations of McCue et al. [27,33,35], in which antibiotic activity could only be observed at low pH (\leq pH 1), in which the compound does not remain intact and presumably the activity is due to oxalate-free titanyl [33,35]. The lack of antibiotic behavior of $\text{Na}_2[\text{Ti}(\text{Salicylate})_3]$ is disappointing given the activity observed by Julius Pick of Ti(IV) monosalicylate and disalicylate in treating tuberculosis, presumably due to the inhibition of *Mycobacterium tuberculosis* [27]. There are a few unknowns given that we did not work with the same compound formulation and did not screen against *Mycobacterium tuberculosis*. In addition, the conditions of Pick's work may have produced a different Ti(IV) speciation especially given the topical and oral administration of the compounds [27]. Interestingly, Ti(IV) salicylate (composition not known to us) is used as a preservative in cosmetics products, although experts have already established insufficient data to determine the compound's safety or true efficacy [64].

Of the solution stable and ligand exchange inert compounds, $\text{Ti}(\text{Deferasirox})_2$ was the only compound to produce a positive hit, effectively inhibiting the proliferation of MRSA at nearly 100% at a MIC of 16 $\mu\text{g/mL}$ or 20.2 μM . Ti has been previously studied as a possible anti-MRSA agent. Kikushi et al. studied pure Ti against MRSA, but the metal alone did not show significant activity [65]. There are various studies that demonstrate TiO_2 NPs as possible MRSA inhibitors [66–68]. In combination with established antibiotics, the NPs are effective in reducing the MRSA resistance against the antibiotics [66,68]. Recently Ajsuvakova et al. evaluated the dose–response effect of $\text{Ti}(\text{citrate})_3^{8-}$ on hemolytic and nonhemolytic *S. aureus* cultures [53]. They found significant inhibition of their growth rate in only the early phase of the bacterial growth cycle but only at the very high concentration of 50 mM, which would likely be toxic to human cells. However, at all concentrations tested (0.5 to 50 mM) $\text{Ti}(\text{citrate})_3^{8-}$ significantly activated biofilm formation, an undesired result [53]. The $\text{Ti}(\text{Deferasirox})_2$ MIC value against MRSA is thus a promising result especially given that at this concentration, the compound displays insignificant toxicity against HEK293 and red blood cells and moderate toxicity against MRC-5 cells [36], which suggests a favorable antibacterial therapeutic index.

$\text{Ti}(\text{Deferasirox})_2$ was designed by us as an anticancer agent who operates intracellularly by transmetalation with the labile iron pool to inhibit Fe bioavailability and enable Ti(IV) release. We have recently proposed that the compound, through its Fe binding and Ti(IV)-induced phosphate hydrolysis of nucleotides, may serve as a dual inhibitor of the iron-dependent human ribonucleotide reductase (RNR class Ia) enzyme, which plays a key role in DNA replication and repair [36,58]. This enzyme is composed of two homodimeric subunits: α_2 and β_2 . The β_2 subunit harbors the essential diferric-tyrosyl radical cofactor that initiates the enzyme's radical chemistry. The Fe binding property of $\text{Ti}(\text{Deferasirox})_2$ may play a significant role in its inhibition of MRSA by disrupting Fe bioavailability. This possibility is supported by the recent preprint work by Sessler et al., which demonstrated that a prochelator form of deferasirox could inhibit MRSA up to 70% at 15 μM [4,69]. $\text{Ti}(\text{Deferasirox})_2$ can be viewed as a prochelator with it displaying high extracellular stability and intracellular Fe-induced transmetalation [36]. $\text{Ti}(\text{Deferasirox})_2$ may also be able to inhibit the bacterial RNR. *S. aureus* contain class Ib and class III RNRs, which are essential for aerobic and anaerobic growth [70,71]. Class Ib RNR is the most commonly observed class and is similar to the class Ia RNR, being composed of two homodimeric subunits [70]. The behavior of $\text{Ti}(\text{Deferasirox})_2$ is very clearly a synergism between titanium and the ligand, as observed by comparing its potency with metal-free deferasirox and the copper(II) compound of deferasirox ($\text{Cu}(\text{Deferasirox})(\text{H}_2\text{O})_2$). The metal-free ligand exhibits significantly less potency than $\text{Ti}(\text{Deferasirox})_2$ with a maximal inhibition of 44.31% at 32 $\mu\text{g/mL}$ or 85.7 μM ; roughly two times the ligand content than $\text{Ti}(\text{Deferasirox})_2$ at its MIC value ($[\text{deferasirox}] = 40.4 \mu\text{M}$) and with less than half the activity. The Cu(II) compound of deferasirox $[\text{Cu}(\text{Deferasirox})(\text{H}_2\text{O})_2]$ is even less potent than either $\text{Ti}(\text{Deferasirox})_2$ or deferasirox, demonstrating a maximal inhibition of 26.9% at 32 $\mu\text{g/mL}$ or 35.3 μM ($[\text{deferasirox}] = 70.6 \mu\text{M}$). The behavior of $[\text{Cu}(\text{Deferasirox})(\text{H}_2\text{O})_2]$ is

consistent with our finding that the compound in solution is very stable, and the ligand has a much higher affinity for Cu(II) than previously reported, competitive with Fe(III) binding [41]. Fe(III)-induced transmetalation likely does not occur for $[\text{Cu}(\text{Deferasirox})(\text{H}_2\text{O})]_2$, and thus the compound would not affect the Fe levels in MRSA. When substituting deferasirox with the analogue BHPT, which lacks the benzoic acid group, the metal-free ligand and the $\text{Ti}(\text{BHPT})_2$ compound are inactive against MRSA. This could be a consequence of poor cellular uptake for the BHPT ligand. Why $\text{Ti}(\text{Deferasirox})_2$ does not display activity against the other microbes in the CO-ADD screen could also be an issue of cellular uptake, and thus the study of its mechanism against MRSA is highly warranted.

5. Conclusions

Having developed a library of compounds and in light of the global need to combat the decreasing pool of effective antimicrobial compounds because of the aggressive resistance of pathogenic agents, an opportunity was sought to explore the potential for Ti(IV) compounds. After the screening of eight Ti(IV) compounds and some of their corresponding ligands by the CO-ADD, $\text{Ti}(\text{deferasirox})_2$ emerged as a very promising antibiotic agent against MRSA with a significant therapeutic index. It is speculated that this compound may operate by the synergistic behavior of the metal and iron chelator to decrease the bioavailability of Fe(III) and inhibit the microbe RNR activity. Future studies are needed to better understand the mechanism of this compound for the design of a more potent and selective derivative.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/antibiotics11020158/s1>, Figure S1: Powder X-ray diffractogram overlay of the simulated $\text{Ti}(\text{salicylate})_3$ obtained from the Cambridge Structural Database deposition number 282262 (bottom, blue) and synthesized $\text{Ti}(\text{salicylate})_3$ (top, orange), Figure S2: Energy dispersive spectra of $\text{Ti}(\text{salicylate})_3$ complex displaying the presence of atoms (carbon and oxygen) present in the ligand and the metal (titanium), Figure S3: FTIR spectrum of salicylic acid (bottom, black) and $\text{Ti}(\text{salicylate})_3$ (top, orange), Table S1: Concentrations of the compounds tested by the CO-ADD converted from $\mu\text{g}/\text{mL}$ to micromolar.

Author Contributions: Conceptualization, I.R., L.F.-V. and A.D.T.; methodology, I.R., L.F.-V., A.N.M.-F., B.S., P.G.-P. and A.D.T.; software, I.R., L.F.-V. and A.D.T.; formal analysis, I.R., L.F.-V. and A.D.T.; investigation, I.R., L.F.-V., A.N.M.-F., B.S., P.G.-P. and A.D.T.; writing—original draft preparation, I.R. and A.D.T.; writing—review, editing, I.R., L.F.-V. and A.D.T.; visualization, I.R., L.F.-V. and A.D.T.; supervision, I.R., L.F.-V. and A.D.T.; project administration, I.R., L.F.-V. and A.D.T.; funding acquisition, I.R., L.F.-V. and A.D.T. All authors have read and agreed to the published version of the manuscript.

Funding: I.R. was supported by the NIH RISE 5R25GM061151-20 grant, NSF STC BioXFEL 1231306 grant, and Puerto Rico Science, Technology & Research Trust Science Communication Grant, A.D.T. and L.F.-V. were supported by the NIH 5SC1CA190504 and the NIH R21CA240997-01A1 (as was A.M.-F.) and the University of Puerto Rico Río Piedras FIPI grant. L.F.-V. was also supported by the University of Puerto Rico Río Piedras PBMA fellowship and the Puerto Rico Science, Technology & Research Trust Living Expenses Grant. A.M.-F. received support from the NSF REU 1757365 grant. P.G.-P. received funding from the NIH MARC 5T34GM007821-40S1 grant. The Rigaku XtaLAB SuperNova X-ray microdiffractometer was acquired through the support of the NSF under the Major Research Instrumentation Program (CHE-1626103). The antimicrobial screening performed by CO-ADD (The Community for Antimicrobial Drug Discovery) was funded by the Wellcome Trust (UK) and The University of Queensland (Australia).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Special thanks to the Materials Characterization Center (MCC) for their technical support, particularly for their assistance in the EDS analysis.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Hunsaker, E.W.; Franz, K.J. Copper potentiates azole antifungal activity in a way that does not involve complex formation. *Dalton Trans.* **2019**, *48*, 9654–9662. [\[CrossRef\]](#) [\[PubMed\]](#)
- Zaengle-Barone, J.M.; Jackson, A.C.; Besse, D.M.; Becken, B.; Arshad, M.; Seed, P.C.; Franz, K.J. Copper Influences the Antibacterial Outcomes of a β -Lactamase-Activated Prochelator against Drug-Resistant Bacteria. *ACS Infect. Dis.* **2018**, *4*, 1019–1029. [\[CrossRef\]](#) [\[PubMed\]](#)
- Jackson, A.C.; Zaengle-Barone, J.M.; Puccio, E.A.; Franz, K.J. A Cephalosporin Prochelator Inhibits New Delhi Metallo- β -lactamase 1 without Removing Zinc. *ACS Infect. Dis.* **2020**, *6*, 1264–1272. [\[CrossRef\]](#) [\[PubMed\]](#)
- Sedgwick, A.; Yan, K.-C.; Mangel, D.N.; Shang, Y.; Steinbrueck, A.; Han, H.H.; Brewster, J.T.; Hu, X.-L.; Snelson, D.W.; Lynch, V.M.; et al. A Fluorescent Pro-Chelator Active Against Antibiotic Resistant Bacteria. *ChemRxiv* 2020, *in press*. [\[CrossRef\]](#)
- Mjos, K.D.; Orvig, C. Metallodrugs in medicinal inorganic chemistry. *Chem. Rev.* **2014**, *114*, 4540–4563. [\[CrossRef\]](#) [\[PubMed\]](#)
- Malfertheiner, P.; Bazzoli, F.; Delchier, J.-C.; Celiński, K.; Giguère, M.; Rivière, M.; Mégraud, F. Helicobacter pylori eradication with a capsule containing bismuth subcitrate potassium, metronidazole, and tetracycline given with omeprazole versus clarithromycin-based triple therapy: A randomised, open-label, non-inferiority, phase 3 trial. *Lancet* **2011**, *377*, 905–913. [\[CrossRef\]](#)
- De Boer, W.A.; Van Etten, R.J.; Van De Wouw, B.A.; Schneeberger, P.M.; Van Oijen, A.H.; Jansen, J.B. Bismuth-based quadruple therapy for Helicobacter pylori—A single triple capsule plus lansoprazole. *Aliment. Pharmacol. Ther.* **2000**, *14*, 85–89. [\[CrossRef\]](#)
- Barillo, D.J.; Barillo, A.R.; Korn, S.; Lam, K.; Attar, P.S. The antimicrobial spectrum of Xeroform®. *Burns* **2017**, *43*, 1189–1194. [\[CrossRef\]](#)
- Frei, A.; Zuegg, J.; Elliott, A.G.; Baker, M.; Braese, S.; Brown, C.; Chen, F.; Dowson, C.G.; Dujardin, G.; Jung, N.; et al. Metal complexes as a promising source for new antibiotics. *Chem. Sci.* **2020**, *11*, 2627–2639. [\[CrossRef\]](#)
- Barras, F.; Aussel, L.; Ezraty, B. Silver and Antibiotic, New Facts to an Old Story. *Antibiotics* **2018**, *7*, 79. [\[CrossRef\]](#)
- Ketikidis, I.; Banti, C.N.; Kourkoumelis, N.; Tsiakoulis, C.G.; Papachristodoulou, C.; Kalampounias, A.G.; Hadjikakou, S.K. Conjugation of Penicillin-G with Silver(I) Ions Expands Its Antimicrobial Activity against Gram Negative Bacteria. *Antibiotics* **2020**, *9*, 25. [\[CrossRef\]](#)
- Sierra, M.A.; Casarrubios, L.; de la Torre, M.C. Bio-Organometallic Derivatives of Antibacterial Drugs. *Chemistry* **2019**, *25*, 7232–7242. [\[CrossRef\]](#) [\[PubMed\]](#)
- Patra, M.; Gasser, G. The medicinal chemistry of ferrocene and its derivatives. *Nat. Rev. Chem.* **2017**, *1*, 0066. [\[CrossRef\]](#)
- Frei, A. Metal Complexes, an Untapped Source of Antibiotic Potential? *Antibiotics* **2020**, *9*, 90. [\[CrossRef\]](#) [\[PubMed\]](#)
- Blaskovich, M.A.T.; Zuegg, J.; Elliott, A.G.; Cooper, M.A. Helping Chemists Discover New Antibiotics. *ACS Infect. Dis.* **2015**, *1*, 285–287. [\[CrossRef\]](#)
- Loza-Rosas, S.A.; Saxena, M.; Delgado, Y.; Gaur, K.; Pandrala, M.; Tinoco, A.D. A ubiquitous metal, difficult to track: Towards an understanding of the regulation of titanium (IV) in humans. *Metallomics* **2017**, *9*, 346–356. [\[CrossRef\]](#) [\[PubMed\]](#)
- Saxena, M.; Loza Rosas, S.; Gaur, K.; Sharma, S.; Perez Otero, S.C.; Tinoco, A.D. Exploring titanium (IV) chemical proximity to iron (III) to elucidate a function for Ti (IV) in the human body. *Coord. Chem. Rev.* **2018**, *363*, 109–125. [\[CrossRef\]](#)
- Zierden, M.R.; Valentine, A.M. Contemplating a role for titanium in organisms. *Metallomics* **2016**, *8*, 9–16. [\[CrossRef\]](#)
- Cini, M.; Bradshaw, T.D.; Woodward, S. Using titanium complexes to defeat cancer: The view from the shoulders of titans. *Chem. Soc. Rev.* **2017**, *46*, 1040–1051. [\[CrossRef\]](#)
- Schur, J.; Manna, C.M.; Deally, A.; Koster, R.W.; Tacke, M.; Tshuva, E.Y.; Ott, I. A comparative chemical-biological evaluation of titanium(IV) complexes with a salan or cyclopentadienyl ligand. *Chem. Commun.* **2013**, *49*, 4785–4787. [\[CrossRef\]](#) [\[PubMed\]](#)
- Elie, B.T.; Fernandez-Gallardo, J.; Curado, N.; Cornejo, M.A.; Ramos, J.W.; Contel, M. Bimetallic titanocene-gold phosphane complexes inhibit invasion, metastasis, and angiogenesis-associated signaling molecules in renal cancer. *Eur. J. Med. Chem.* **2019**, *161*, 310–322. [\[CrossRef\]](#)
- Schilling, T.; Keppler, K.B.; Heim, M.E.; Niebch, G.; Dietzfelbinger, H.; Rastetter, J.; Hanauske, A.-R. Clinical phase I and pharmacokinetic trial of the new titanium complex budotitan. *Investig. New Drugs* **1996**, *13*, 327–332. [\[CrossRef\]](#) [\[PubMed\]](#)
- Lummen, G.; Sperling, H.; Luboldt, H.; Otto, T.; Rubben, H. Phase II trial of titanocene dichloride in advanced renal-cell carcinoma. *Cancer Chemother. Pharmacol.* **1998**, *42*, 415–417. [\[CrossRef\]](#) [\[PubMed\]](#)
- Sharma, S.; Sharma, R.K.; Gaur, K.; Cálata Torres, J.F.; Loza-Rosas, S.A.; Torres, A.; Saxena, M.; Julin, M.; Tinoco, A.D. Fueling a Hot Debate on the Application of TiO₂ Nanoparticles in Sunscreen. *Materials* **2019**, *12*, 2317. [\[CrossRef\]](#) [\[PubMed\]](#)
- Adams, L.K.; Lyon, D.Y.; Alvarez, P.J. Comparative eco-toxicity of nanoscale TiO₂, SiO₂, and ZnO water suspensions. *Water Res.* **2006**, *40*, 3527–3532. [\[CrossRef\]](#) [\[PubMed\]](#)
- Yeung, K.L.; Leung, W.K.; Yao, N.; Cao, S. Reactivity and antimicrobial properties of nanostructured titanium dioxide. *Catal. Today* **2009**, *143*, 218–224. [\[CrossRef\]](#)
- Schwietert, C.W.; McCue, J.P. Coordination compounds in medicinal chemistry. *Coord. Chem. Rev.* **1999**, *184*, 67–89. [\[CrossRef\]](#)
- Kubo, M.; Onodera, R.; Shibasaki-Kitakawa, N.; Tsumoto, K.; Yonemoto, T. Kinetics of ultrasonic disinfection of *Escherichia coli* in the presence of titanium dioxide particles. *Biotechnol. Prog.* **2005**, *21*, 897–901. [\[CrossRef\]](#) [\[PubMed\]](#)
- George, S.A.; Raj, M.S.; Solomon, D.; Roselin, P. A Comparative Study of the Anti-Fungal Activity of Zinc Oxide and Titanium Dioxide Nano and Bulk Particles with Anti-Fungals against Fungi Isolated from Infected Skin and Dandruff Flakes. *Res. Rev. J. Microbiol. Biotechnol.* **2014**, *3*, 23–30.

30. Ahmad, N.S.; Abdullah, N.; Yasin, F.M. Antifungal Activity of Titanium Dioxide Nanoparticles against *Candida albicans*. *Bioresources* **2019**, *14*, 8866–8878.
31. Kermani, S.A.; Salari, S.; Almani, P.G.N. Comparison of antifungal and cytotoxicity activities of titanium dioxide and zinc oxide nanoparticles with amphotericin B against different *Candida* species: In vitro evaluation. *J. Clin. Lab. Anal.* **2021**, *35*, e23577. [\[CrossRef\]](#)
32. Duffy, B.; Schwietert, C.; France, A.; Mann, N.; Culbertson, K.; Harmon, B.; McCue, J.P. Transition metals as protease inhibitors. *Biol. Trace Elem. Res.* **1998**, *64*, 197–213. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Yaghoubi, S.; Schwietert, C.W.; McCue, J.P. Biological roles of titanium. *Biol. Trace Elem. Res.* **2000**, *78*, 205–217. [\[CrossRef\]](#)
34. Van de Velde, G.M.H. The oxalato complexes of titanium (IV)—I: Mononuclear $\text{Ti}(\text{OH})_2(\text{C}_2\text{O}_4)_2^{2-}$ in solution. *J. Inorg. Nucl. Chem.* **1977**, *39*, 1357–1362. [\[CrossRef\]](#)
35. Schwietert, C.W.; Yaghoubi, S.; Gerber, N.C.; McSharry, J.J.; McCue, J.P. Dietary titanium and infant growth. *Biol. Trace Elem. Res.* **2001**, *83*, 149–167. [\[CrossRef\]](#)
36. Loza-Rosas, S.A.; Vázquez-Salgado, A.M.; Rivero, K.I.; Negrón, L.J.; Delgado, Y.; Benjamín-Rivera, J.A.; Vázquez-Maldonado, A.L.; Parks, T.B.; Munet-Colón, C.; Tinoco, A.D. Expanding the therapeutic potential of the iron chelator deferiasirox in the development of aqueous stable Ti(IV) anticancer complexes. *Inorg. Chem.* **2017**, *56*, 7788–7802. [\[CrossRef\]](#)
37. Tinoco, A.D.; Thomas, H.R.; Incarvito, C.D.; Saghatelian, A.; Valentine, A.M. Cytotoxicity of a Ti (IV) compound is independent of serum proteins. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 5016–5021. [\[CrossRef\]](#)
38. Tinoco, A.D.; Eames, E.V.; Incarvito, C.D.; Valentine, A.M. Hydrolytic Metal with a Hydrophobic Periphery: Titanium (IV) Complexes of Naphthalene-2,3-diolate and Interactions with Serum Albumin. *Inorg. Chem.* **2008**, *47*, 8380–8390. [\[CrossRef\]](#)
39. Dey, R.; Mukharjee, K.; Langer, V.; Roychowdhury, P. A titanium salicylate, $\text{Na}_4[\text{Ti}(\text{C}_7\text{H}_4\text{O}_3)_3]_2 \cdot 11\text{H}_2\text{O}$. *Acta Crystallogr. Sect. E Struct. Rep. Online* **2005**, *61*, m1495–m1497. [\[CrossRef\]](#)
40. Zhou, Z.H.; Deng, Y.F.; Jiang, Y.Q.; Wan, H.L.; Ng, S.W. The first structural examples of tricitratotitanate $[\text{Ti}(\text{H}_2\text{cit})_3]^{2-}$ dianions. *Dalton Trans.* **2003**, *13*, 2636–2638. [\[CrossRef\]](#)
41. Fernandez-Vega, L.; Loza Rosas, S.A.; Maser-Figueroa, A.N.; Rodriguez, I.; Alvarez-Carillo, V.; Morales-Rodriguez, S.; Zayas-Ortiz, A.; Mendez-Fernandez, A.P.; Vazquez-Maldonado, A.L.; Ebenki, V.B.; et al. A potential copper (II) contribution to the anticancer transmetallation mechanism of $\text{Ti}(\text{Deferiasirox})_2$. 2022, *Unpublished work*.
42. Tinoco, A.D.; Valentine, A.M. Ti (IV) Binds to Human Serum Transferrin More Tightly Than Does Fe (III). *J. Am. Chem. Soc.* **2005**, *127*, 11218–11219. [\[CrossRef\]](#)
43. Tinoco, A.D.; Saxena, M.; Sharma, S.; Noinaj, N.; Delgado, Y.; Gonzalez, E.P.Q.; Conklin, S.E.; Zambrana, N.; Loza-Rosas, S.A.; Parks, T.B. Unusual synergism of transferrin and citrate in the regulation of Ti (IV) speciation, transport, and toxicity. *J. Am. Chem. Soc.* **2016**, *138*, 5659–5665. [\[CrossRef\]](#)
44. Van Meerloo, J.; Kaspers, G.J.; Cloos, J. Cell sensitivity assays: The MTT assay. *Methods Mol. Biol.* **2011**, *731*, 237–245. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Toney, J.H.; Marks, T.J. Hydrolysis chemistry of the metallocene dichlorides $\text{M}(\eta^5\text{-C}_5\text{H}_5)_2\text{Cl}_2$, M = titanium, vanadium, or zirconium. Aqueous kinetics, equilibria, and mechanistic implications for a new class of antitumor agents. *J. Am. Chem. Soc.* **1985**, *107*, 947–953. [\[CrossRef\]](#)
46. Hall, M.D.; Telma, K.A.; Chang, K.-E.; Lee, T.D.; Madigan, J.P.; Lloyd, J.R.; Goldlust, I.S.; Hoeschele, J.D.; Gottesman, M.M. Say no to DMSO: Dimethylsulfoxide inactivates cisplatin, carboplatin, and other platinum complexes. *Cancer Res.* **2014**, *74*, 3913–3922. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Plumb, J.A.; Milroy, R.; Kaye, S.B. Effects of the pH dependence of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide-formazan absorption on chemosensitivity determined by a novel tetrazolium-based assay. *Cancer Res.* **1989**, *49*, 4435–4440. [\[PubMed\]](#)
48. Tinoco, A.D.; Incarvito, C.D.; Valentine, A.M. Calorimetric, Spectroscopic, and Model Studies Provide Insight into the Transport of Ti (IV) by Human Serum Transferrin. *J. Am. Chem. Soc.* **2007**, *129*, 3444–3454. [\[CrossRef\]](#) [\[PubMed\]](#)
49. Parks, T.B.; Cruz, Y.M.; Tinoco, A.D. Applying the Fe (III) Binding Property of a Chemical Transferrin Mimetic to Ti(IV) Anticancer Drug Design. *Inorg. Chem.* **2014**, *53*, 1743–1749. [\[CrossRef\]](#)
50. Collins, J.M.; Uppal, R.; Incarvito, C.D.; Valentine, A.M. Titanium (IV) Citrate Speciation and Structure under Environmentally and Biologically Relevant Conditions. *Inorg. Chem.* **2005**, *44*, 3431–3440. [\[CrossRef\]](#)
51. Panagiotidis, P.; Kefalas, E.T.; Raptopoulou, C.P.; Terzis, A.; Mavromoustakos, T.; Salifoglou, A. Delving into the complex picture of Ti (IV)-citrate speciation in aqueous media: Synthetic, structural, and electrochemical considerations in mononuclear Ti(IV) complexes containing variably deprotonated citrate ligands. *Inorg. Chim. Acta* **2008**, *361*, 2210–2224. [\[CrossRef\]](#)
52. Tinoco, A.D.; Eames, E.V.; Valentine, A.M. Reconsideration of serum Ti (IV) transport: Albumin and transferrin trafficking of Ti(IV) and its complexes. *J. Am. Chem. Soc.* **2008**, *130*, 2262–2270. [\[CrossRef\]](#)
53. Gritsenko, V.A.; Ajsuvakova, O.P.; Tinkov, A.A.; Bezryadin, S.G.; Gatiatulina, E.R.; Ivanova, V.Y.; Chevela, V.V.; Nikonorov, A.A. The effect of the Ti(IV)-citrate complex on *Staphylococcus aureus* growth and biofilm formation. *Arch. Biol. Sci.* **2015**, *67*, 981–992. [\[CrossRef\]](#)
54. Van De Velde, G.; Harkema, S.; Gellings, P.J. The crystal and molecular structure of ammonium titanyl oxalate. *Inorg. Chim. Acta* **1974**, *11*, 243–252. [\[CrossRef\]](#)

55. Haddad, M.; Brisse, F. Sels alcalins des complexes oxalato titanates (IV); 2, La structure cristalline de l'oxalato titanate (IV) de potassium hydrate $K_2[TiO(CO_2O_4)_2] \cdot 2.25 H_2O$. *Can. Miner.* **1978**, *16*, 379–385.
56. Fester, A.; Bensch, W.; Trömel, M. Dipotassium bis(oxalato)oxotitanate(IV) dihydrate. *Acta Crystallogr. Sect. C Cryst. Struct. Commun.* **1994**, *50*, 850–852. [[CrossRef](#)]
57. Masato, K.; Makoto, K.; Koji, T.; Valery, P. Application of Water-Soluble Titanium Complexes as Precursors for Synthesis of Titanium-Containing Oxides via Aqueous Solution Processes. *Bull. Chem. Soc. Jpn.* **2010**, *83*, 1285–1308. [[CrossRef](#)]
58. Gaur, K.; Pérez Otero, S.C.; Benjamín-Rivera, J.A.; Rodríguez, I.; Loza-Rosas, S.A.; Vázquez Salgado, A.M.; Akam, E.A.; Hernández-Matías, L.; Sharma, R.K.; Alicea, N.; et al. Iron Chelator Transmetalative Approach to Inhibit Human Ribonucleotide Reductase. *JACS Au* **2021**, *1*, 865–878. [[CrossRef](#)]
59. Fu, Y.-L.; Xu, Z.-W.; Ren, J.-L.; Ng, S.W. Bis(dimethylammonium)mer-tris(salicylato- κ^2O,O') titanate(IV). *Acta Crystallogr. Sect. E Struct. Rep. Online* **2005**, *61*, m1730–m1732. [[CrossRef](#)]
60. Gigant, K.; Rammal, A.; Henry, M. Synthesis and Molecular Structures of Some New Titanium (IV) Aryloxides. *J. Am. Chem. Soc.* **2001**, *123*, 11632–11637. [[CrossRef](#)]
61. Borgias, B.A.; Cooper, S.R.; Koh, Y.B.; Raymond, K.N. Synthetic, structural, and physical studies of titanium complexes of catechol and 3,5-di-tert-butylcatechol. *Inorg. Chem.* **1984**, *23*, 1009–1016. [[CrossRef](#)]
62. Faraji, M.; Farajtabar, A.; Gharib, F.; Ghasemnejad-Borsa, H. Deprotonation of salicylic acid and 5-nitrosalicylic acid in aqueous solutions of ethanol. *J. Serb. Chem. Soc.* **2011**, *76*, 1455–1463. [[CrossRef](#)]
63. Perrin, D.D. Stability of Metal Complexes with Salicylic Acid and Related Substances. *Nature* **1958**, *182*, 741–742. [[CrossRef](#)] [[PubMed](#)]
64. Johnson, W., Jr. *The 2019 Cosmetic Ingredient Review Expert Panel: Safety Assessment of Titanium Complexes as Used in Cosmetics*; Personal Care Products Council: Washington, DC, USA, 2019.
65. Yasuyuki, M.; Kunihiro, K.; Kurissery, S.; Kanavillil, N.; Sato, Y.; Kikuchi, Y. Antibacterial properties of nine pure metals: A laboratory study using *Staphylococcus aureus* and *Escherichia coli*. *Biofouling* **2010**, *26*, 851–858. [[CrossRef](#)] [[PubMed](#)]
66. Ullah, K.; Khan, S.A.; Mannan, A.; Khan, R.; Murtaza, G.; Yameen, M.A. Enhancing the Antibacterial Activity of Erythromycin with Titanium Dioxide Nanoparticles against MRSA. *Curr. Pharm. Biotechnol.* **2020**, *21*, 948–954. [[CrossRef](#)] [[PubMed](#)]
67. Chow, W.L.; Tin, A.S.; Lim, W.W.; Lim, J.; Kurup, A.; Ling, M.L.; Tan, A.L.; Ong, B.C. Efficacy of titanium dioxide compounds in preventing environmental contamination by meticillin resistant *Staphylococcus aureus* (MRSA). *Int. J. Infect. Control.* **2013**, *9*, 1–9. [[CrossRef](#)]
68. Roy, A.S.; Parveen, A.; Koppalkar, A.R.; Prasad, M.V.N.A. Effect of Nano—Titanium Dioxide with Different Antibiotics against Methicillin-Resistant *Staphylococcus aureus*. *J. Biomater. Nanobiotechnol.* **2010**, *1*, 37–41. [[CrossRef](#)]
69. Sedgwick, A.C.; Yan, K.C.; Mangel, D.N.; Shang, Y.; Steinbrueck, A.; Han, H.H.; Brewster, J.T., 2nd; Hu, X.L.; Snelson, D.W.; Lynch, V.M.; et al. Deferasirox (Exjade): An FDA-Approved AIEgen Platform with Unique Photophysical Properties. *J. Am. Chem. Soc.* **2021**, *143*, 1278–1283. [[CrossRef](#)]
70. Masalha, M.; Borovok, I.; Schreiber, R.; Aharonowitz, Y.; Cohen, G. Analysis of transcription of the *Staphylococcus aureus* aerobic class Ib and anaerobic class III ribonucleotide reductase genes in response to oxygen. *J. Bacteriol.* **2001**, *183*, 7260–7272. [[CrossRef](#)]
71. Rabinovitch, I.; Yanku, M.; Yeheskel, A.; Cohen, G.; Borovok, I.; Aharonowitz, Y. *Staphylococcus aureus* NrdH redoxin is a reductant of the class Ib ribonucleotide reductase. *J. Bacteriol.* **2010**, *192*, 4963–4972. [[CrossRef](#)]