Light-Activated Rhenium Complexes with Dual Mode of Action against Bacteria

Angelo Frei, Maite Amado, Matthew Cooper, Mark A. T. Blaskovich

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New antibiotics and innovative approaches to kill drug-resistant bacteria are urgently needed. Metal-complexes offer access to potential alternative modes of action and have only sparingly been investigated in antibacterial drug discovery. We have developed a light-activated rhenium complex with activity against drug resistant bacteria. The activity profile against mutant strains combined with assessments of cellular uptake and synergy suggest two distinct modes of action.

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Abstract
New antibiotics and innovative approaches to kill drug-resistant bacteria are urgently needed. Metal-complexes offer access to potential alternative modes of action and have only sparingly been investigated in antibacterial drug discovery. We have developed a light-activated rhenium complex with activity against drug resistant bacteria. The activity profile against mutant strains combined with assessments of cellular uptake and synergy suggest two distinct modes of action.

Introduction
The rise of widespread antimicrobial resistance has been designated as one of the biggest threats to global health and food security by the World Health Organization (WHO).\(^{[1]}\) Most major pharmaceutical companies have shut down their antibiotic drug discovery programs, leaving academic researchers as the source of new classes of compounds, especially for the notoriously more difficult to treat Gram-negative pathogens.\(^{[2]}\) Most of these efforts focus on the investigation of purely organic compounds as antibiotics. Metal complexes provide a distinct alternative, and have proven to be promising candidates for the treatment of diseases such as malaria, Parkinson’s and cancer, with several metal-based compounds currently in clinical trials.\(^{[3]}\) However, they have only sparingly been investigated for their application against bacterial infections. Several interesting metal complexes with antibacterial activity were summarized in recent
reviews.[4] In 2019 several studies on promising, highly positively charged ruthenium compounds with excellent antibacterial activity have been reported.[5] Rhenium has long been overshadowed by metals such as iron and ruthenium when it comes to medicinal applications. However, there has been a continuous stream of reports on the biological applications of rhenium complexes, mainly for anticancer applications. These reports have been summarized very recently in excellent review articles by Wilson and Crans.[6] Seminal work on the antibacterial potential of rhenium compounds was reported in a series of studies led by Metzler Nolte, where the structure-activity relationship of a tri-metallic antimicrobial peptide and its derivatives was examined (Figure 1, top).[7] The authors concluded that the [(dpa)Re(CO)₃] moiety was crucial for the overall activity of the compound. In recent years several other reports on rhenium complexes with some activity against Gram-positive bacteria have been released.[8]

![Chemical structure of tri-metallic antibacterial compound reported by Patra et al.](image)

**Figure 1.** Structure of tri-metallic antibacterial compound reported by Patra et al. (top). Structures of compounds 1-3 reported in this work.

A further point of inspiration for the present work was the report that bisquinoline rhenium tricarbonyl-type complexes can produce reactive oxygen species (ROS), namely singlet oxygen upon light irradiation.[9] Singlet oxygen and ROS in general are highly reactive species that are used in photodynamic therapy (PDT) to selectively kill either cancer cells or bacteria. As singlet oxygen is only generated upon light irradiation, the treatment allows for spatial and temporal control of its generation, providing a targeted therapy with reduced side-effects. Antimicrobial PDT (aPDT) has gained more attention in recent years because the generated ROS do not have a specific target in the bacteria, such as most antibiotics, which makes it very difficult to develop resistance against this treatment.[10] Extensive studies have shown that organic cationic aPDT agents favourably target the highly negatively charged bacterial surface.[11] Very
recently Feng et al. reported on highly positively charged ruthenium complexes that were effective at photoinactivating MRSA while possessing low toxicity and haemolytic properties.\textsuperscript{[5b]}

Based on the evidence that the Re(CO)\textsubscript{3} core is important for non-light mediated antibiotic activity, coupled with the potential for additional bactericidal activity through light-mediated singlet oxygen generation, we sought to combine these two features. We now report the synthesis and antibacterial profiling of three rhenium bisquinoline complexes (1-3). Compounds 1-3 were found to possess two modes of activity against both Gram-positive and -negative strains including methicillin resistant \textit{S. aureus} (MRSA) and colistin-resistant \textit{E. coli}.

\textbf{Results and Discussion}

The bisquinoline scaffold was chosen as the ligand system with the goal of preparing complexes capable producing singlet oxygen upon light irradiation. The results of Patra \textit{et al.} inspired us to choose a terminal alkyne group.\textsuperscript{[7a, 9]} The alkyne group has the added benefit of enabling further functionalization of the system via click chemistry at a later stage. We also prepared amine (2) and alkyl (3) analogues to explore the role of the terminal functional group on the activity of the compounds. Complexes 1-3 were prepared by reacting ligands L1-L3 (see SI) with either Re(CO)\textsubscript{5}Br or [NEt\textsubscript{4}]\textsubscript{2}ReBr\textsubscript{3}(CO)\textsubscript{3} in MeOH under microwave irradiation for 30 min (deprotecting the amine of 2 after complexation). The compounds were purified by preparative HPLC and characterized by \textsuperscript{1}H and \textsuperscript{13}C NMR as well as HR-MS.

The antibacterial activity of the complexes was assessed by a broth microdilution minimum inhibitory concentration (MIC) assay, initially against ATCC strains of the Gram-positive \textit{S. aureus} and the Gram-negative \textit{E. coli}. To investigate the effect of light irradiation, the 96-well plates containing bacteria and freshly added compound were irradiated with a UV lamp at 375 nm for 1 h (\textasciitilde 3 J/cm\textsuperscript{2}) before standard overnight incubation. The UV light alone had no measurable effect on bacterial growth. In general, all three compounds showed activity with and without light against \textit{S. aureus}, with 1 and 2 having nanomolar MIC values (Table 1, top). The MIC with light was 4- to 16-fold lower than without light, indicating enhanced activity upon light irradiation for all three compounds. No activity (up to 64 μg/ml) was found against \textit{E. coli} in the absence of light. However, upon light irradiation, compound 1 gave MIC values as low as 5.8 μM (4 ug/ml), with some activity also seen for compounds 2 and 3. The control antibiotics (vancomycin for \textit{S. aureus}, polymyxin B for \textit{E. coli}) showed no variation in activity when exposed to light. To investigate if 1 could also be effective against antibiotic resistant strains we determined the MIC against both methicillin resistant \textit{S. aureus} (MRSA) and colistin resistant \textit{E. coli} (Table 1, top-middle). Compound 1 did not show any reduction in activity against these resistant strains suggesting that it circumvents common resistance mechanisms. To the best of our knowledge this makes 1 the first rhenium-based compound that is active against both Gram-positive and -negative pathogens including ones with inherent antibiotic resistance.

Selectivity for bacterial cells over mammalian cells is critical for any potential antibiotic. Cytotoxicity against human cells and haemolytic properties of the rhenium complexes were assessed. Compound 1 showed some toxicity against human embryonic kidney (HEK) cells with a CC\textsubscript{50} of 38.1 ± 0.5 μM, but no haemolysis
up to 300 μM. Compound 2 displayed minimal cytotoxicity and no detectable haemolysis, while 3 had a CC₅₀ of 14.9 ± 0.1 μM and some degree of haemolysis (IC₅₀ = 139.5 ± 0.1 μM). It is reasonable to assume, that the cytotoxicity is increased upon light irradiation. However, the premise of aPDT is that only areas affected by the bacterial infection are irradiated to reduce the damage to healthy cells to a minimum. Therefore, the dark-cytotoxicity is the more important factor. Overall, the activity profile for 1 was the most promising with good activity against Gram-positive and -negative strains, moderate cytotoxicity, and no haemolysis. The fact that activity is seen both in the dark and with light suggests two distinct modes of action. Gram-negative bacteria are notoriously harder to kill than Gram-positive bacteria.

| MICs against Gram(+) and Gram(-) | S. aureus (ATCC25923) |  | 365 nm |
|----------------------------------|------------------------|----------------|--------|
|                                  | dark [μg/ml]            | [μM] | [μg/ml] | [μM] |
| Vanₐ                             | 1                      | 0.7  | 1       | 0.7  |
| 1                                | 8                      | 11.6 | 0.5-1   | 0.72-1.45 |
| 2                                | 32                     | 46.2 | 4-8     | 5.8-11.6 |
| 3                                | 2                      | 2.9  | 0.25-0.5| 0.36-0.72 |
| **E. coli (ATCC 25922)**         |                        |      |         |        |
| Pmxᵇ                             | 1                      | 0.8  | 1       | 0.8  |
| 1                                | >64                    | >93.1| 4-8     | 5.8-11.6 |
| 2                                | >64                    | >92.4| 32      | 46.2  |
| 3                                | >64                    | >92.5| 16      | 23.1  |

| MICs against resistant Gram(+) and Gram(-) | S. aureus (ATCC43300; MRSA) |  | 365 nm |
|---------------------------------------------|-----------------------------|----------------|--------|
| Vanₐ                                        | 2                           | 1.4 | 2       | 1.4  |
| 1                                           | 4-8                         | 5.8-11.6 | 2     | 2.9  |
| **E. coli (mcr-1)**                         |                             |      |         |      |
| Pmxᵇ                                        | 4                           | 3.2 | 4       | 3.2  |
| 1                                           | >64                         | >93.1| 8     | 11.6 |

| MICs against mutant E. coli strains | E. coli (MB4827; Control for mutants) |  | 365 nm |
|------------------------------------|---------------------------------------|----------------|--------|
| Pmxᵇ                               | 0.13-0.25                             | 0.1-0.2 | 0.13-0.25 | 0.1-0.2 |
| 1                                  | >64                                   | >93.1 | 8-16    | 11.6-23.3 |
| **E. coli (MB4902; lpxC)**         |                                        |      |         |        |
| Pmxᵇ                               | 0.06                                  | 0.05  | 0.06    | 0.05 |
| 1                                  | >64                                   | >93.1 | 1-4     | 1.5-5.8 |
| **E. coli (MB5747; tolC)**          |                                        |      |         |        |
| Pmxᵇ                               | 0.13                                  | 0.1   | 0.13    | 0.1 |
| 1                                  | 8                                     | 11.6  | 2       | 2.9  |

| **E. coli (MB5746; lpxC, tolC)**     |                                        |      |         |        |
The presence of an additional outer membrane, an abundance of efflux pumps, and highly selective porins all make it more difficult for compounds to reach an intracellular target. Indeed, compound 1 showed no activity up to 128 μg/ml against multi-drug resistant K. pneumoniae with and without light as well as only limited light activity (MIC = 16-32 μg/ml and 64 μg/ml) against A. baumannii and P. aeruginosa. We hypothesized that the lack of activity in the dark of complex 1 against the Gram-negative strains stems from its inability to effectively accumulate inside the cells. We thus conducted MIC assays against lpxC and tolC deficient mutant E. coli strains, which have non-efficient lipid A production leading to a more permeable membrane, or a deficient efflux pump respectively. In general, these strains should allow for more Re-complex to accumulate inside of the bacteria.

The lpxC mutant resulted in a lower MIC upon light irradiation, potentially due to greater susceptibility of the mutant to the oxidative stress generated upon irradiation, but no noticeable effect in the absence of light.

Table 1. MIC values of 1-3 against E. coli and S. aureus (top), MIC values of 1 against methicillin resistant S. aureus and colistin resistant E. coli (top-middle), MIC values of complex 1 against various mutant strains of E. coli (bottom-middle), and against different mutants of P. aeruginosa.*Gram(+) = Gram-positive, Gram(-) = Gram-negative.

| MICs against mutant P. aeruginosa strains | | |
|----------------------------------------|---|---|---|---|
| P. aeruginosa (PAO1)                   | | | | |
| Pmxb                                  | 1 | 0.8 | 1 | 0.8 |
| 1                                     | >128 | >186.2 | 64 | 93.1 |
| P. aeruginosa (PAO397)                 | | | | |
| Pmxb                                  | 1 | 0.8 | 1 | 0.8 |
| 1                                     | 128 | 186.2 | 4-8 | 5.8-11.6 |

Figure 2. Cellular uptake and distribution of rhenium in three different E. coli strains after 60 min. Percentage of rhenium in different fractions given as percentage of total detected rhenium.
Conversely, the tolC strain showed slightly improved MIC values upon irradiation but a more drastic effect without light, where the MIC decreased from >64 μg/ml (>93.1 μM) to 8 μg/ml (11.6 μM). This suggests that reduced efflux activity of the main efflux-pump (allows the compound to reach intracellular concentrations high enough for it to exhibit the same non-light mediated mode of action that was previously only observed in the Gram-positive S. aureus. Screening against a strain with both mutations gave a remarkably low MIC of ≤0.2 μM upon light irradiation, while the value remained unchanged from the tolC strain result in the absence of light. This further order of magnitude increase in activity with light indicates, that in this double mutant, high intracellular concentrations of 1 can be achieved and that the combination of low efflux and more permeable membrane render the strain more vulnerable to the oxidative stress generated.

To better understand these results, we measured the cellular uptake of compound 1 to see if a correlation could be drawn between MIC and cellular accumulation. We initially attempted to adapt an assay for bacterial uptake based on fluorescence,[14] but this was not successful due to overlap in the fluorescence and absorption between tryptophan and our compound. Instead, we employed inductively coupled plasma mass spectrometry (ICP-MS) to detect the Re content. ICP-MS is an established technique that is routinely used to determine the accumulation of metal-based drugs into cancer cells.[15] However, this technique has not yet been widely adopted for this purpose for bacteria, with one study applying an ICP-based technique (ICP-AES) to measure the uptake of a ruthenium complex in bacteria.[5a] Another study coupled liquid chromatography and gel electrophoresis with ICP-MS (LC-GE-ICP-MS) to investigate the molecular mechanism of silver ions in bacteria.[16] As with ruthenium and silver, rhenium does not occur naturally in bacteria, hence the metal can be used as a unique probe to measure the precise amount of a specific rhenium complex in a given sample. For our study, bacteria were incubated with compound 1 for either 5 or 60 min. After centrifugation, the supernatant was removed and the remaining cells (containing free intracellular or membrane/protein bound 1) were exposed to a lysing buffer overnight. The lysate was then separated from the remaining pellet by centrifugation. After freeze-drying and subsequent digestion in concentrated HNO₃, the rhenium content was measured for the lysate, the remaining pellet and the initially removed supernatant. Separating the lysate and pellet allows for differentiation of complex that is “free” in the intracellular cytoplasm from complex that is bound to the membrane in some way. The cellular uptake was measured for three different bacterial strains: wild type, lpxC, and tolC mutant E. coli. Figure 2 shows the distribution of rhenium between the fractions in the three strains.

Overall, the apparent intracellular concentrations correlate with the measured MIC values. No significant difference in accumulation could be detected between 5 and 60 min incubation. There was a small increase in total accumulation in the lpxC strain compared to the wild type strain, which did not significantly alter the effect of the compound on the cells as reflected in the MIC values. A strong increase of uptake was seen in the tolC mutant, which mirrored the observed decrease in MIC. The percentage of rhenium found in the lysate relative to the pellet was similar between the tolC mutant E. coli and the S. aureus strain (Figure S1),
consistent with the measured MIC values for these strains. Throughout the *E. coli* mutants, an increase in “free” (i.e. found in the lysate fraction) compound mostly correlated with an increase in membrane bound complex (i.e. found in the pellet fraction). These results suggest that 1 is indeed active against *E. coli* both in the dark and with light, but also that 1 is substrate for the tolC efflux pump and hence cannot reach concentrations high enough for its non-light dependent mode of action in the wild-type strain. This conclusion is further supported by analogous results in another Gram-negative species, *P. aeruginosa*. In this case 1 showed no significant activity in the dark (MIC > 128 μg/ml) or light irradiation (MIC = 64 μg/ml) in a wild-type strain (PAO1, expressing MexAB-OprM). Conversely, an MIC of 4-8 μg/ml was obtained with light irradiation in an multiple efflux pump deficient mutant (ΔmexAB-oprM, ΔmexCD-oprJ, ΔmexEF-oprN, ΔmexJKL, ΔmexXY, ΔopmH; MIC<sub>dark</sub> = 128 μg/ml, Table 1 bottom). In this case, the uptake in the wild-type was too low for even the light-mediated mode of action to occur to any significant extent. In the mutant strain, a lack of efflux pump activity allowed for sufficient accumulation of 1 inside the cells for the light-mediated activity to kill the bacteria.[17]

As cellular uptake seemed to be the limiting factor for the efficacy of 1, we surmised that combining the compound with antibiotics with known effects on the integrity of bacterial membranes could potentiate its activity. To investigate this we measured the MIC of 1 in combination with sublethal levels of the antibiotics polymyxin B, octapeptin C4, gentamicin and meropenem. Polymyxin B and octapeptin C4 both target the bacterial membrane and have been shown to increase membrane permeability, with polymyxin analogues widely used to potentiate other antibiotics.[18] Gentamicin and meropenem were used as non-membrane targeting controls, with gentamycin an aminoglycoside acting on the bacterial ribosome, and meropenem a carbapenem that inhibits bacterial cell wall synthesis.[19] As seen in Table 2, 1 reaches lower (more potent) MIC values in the dark and partially with light when used in combination with the membrane targeting antibiotics, but not gentamicin and meropenem. This indicated that polymyxin and octapeptin, but not gentamycin and meropenem, increased the cellular uptake of 1, allowing it to reach higher intracellular concentrations and hence exert both its modes of action. Interestingly, a significant increase in light-mediated activity was observed in combination with gentamicin but not with meropenem. Possibly the oxidative stress generated by 1 synergises with the non-lethal ribosome inhibition of gentamicin, or by the ROS effects leading to destabilisation of Fe-S cluster and increased aminoglycoside uptake.[20]

**MICs of synergy studies against *E. coli***

|  |  |  |  |  |
|---|---|---|---|---|
|  |  |  |  |  |
| **E. coli** (ATCC 25922) |  |  |  |  |
|  | dark |  | 365 nm |  |
|  | [μg/ml] | [μM] | [μg/ml] | [μM] |
| 1 | >64 | >93.1 | 4-8 | 5.8-11.6 |
| 1 + Gen<sup>a</sup> | >64 | >93.1 | 1 | 1.5 |
| 1 + Mer<sup>b</sup> | >64 | >93.1 | 8-16 | 11.6-23.3 |
| 1 + Oct<sup>c</sup> | 16-32 | 23.3-46.5 | 1-2 | 1.5-2.9 |
| 1 + Pmx<sup>d</sup> | 16-32 | 23.3-46.5 | 4-8 | 5.8-11.6 |
Table 2. MIC values of 1 in combination with sublethal concentrations of other antibiotics.  

| Antibiotic                        | Concentration | MIC Range | 
|-----------------------------------|---------------|-----------|
| Gentamicin (0.25 μg/ml)           |               | 0.5 - 2 μg/ml |
| Meropenem (0.007 μg/ml)           |               | 0.03 - 0.06 μg/ml |
| Octapeptin C4 (1 μg/ml)           |               | 2 - 4 μg/ml |
| Polymyxin B (0.03 μg/ml)          |               | 0.125 - 0.5 μg/ml |

In summary, 1 is the first reported rhenium-based compound with antibacterial activity against both Gram-positive and Gram-negative bacteria as well as drug resistant strains. We have shown that 1 has two distinct potential modes of action, one more potent activity mechanism mediated by UV-light irradiation and a second mechanism independent of light irradiation. While the light-mediated activity was demonstrated against both types of bacteria, we demonstrated that efflux pumps in the Gram-negative strains E. coli and P. aeruginosa prevent enough accumulation of compound 1 in the cells for the light-independent mechanism to occur. We have demonstrated that this defense mechanism can be circumvented by co-administering 1 with sub-lethal concentrations of the membrane-targeting antibiotics polymyxin B or octapeptin C4, leading to improved antibacterial activity. The dual mode of action should improve the resilience of these types of compounds against the development of resistance, and offers the prospect of an infection-site targeted light-based therapy. Future work will be focused on reducing the dark-cytotoxicity of this compound class and investigating their ADMET (absorption, distribution, metabolism, and excretion) properties, which is a requirement for further development as an antibiotic. Furthermore, approaches to improve bacterial uptake of these rhenium complexes are currently being investigated in our lab.

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Conflicts of interest
There are no conflicts to declare.

Experimental methods
General
All materials, unless otherwise noted, were obtained from commercial suppliers and used without further purification. [NEt₄]₂[ReBr₃(CO)₃] and Re(CO)₅Br was kindly provided by Prof. Roger Alberto at the University of Zurich. ¹H (600 MHz) and ¹³C (125 MHz) NMR spectra were obtained using a Bruker Avance-600 spectrometer equipped with a TXI Cryoprobe. Chemical shifts are reported relative to the residual solvent signals in parts per million (δ). High-resolution mass spectrometry (HRMS) was performed on a Bruker Micro TOF mass spectrometer using (+)-ESI calibrated to NH₄-OAc. Analytical LC-MS was
performed on a Shimadzu LCMS-2020 using 0.05% formic acid in water (solvent A) and 0.05% formic acid in acetonitrile (solvent B) as mobile phase.

LC-MS Method A: column Zorbax Eclipse XDB-Phenyl, 3.0×100 mm, 3.5 μm. Column temperature: 40 °C; flowrate: 1 mL/min; gradient timetable: 0.00 min, 5% B; 0.50 min, 5% B; 3.00 min, 100% B; 4.2 min, 100% B; 5.00 min, 5% B. HPLC purification was performed on a Gilson PLC 2020 system using water (solvent A) and acetonitrile (solvent B) as mobile phase.

HPLC Method A: column XTerra Prep RP18 OBD 5 μm, 19x100 mm. Flowrate: 20 ml/min. Gradient timetable: 0.00 min, 10% B; 22.00 min, 80% B; 24.00 min, 80% B; 25.00 min, 10% B; 27.00 min, 10% B.

All final products were >95% pure as determined by LC-MS using UV at 254 nm, ELSD and APCI/ESI-MS.

**General procedure for rhenium complexes**

The rhenium complexes 1, 2 and 3 were prepared from either Re(CO)₅Br or [NEt₄][ReBr₃(CO)₃].

Ligand (L₁, L₂ or L₃, 1 eq.) was dissolved in MeOH (3 ml) in a Biotage microwave vial (2-5 ml). The respective rhenium precursor was added and the solution was heated by microwave to 120 °C for 30 min ([NEt₄][ReBr₃(CO)₃]) or 60 min (Re(CO)₅Br). The solvent was evaporated and the crude product was re-dissolved in a 1:1 CH₃CN/H₂O mixture and purified by preparative HPLC (Method A). The complexes were obtained as brown-ish powders.

**Compound 1.** Yield: 21% from L₁. HPLC(Gradient 1): RT = 3.13 min; ¹H NMR (400 MHz, CDCl₃): δ (ppm) 2.70 (m, 1H), 4.59 (d, J = 1.6 Hz, 2H), 5.21 (d, J = 17.7 Hz, 2H), 5.50 (d, J = 17.7 Hz, 2H), 7.66 (t, J = 7.5 Hz, 2H), 7.74 (d, J = 8.3 Hz, 2H), 7.82 – 7.91 (m, 2H), 7.90 (d, J = 8.0 Hz, 2H), 8.35 (d, J = 8.2 Hz, 2H), 8.49 (d, J = 8.8 Hz, 2H). ¹³C NMR (125 MHz, CDCl₃): δ (ppm) 56.86, 69.07, 76.49, 79.62, 120.40, 128.32, 128.68, 129.70, 133.11, 141.55, 147.13, 164.93, 193.91, 195. HR-ESI mass spectrum (CH₃CN/H₂O): found 609.1077; calcd. for [C₂₆H₂₀N₃O₃Re] 609.1057.

**Compound 2.** Yield: 11% from N-Boc-1,2-diaminoethane. HPLC(Gradient 1): RT = 3.03 min; ¹H NMR (400 MHz, CDCl₃): δ (ppm) 3.04 – 3.07 (m, 1H), 3.17 – 3.24 (m, 1H), 3.46 – 3.48 (m, 1H), 3.81 – 3.83 (m, 1H), 4.56 – 4.58 (m, 1H), 4.96 – 5.08 (m, 3H), 5.42 – 5.45 (m, 1H), 6.64 – 6.70 (m, 1H), 7.45 (d, J = 8.3 Hz, 1H), 7.58 (d, J = 8.3 Hz, 1H), 7.65 – 7.68 (m, 2H), 7.81 – 7.88 (m, 2H), 7.91 – 7.92 (m, 1H), 7.95 – 7.97 (m, 1H), 8.14 (d, J = 8.4 Hz, 1H), 8.33 (d, J = 8.34 Hz, 1H), 8.38 (d, J = 8.34 Hz, 1H), 8.60 (d, J = 8.8 Hz, 1H); ¹³C NMR (125 MHz, CDCl₃): δ (ppm) 45.96, 56.42, 68.09, 68.76, 77.15, 110.15, 119.41, 123.15, 127.66, 128.50, 128.96, 129.69, 130.76, 133.10, 137.77, 141.49, 147.92, 153.47, 161.99, 193.81, 194.66, 195.60; HR-ESI mass spectrum (CH₃CN/H₂O): found 613.1294; calcd. for [C₂₅H₂₂N₄O₃Re] 613.1245.

**Compound 3.** Yield: 28% from L₃. HPLC(Gradient 1): RT = 3.20 min; ¹H NMR (400 MHz, CDCl₃): δ (ppm) 1.10 (t, J = 7.3 Hz, 3H), 2.11 – 2.15 (m, 2H), 3.78 – 3.81 (m, 2H), 5.00 – 5.03 (m, 2H), 6.15 – 6.18 (m, 2H),
7.60 – 7.63 (m, 2H), 7.77 – 7.85 (m, 2H), 7.83 – 7.85 (m, 2H), 7.94 (d, J = 8.43, 2H), 8.29 (d, J = 8.42, 2H), 8.45 (d, J = 8.8 Hz, 2H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): \(\delta\) (ppm) 11.39, 20.48, 69.06, 70.82, 121.04, 128.20, 128.43, 128.59, 129.65, 132.87, 141.38, 165.25, 194.52, 195.92; HR-ESI mass spectrum (CH\(_3\)CN/H\(_2\)O): found 612.1292; calcd. for [C\(_{26}\)H\(_{23}\)N\(_3\)O\(_3\)Re] 611.1291.

**Bacteria strains**

Control strains of *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were used to determine the activity of the rhenium complexes. Also methicillin resistant *S. aureus* (MRSA, ATCC 43300) and colistin resistant *E. coli* (mcr-1, faecal clinical isolate\[^{21}\]) were used to assess if 1 holds its activity against these strains. In order to characterize the cellular uptake of 1 three *E. coli* mutants (MB4902, lpxC outer-membrane permeable strain; MB5747, tolC efflux-negative strain; and MB5746 lpxC and tolC permeable efflux-negative) and their wild type parent strain (MB4827) were used.\[^{13}\] The mutant tolC, lpxC and tolC + lpxC *E. coli* strains MB4902, MB5747, MC5746 and control strain MC4827 were generously supplied by Merck Sharp & Dohme (Kenilworth, NJ). The mcr-1 *E. coli* strain was generously provided by Dr. Bone Siu-Fai Tang from the Hong Kong Sanatorium & Hospital. *Pseudomonas aeruginosa* mutants (PA01, PA0750, PAO397) were kindly provided by Herbert Schweizer at Colorado State University.\[^{17}\]

**Minimum inhibitory concentration (MIC)**

Bacteria were cultured in Cation adjusted Mueller Hinton broth (CaMHB; BD, Cat. # 212322) at 37 °C overnight with shaking (~200 rpm). A sample of each culture was then diluted 40-fold in fresh CaMHB and incubated at 37 °C for 1.5-3 h with shaking (~200 rpm). Compound stock solutions were prepared as 1.28 mg/mL in water or 20% DMSO. The compounds were serially diluted two-fold across the wells of 96-well plates (Polystyrene, Corning, catalogue No. 3370) in quadruplets. Mid-log phase bacterial cultures were diluted to 1 x 10\(^8\) colony forming units (CFU)/mL and 50 \(\mu\)L was added to each well of the compound containing plates giving a compound concentration range of 64 \(\mu\)g/mL to 0.03 \(\mu\)g/mL. Plates were immediately irradiated (60 min at 365 nm) or kept in the dark. We found that the results of the assay were influenced by a number of factors. Using 384 well plates resulted in higher (less potent) MICs. Removing the lid covering the plates gave better results, probably due to light absorption. Finally, the orientation of the well and distance from the lamp was optimized to obtain the best possible results. After treatment they were incubated at 37 °C for 18-24 h. MICs were determined visually as the lowest compound concentration at which no bacterial growth was visible.

In order to study the effect of different antibiotics in combination with the rhenium metal complex, the same procedure was followed with the addition of a fix sub-MIC concentration (1/4 MIC) of polymyxin B, octapepetin C4, meropenem and gentamicin.
Rhenium complex accumulation in bacteria

Bacteria were cultured in Luria-Bertani broth (LB; Difco, Cat. #244620) at 37 °C overnight with shaking (~200 rpm). A sample of each culture was diluted 40-fold in fresh LB and incubated at 37 °C shaking (~200 rpm) until mid-exponential (OD$_{600}$=0.6, which corresponds to ~0.6 x 10$^8$ CFU/mL) phase was reached. Then cells were harvested at 3000 g for 15 min at 20 °C and resuspended in phosphate buffered saline (PBS) to OD$_{600}$=6 (~6 x 10$^9$ CFU/mL). Washed cells were transferred to a glass vial containing compound (final concentration of 50 µM) or buffer, and incubated for 60 min in water bath at 37 °C. After incubation, cell were spun down (18000 g, 5 min) and the supernatant collected. Lysing buffer (0.1M glycine-HCl buffer, pH 3) was added to the pellet, mixed well and incubated overnight at room temperature. After lysis, the tubes were centrifuged (18000 rpm, 5 min) to separate cell debris (pellet) from cytoplasmic and periplasmic content. The three separate samples: supernatant after incubation (supernatant), supernatant after lysis (Lysate), and cell debris after lysis (pellet). All samples were freeze dried to remove all remaining solvents. Ultra-pure nitric acid (200 µL for pellet and lysate and 500 µL for supernatant) was added and the Eppendorf vials were shaken at 40 °C for 24 h. The samples were then diluted (dilution was adjusted so that final rhenium content would not be higher than 30 ppb due to detection limits of the instrument) to a final volume of 10 mL and a final HNO$_3$ content of 2% before ICP-MS measurement of rhenium content.

ICP-MS measurements.

ICP-MS experimental work was performed at the Environmental Geochemistry Laboratory of the School of Earth and Environmental Sciences, The University of Queensland.

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Supporting Information

Light-activated Rhenium Complexes with Dual Mode of Action against Gram-positive and Gram-negative Bacteria

Angelo Frei*, Maite Amado, Matthew A. Cooper, Mark A. T. Blaskovich*

Experimental

Scheme S1. Synthetic route to L1 complex 1.

Ligand L1 was prepared according to reported procedure and purified by chromatography on silica (3:1 Hexane/EtOAc) as reported.[1]
Scheme S2. Synthetic route to L2 and complex 2.

N-Boc-1,2-diaminoethane was prepared as reported previously.[2] L2 was prepared using an analogous procedure as for L1 and used without further purification to prepare 2.1 according to the general procedure for rhenium complexes.[1] The crude of 2.1 was dissolved in MeOH (2 ml) and cooled by means of an ice bath. Then 35% HCl (1 ml) was slowly added. The next day, the pH was adjusted to 12 with NaOH and the solvent was evaporated. The solids were taken up in MeOH to remove excess NaCl and evaporated again after filtration. The crude of 2.1 was then purified by preparative HPLC (method A).

Scheme S3. Synthetic route to L3 and complex 3.

L3 was prepared using an analogous procedure as for L1 and then used without further purification for the preparation of 3 according to the general procedure for rhenium complexes.[1]

ICP-MS measurements.

ICP-MS experimental work was performed at the Environmental Geochemistry Laboratory of the School of Earth and Environmental Sciences, The University of Queensland.

Analysis

Analysis by ICP-MS (Agilent 7900) is done in collision mode through the He collision cell. Instrument parameters are as follows: RF power: 1550 W, Carrier gas: 1.08 L/min, Nebuliser pump: 0.10 rps He Flow: 5 mL/min, $^{115}$In was used as an internal standard to monitor instrumental drift during the experiment. The ICP-MS is a highly sensitive instrument and easily damaged. Its working range is around 10 ppb, and concentrations above 30 ppb should be avoided.

Experimental Procedure

Aqueous samples are arranged in auto-sampler tubes in a numbered rack in the auto-sampler. Calibration standards are prepared to enclose the range of expected concentrations and also loaded into the auto-sampler. A separate set of standards are made up from different primary standards to that used for preparing the calibration standards, to serve as controls for the
calibration and the instrument performance. These are used to test the accuracy of the analytical process, designated as the standards as unknowns. Instrument blanks (that is, solvent blanks consisting of 2% ultrapure nitric acid) are analysed at regular intervals during the experiment to determine the detection limits for the experiment. At least 7 blanks are required for a good detection limit determination. The internal standard is automatically added to each sample during analysis to correct for internal drift. A monitoring sample, similar to the samples being analysed, is also analysed at regular intervals during the experiment, to monitor any unexpected variations in instrument performance (external drift) and used to correct for this. Duplicate analysis are taken of every 10th sample at the end of the experimental run, to determine precision of analysis.

Data Reduction

Data reduction is completed after exporting the raw data to Excel and completing drift corrections, calibration and subsequent calculations in Excel. The data is processed as follows:

1. Export the raw data from the instrument to a csv or Excel file.
2. Pre-treat the data. Pre-treatment may include transformation, blank subtraction and internal and external drift correction. The degree of pre-treatment is dependent on the software associated with the analytical instrument.
3. Construct calibration curves for the analysed data, using the known concentrations of the standards and the instrument response to each analyte.
4. Determine whether the calibrations are linear or non-linear. Accurate calculations can only be done on linear calibrations. If necessary, remove the most concentrated standards from the calibration line until a linear result is achieved.
5. Determine whether the analyte content for all samples fall within calibration range. If there are analytes beyond calibration range, the analysis will have to be repeated, using either a more concentrated standard or a more diluted sample. For ICP-MS, a more diluted sample is generally required.
6. Once suitable calibration figures have been established, test the legitimacy of the calibration by calculating the concentrations of the calibration standards from the intensity data and the newly-constructed calibration curves. The concentration of the standards should be within 90-110% of the actual, known concentrations. For values in the ppb range, concentrations within 80%-120% of the known value, are acceptable. If not, the calibration curves will need to be refined until acceptable values are obtained. This step is known as recovery.
7. Calculate the standards as unknowns. Their values should be within 5-10% of their expected value.
8. Calculate the detection limits by finding the standard deviations of the intensity values for the instrument blanks and multiplying the value with 3. Then calculate the associated concentration (the detection limit) by using this value and the calibration equation for each analyte.

9. Calculate the precision or reproducibility of the analysis by calculating the average and standard deviation on each set of duplicates. Multiply the standard deviation with 100 and divide by the average. This figure is the percentage relative standard deviation. For trace elements, they should not exceed 5%.

**Figure S1.** Cellular uptake and distribution of rhenium in *S. Aureus*. Percentage of rhenium in different fractions given as percentage of total detected rhenium.
Spectral Data for Complexes 1-3.

Figure S2. $^1$H NMR of Compound 1 in CDCl$_3$. 
Figure S3. $^{13}$C NMR of Compound 1 in CDCl$_3$.

Figure S4. HR-ESI MS of compound 1 ($\text{H}_2\text{O/CH}_3\text{CN}$).
Figure S5. HPLC trace for complex 1 at 254 nm.

Figure S6. $^1$H NMR of Compound 2 in CDCl$_3$. 
Figure S7. $^{13}$C NMR of Compound 2 in CDCl$_3$.

Figure S8. HR-ESI MS of compound 2 (H$_2$O/CH$_3$CN).
Figure S9. HPLC trace for complex 2 at 254 nm.

Figure S10. $^1$H NMR of Compound 3 in CDCl$_3$. 
**Figure S11.** $^{13}$C NMR of Compound 3 in CDCl$_3$.

**Figure S12.** HPLC trace for complex 3 at 254 nm.
Figure S13. HR-ESI MS of compound 3 (H$_2$O/CH$_3$CN).

| MICs against Gram(-)          |
|-------------------------------|
| K. pneumoniae (ATCC700603) MDR|

|         | 365 nm | dark |
|---------|--------|------|
| Pmx'    |        |      |
| 0.25    | 0.2    |      |
| 1       | >128   | >186.2|
| 0.25-5  | 0.2-0.4|      |
| 128     | 186.2  | 16-32|

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