Indole-containing arene-ruthenium complexes with broad spectrum activity against antibiotic-resistant bacteria

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A B S T R A C T

Antimicrobial resistant (AMR) bacteria are emerging and spreading globally, threatening our ability to treat common infectious diseases. The development of new classes of antibiotics able to kill or inhibit the growth of such AMR bacteria through novel mechanisms of action is therefore urgently needed. Here, a new family of indole-containing arene ruthenium organometallic compounds are screened against several bacterial species and drug resistant strains. The most active complex [(p-cym)Ru(O-cyclohexyl-1H-indole-2-carbothioate)](3) shows growth inhibition and bactericidal activity against different organisms (Acinetobacter baumannii, Mycobacterium abscessus, Mycobacterium tuberculosis, Staphylococcus aureus, Salmonella enterica serovar Typhi and Escherichia coli), demonstrating broad-spectrum inhibitory activity. Importantly, this compound series exhibits low toxicity against human cells. Owing to the novelty of the antibiotic family, their moderate cytotoxicity, and their inhibitory activity against Gram positive, Gram negative and acid-fast, antibiotic resistant microorganisms, this series shows significant promise for further development.

Introduction

Antibiotic resistance is a worldwide threat to public health, food security, and economic and societal developments. (1) The prevalence of antimicrobial resistance (AMR) among common pathogens is rapidly increasing, which leads to widespread diseases becoming harder, or impossible, to cure. AMR organisms of interest include Acinetobacter baumannii, Escherichia coli, Salmonella sp. and several other multidrug-resistant Gram-negative organisms. (Wang et al., 2019) In the last decades, a global effort has allowed the development of new anti-biotic drugs with activity against common AMR organisms, (Cox and Worthington, 2017) but resistance against such agents is already emerging. There is therefore an urgent need to find new families of compounds with high levels of antibacterial activity, novel mechanisms of action and low frequencies of antibiotic resistance.

Inorganic metallotherapeutics offer potential for unique mechanisms of drug action based on the choice of the metal, its oxidation state, the types and number of coordinated ligands and the coordination geometry. (Barry and Sadler, 2013, Barry and Sadler, 2014) As such, medicinal inorganic chemistry provides a rich platform in the pharmacological space for structural and electronic diversity. (Silva et al., 2021) Medicinal inorganic chemistry has been stimulated by the success of platinum anticancer drugs (used as a component of nearly 50% of all cancer chemotherapy treatments), by the use of gadolinium(III) complexes as MRI contrast agents (used in about 20 million doses administered per year), and of the radionuclide 99m-technetium radiopharmaceuticals for γ-ray imaging (used in about 20 million radio diagnostic procedures each year). (Biancalana et al., 2020, Farley et al., 2021, Hanif et al., 2020, Rafols et al., 2021) However, the involvement of metals in many other diseases and conditions is of current interest in relation to their causes, their treatment or detection, including neurodegeneration, (Anthony et al., 2020) fungal (Golbaghi et al., 2020), parasitic, (Mimori et al., ...
The antimicrobial activity of some half-sandwich “piano-stool” complexes of precious metals (Ru, Os, Rh, Ir) has very recently proven to be highly promising in our current fight against AMR, with demonstrated potency against drug-resistant strains of *Mycobacterium tuberculosis* (Coverdale et al., 2021). Half-sandwich complexes are versatile and have been widely used as antibacterial, antifungal, anti-inflammatory, antihistaminic, and anticancer drugs. (Wan et al., 2019, Dadashpour and Emami, 2018) Examples of such compounds currently in clinical use are the non-steroidal anti-inflammatory drug indomethacin (Lal and Snape, 2012) or the antiretroviral delavirdine. (Xu and Lv, 2009)

Herein, we report the synthesis and characterisation of a new family of four ruthenium half-sandwich complexes containing O-R-IH-indole-2-carbothioate (O-R-ind-th; R = methyl, ethyl, cyclohexyl, phenyl) ([p-cym]Ru(O-Me-ind-th)Cl] (1), [p-cym]Ru(O-Et-ind-th)Cl] (2), [p-cym]Ru(O-Cy-ind-th)Cl] (3), [p-cym]Ru(O-Ph-ind-th)Cl] (4) (Scheme 1). Their stability in solution, hydrolysis rates and acid dissociation constants are studied, along with toxicity data on three human cell lines for both ligands and complexes. The bactericidal activities of the four ligands and complexes against several drug resistant isolates of *Escherichia coli* are reported, including *E. coli J53 2138*, a clinical isolate producing the extended spectrum β-lactamase (ESBL) OXA-1, which confers resistance to ampicillin, ticarcillin, piperacillin and cephalosporins, and *E. coli J53 2140E*, producing the ESBL OXA-3.

**Results and discussions**

**Synthesis, stability in solution, aquation and pKa determination**

The indole 2-carboxylate ligands L2 – L5 were synthesised by esterification of 1H-indole-2-carboxylic acid (L1) with the corresponding alcohol (MeOH, EtOH, CyOH, PhOH, respectively). Thiolation of the carboxylic group of ligands L2 – L5 was performed by refluxing the corresponding ligands with the Lawesson’s reagent in toluene to yield the O-R-IH-indole-2-carboxothioate ligands L6 – L9 (R = methyl, ethyl, cyclohexane, benzene; Scheme 1; Experimental Section). Complexes 1 – 4 were then prepared by stirring the dichloro(p-cymene) ruthenium(II) dimer with the corresponding ligand (L6 – L9) in dry dichloromethane at ambient temperature and in the presence of triethylamine. All ligands and complexes were characterised by 1H and 13C NMR spectroscopy, and high-resolution ESI-MS (Experimental Section). 1H and 13C NMR spectra can be found in the Supporting Information (Figs. S1 – S24), as well as the HR mass spectra (Figs. S25 – S36). As an example, Fig. 1 shows the 1H NMR spectra of L4 (bottom), L8 (middle), and complex 3 (top) in CDCl3. The lowest field region (9.5 – 7.0 ppm) shows the aromatic protons of the indole-based substituent, followed by the region of the coordinated arene (5.5 – 4.0 ppm), and by the aliphatic region (4.0 – 0.5 ppm). Chemical shifts for the protons located near the ligand heteroatoms that bind to the metal can be observed, for example, the proton of the CH cyclohexyl bound to the oxygen/sulfur (Fig. 1) can be seen to shift upfield from the free ligand L8 to the metal complex 3. Such shifts can be explained by the stereo-electronic effects due to the coordination to the metal moiety, as previously observed. (Soldevilla-Barreda et al., 2020) The signal for the NH proton is also no more visible after the metal complexation, as previously observed. (Soldevilla-Barreda et al., 2020)

**Scheme 1. Preparation of the indole-based ligands L6 – L9 and complexes 1 – 4.**
[(p-cym)Ru(O-Cy-ind-th)Cl], and 4 [(p-cym)Ru(O-Ph-ind-th)Cl], suitable for X-ray structure determination, were obtained by slow diffusion of hexane into a saturated dichloromethane solution at 20 °C. The crystallographic data and selected bond lengths and angles are given in Tables S1 – S12, and the crystal structures are shown in Fig. 2 and Fig. S40. Complexes 2-4 adopt a pseudo-octahedral structure with Ru\textsuperscript{II} bound to a η\textsuperscript{6}-para-cymene ring, a N,S-chelated indole and chloride as ligands to form an 18-electron complex with "piano-stool" geometry.

To investigate the stability of complexes 1 – 4, the compounds were dissolved in a mixture (1:1) (v/v) of DMSO/RPMI and UV-Vis spectra were recorded over 24 h (Fig. 3). The absorption band at 420 nm gradually decreases while the one at 350 nm concomitantly increases, intersecting at the isosbestic point, which indicates that only the chloride and the hydrolysed forms of the complex contribute to the observed absorptions. (Bacac et al., 2004, Peacock et al., 2007, Peacock et al., 2007, Scolaro et al., 2008) The time dependence of the absorbance allows for the determination of the rate constants by plotting the absorbance versus time at fixed wavelength for each compound (Table 1). The hydrolysis rate depends on the ester group of the indole ligand. Complex 4 (the only complex containing an aromatic R substituent) presents the fastest hydrolysis. The rate of hydrolysis of complexes 1 – 3, with aliphatic R groups, suggests that the bulkier the R substituent is, the slower the aquation process is.

Aquation of the monodentate ligand X is a common behaviour for

![Fig. 1. 1H NMR spectra of L4 (bottom), L8 (middle), and complex 3 (top) in CDCl\textsubscript{3} (400 MHz). Residual solvents are marked with a cross.](image)

![Fig. 2. Structures of ligand L5 (left) and Ru\textsuperscript{II} complex 4 (right). Thermal ellipsoids are drawn at the 50% probability level.](image)
half-sandwich complexes of the type [(arene)M(NUSX)] and is usually considered an activation step, which allows further reactions with the corresponding targets. (Liu and Sadler, 2014, Soldevila-Barreda and Metzler-Nolte, 2019, Meier-Menches et al., 2018, Rilak Simović et al., 2019) Solutions ranging from pH 7 to 12 were prepared using 0.1 M NaOH solution, and complexes 1 – 4 (previously dissolved in pure acetonitrile) were added to the corresponding solution with a known pH and mixed for 10 min. Spectra were recorded by UV-visible spectroscopy. The pKa values of the complexes were calculated using Origin 2019 by plotting the absorbance at the corresponding wavelength against the pH and fitting it to the Boltzmann equation to obtain the inflection point. For complexes 1 – 4, the pKa values were calculated to be around 10 (Experimental section and Table 1). These pKa values are high, although such decrease in acidity has been previously reported (generally attributed to an increased electron density on the metal center). (Liu and Sadler, 2014, Peacock et al., 2006, Cross et al., 2016) As a consequence of such high pKa values, only the aqua adduct of the metal complexes is expected to be present at physiological pH (7.4), thus favouring the reaction between the metal complexes with possible ligands such as nucleobases or proteins.

Toxicity studies against human cells

Toxicity data against human cells is important information for the identification of a novel family of antibiotic drug candidates. Initially developed for anticancer activity, complexes 1 – 4 (and ligands L6 – L9) were tested against human ovarian adenocarcinoma (A2780), cisplatin-resistant variant of A2780 (A2780cisR) and normal human prostate epithelial (PNT2) cell lines. Half-maximal inhibitory concentrations (IC50) were determined using a 24 h MTT assay with 48 h recovery period. The IC50 values are shown in Table 2 and the IC50 graphs for ligands L6 – L9 and complexes 1 – 4 against PNT2, A2780, and A2780cisR can be found in the Supporting Information (Figs. S37 – S38).

Table 1

| Compound                          | pKa          | $K_{\text{aquation}}$ (s$^{-1}$) |
|-----------------------------------|--------------|----------------------------------|
| ([p-cym]Ru(O-Me-ind-th)Cl] (1)    | 10.34 ± 0.07 | 2.0·10$^{-4}$ ± 6·10$^{-5}$       |
| ([p-cym]Ru(O-Et-ind-th)Cl] (2)    | 10.24 ± 0.09 | 1.8·10$^{-4}$ ± 4·10$^{-5}$       |
| ([p-cym]Ru(O-cy-ind-th)Cl] (3)    | 9.81 ± 0.07  | 1.2·10$^{-4}$ ± 3·10$^{-5}$       |
| ([p-cym]Ru(O-pH-ind-th)Cl] (4)    | 10.07 ± 0.05 | 2.4·10$^{-4}$ ± 4·10$^{-5}$       |

Fig. 3. UV–vis spectra of complex 2 in the mixture DMSO/RPMI over time (10$^{-5}$ M, 298 K, 24 h).

Table 2

| IC50 values (µM) in A2780, A2780 cisplatin resistant and PNT2 cells for ligands L6 – L9 and for complexes 1 – 4. |
|-------------------------------------------------|
| Compound                              | IC50 values (µM) |
|---------------------------------------|------------------|
| A2780                                 |                 |
| L6                                    | >100            |
| L7                                    | >100            |
| L8                                    | 72 ± 2          |
| L9                                    | 21 ± 1          |
| 1                                     | 22 ± 1          |
| 2                                     | 12 ± 2          |
| 3                                     | 10.7 ± 0.6      |
| 4                                     | 68 ± 4          |
| Cisplatin                             | 5.9 ± 0.4       |
| A2780cisR                             | 10.24 ± 0.18    |
| PNT2                                  | >100            |
| A2780                                 | >100            |
| L7                                    | >100            |
| L8                                    | 76 ± 5          |
| L9                                    | 20 ± 2          |
| 1                                     | 28 ± 1          |
| 2                                     | 32 ± 2          |
| 3                                     | 37 ± 2          |
| 4                                     | 20.8 ± 0.9      |
| Cisplatin                             | 11.8 ± 0.8      |

Half-maximal inhibitory concentrations (IC50) were determined using a 24 h MTT assay with 48 h recovery period. The IC50 values are shown in Table 2 and the IC50 graphs for ligands L6 – L9 and complexes 1 – 4 against PNT2, A2780, and A2780cisR can be found in the Supporting Information (Figs. S37 – S38).

Complexes 1, 2 and 3 are moderately cytotoxic against the tested cell lines and showed 2 – 3× higher IC50 values towards normal prostate cells in comparison to cancer cells. They are also less toxic than cisplatin against all cell lines. Complex 4 exhibits only low toxicity against the three cell lines.

Antibiotic activity

A concentration range of complexes 1 – 4 were tested and activity was observed for a range of organisms, including Mycobacterium abscessus NCTC 13031, Escherichia coli ATCC 11775, I469 ESBL, J53 2138E, J53 2140E, Staphylococcus aureus ATCC 29213, Acinetobacter baumannii NCTC 12156, Salmonella enterica serovar Typhi and Mycobacterium tuberculosis H37Rv. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) activity of all four complexes were determined (Tables 3 and 4). Overall, complex 3 was the most effective, inhibiting nine out of the 12 organisms tested.

Table 3

| Organism                        | Minimum Inhibitory Concentration (MIC) µg/mL |
|---------------------------------|----------------------------------------------|
|                                 | Complex 1 | Complex 2 | Complex 3 | Complex 4 |
| Acinetobacter baumannii NCTC 12156 | >100      | >100      | 50 (94.5) | >100      |
| Escherichia coli ATCC 11775     | >100      | 100 (210.5) | 50 (94.5) | >100      |
| Escherichia coli I469 ESBL      | >100      | >100      | 50 (94.5) | >100      |
| Escherichia coli J53 2138E      | >100      | 50 (105.2) | 25 (47.25)| >100      |
| Escherichia coli J53 2140E      | >100      | 100 (210.5)| 25 (47.25)| >100      |
| Klebsiella pneumoniae H467 KPC  | >100      | >100      | >100      | >100      |
| Mycobacterium tuberculosis H37Rv NCTC 13031 | 50 (108.4) | 25 (52.6) | 12.5 (23.6)| >100      |
| Mycobacterium tuberculosis H37Rv NCTC 8309 | >100      | >100      | >100      | >100      |
| Proteus mirabilis               | >100      | >100      | >100      | >100      |
| Pseudomonas aeruginosa ATCC 10145 | >100      | >100      | >100      | >100      |
| Salmonella enterica serovar Typhi | 50 (108.4) | 25 (52.6) | 6.25 (11.8)| >100      |
| Staphylococcus aureus ATCC 29213 | 6.25 (13.5)| 12.5 (26.3)| 1.56 (2.94)| 12.5 (23.9)|

The minimum inhibitory concentrations observed for all organisms tested.
Absence of bacterial growth was observed after exposure to complex 1. 5.

**Fig. 4.** The minimum bactericidal concentrations (MBC) for complex 3 identified by absence of growth. MBCs were defined by minimum concentration at which an absence of bacterial growth was observed after exposure to complex 3. A, Acinetobacter baumannii ATCC 12156 had an MBC of 50 µg/mL (n=3). B, Escherichia coli ATCC 11775 and G, E. coli ESBL had an MBC of 50 µg/mL (n=3). D, E. coli 2138E and E, E. coli 2140E both had MBCs of 25 µg/mL (n=3). F, Salmonella enterica serovar Typhi had an MBC of 6.25 µg/mL (n=3). G, Staphylococcus aureus exhibited an MBC of 3.125 µg/mL (n=3). H, plate map showing concentrations of complex 3.

**Table 4**
The minimum bactericidal concentrations observed for all organisms tested.

| Organism                        | Minimum Bactericidal Concentration (MBC) µg/mL |
|---------------------------------|-----------------------------------------------|
|                                 | Complex 1         | Complex 2         | Complex 3         | Complex 4         |
| Acinetobacter baumannii ATCC    | >100             | >100             | 50 (94.5)         | >100             |
| 12156                           | <100 µM          | <100 µM          | <100 µM           | <100 µM           |
| Escherichia coli ATCC 11775     | >100             | >100             | 50 (94.5)         | >100             |
|                                 | <100 µM          | <100 µM          | <100 µM           | <100 µM           |
| Escherichia coli ATCC 1469 ESBL | >100             | >100             | 50 (94.5)         | >100             |
|                                 | <100 µM          | <100 µM          | <100 µM           | <100 µM           |
| Escherichia coli JS3 2138E      | >100             | >100             | 25 (47.25)        | >100             |
|                                 | <100 µM          | <100 µM          | <100 µM           | <100 µM           |
| Escherichia coli JS3 2140E      | >100             | >100             | 25 (47.25)        | >100             |
|                                 | <100 µM          | <100 µM          | <100 µM           | <100 µM           |
| Klebsiella pneumoniae ATCC 1467 | >100             | >100             | 50 (95.6)         | >100             |
|                                 | <100 µM          | <100 µM          | <100 µM           | <100 µM           |
| Mycobacterium abscessus ATCC    | >100             | >100             | >100              | >100             |
| 13031                           | <100 µM          | <100 µM          | <100 µM           | <100 µM           |
| Mycobacterium tuberculosis H37Rv| 50 (10.8)        | >100             | >100              | 50 (95.6)         |
|                                 | <100 µM          | <100 µM          | <100 µM           | <100 µM           |
| Proteus mirabilis NCTC 8309     | >100             | >100             | >100              | >100             |
|                                 | <100 µM          | <100 µM          | <100 µM           | <100 µM           |
| Pseudomonas aeruginosa ATCC 10145| >100             | >100             | >100              | >100             |
|                                 | <100 µM          | <100 µM          | <100 µM           | <100 µM           |
| Salmonella enterica serovar Typhi| 50 (10.8)        | 25 (52.6)        | 6.25 (11.8)       | >100             |
|                                 | <100 µM          | <100 µM          | <100 µM           | <100 µM           |
| Staphylococcus aureus ATCC 29213| 12.5 (27.11)     | 12.5 (26.3)      | 3.125 (5.9)       | 50 (95.6)         |
|                                 | <100 µM          | <100 µM          | <100 µM           | <100 µM           |

Complex 3 also had the lowest MIC observed for all complexes tested, at 1.56 µg/mL against *S. aureus*. Bactericidal activity was also observed against seven out of the 12 organisms tested (Fig. 4). The two organisms that did not have an MBC were both mycobacterial species, *M. abscessus* and *M. tuberculosis* (Fig. 4 and 6). However, complex 3 was bacteriostatic against both species of mycobacteria inhibiting at a concentration of 12.5 µg/mL and 100 µg/mL, respectively. Complex 2 had similar inhibitory activity to complex 3, however no activity was observed for *A. baumannii* or *E. coli* 1469 EBL (Table 3). Bactericidal activity for complex 2 was greatly reduced compared to complex 3 with an MBC observed for only *S. typhi* and *S. aureus*.

Complex 1 was less effective, only inhibiting *M. abscessus*, *M. tuberculosis*, *S. typhi* and *S. aureus*. No bactericidal activity was observed for *M. abscessus* but bactericidal activity was observed for *M. tuberculosis*, *S. typhi* and *S. aureus*. Arguably complex 4 was the least effective, with an MIC and MBC observed for only *S. aureus* and *M. tuberculosis* (Fig. 6). However, *M. tuberculosis* is a major human pathogen that is often highly drug resistant. Therefore, complex 4 is still of great potential importance and could be effective against other pathogens not yet tested.

Growth curve data was obtained for *M. abscessus* (Fig. 5) and analysed with an ANOVA. A significant difference between the different concentrations and controls was observed for complexes 1, 2 and 3 with p values of <0.0001 for all three complexes. No significant difference between concentrations and control were observed for complex 4. A multiple comparison was conducted for complexes 1, 2 and 3, which identified a significant difference between all concentrations and control (p value <0.0001) for all, apart from complex 1 at 3.125 µg/mL and the *M. abscessus* only control (p value 0.0064). This identifies that complexes 1, 2 and 3 are bacteriostatic against *M. abscessus* and have a significant impact on the growth.

All four of the complexes inhibited a variety of microorganisms tested, with complex 3 being the most effective. The Gram-positive *S. aureus* was the most affected organism with the lowest MIC and MBC for all of the complexes tested, as well as being the only one, other than *M. tuberculosis*, affected by complex 4. Growth of *M. tuberculosis* was also inhibited by each complex, but at higher concentrations than...
for *S. aureus*. However, unlike *S. aureus*, complexes 2 and 3 had no bactericidal activity against *M. tuberculosis*. *M. abscessus* was largely inhibited by three out of the four complexes but had no bactericidal activity. A range of activity was identified for the Gram-negative organisms *E. coli* and *S. typhi* against complexes 1, 2 and 3, however no activity was observed for *Proteus mirabilis*, *Pseudomonas aeruginosa* or *Klebsiella pneumoniae*.

**Conclusions**

In conclusion, four half-sandwich metal complexes containing a bioactive indole moiety were synthesised and characterised. They were progressed to *in vitro* screening to gain a first estimation of their cytotoxicity profile. The complexes only show moderate toxicity against A2780 ovarian cancer, A2780 cisplatin resistant, and PNT2 cell lines. As such, the bactericidal activity of the four compounds was investigated against several drug resistant isolates of Gram negative *Escherichia coli* and *Salmonella enterica* serovar *Typhi* as well as against isolates of Gram positive bacteria.

All complexes showed growth inhibition and bactericidal activity against a variety of bacteria, including the notable pathogens *M. tuberculosis*, *M. abscessus* and *E. coli*. Complexes 2 and 3 exhibit the most promising antibacterial activities, having the lowest minimum inhibitory concentrations and exhibiting bactericidal activity. It appears that steric hindrance of the R group on the indole has an influence on the aquation rate, the cytotoxicity, and the antibacterial properties, which
will be confirmed/infirmed by future determination of the antibacterial mechanism of action. This series shows significant promise for further hit-to-lead medicinal chemistry, and future work will include further cytotoxicity studies, collection of in vivo data and mechanisms of action elucidation (target identification).

Materials and methods

Hydrated metallic chlorides were purchased from Precious Metals Online. All other chemicals were purchased from Sigma-Aldrich (UK). Non-dried solvents were purchased from Fischer Scientific and used as received. Dichloromethane, tetrahydrofuran and toluene were dried over molecular sieves (3 Å). All compounds were prepared under a purified dinitrogen atmosphere using standard Schlenk and vacuum line procedures. pH* was adjusted using EDT direction non-glass pocket pH meter with an ISFET silicon chip pH sensor. pH* values (pH readings without correction for the effect of deuterium) of NMR samples were adjusted using KOD solutions in D2O.

Purified dinitrogen atmosphere using standard Schlenk and vacuum line procedures. All compounds were prepared under a purified dinitrogen atmosphere using standard Schlenk and vacuum line procedures. All non-dried solvents were purchased from Fischer Scientific and used as received. Online. All other chemicals were purchased from Sigma-Aldrich (UK).

Cytoxicity studies, collection of in vivo data and mechanisms of action elucidation (target identification).

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The synthesis and characterisation are in accordance with the literature. (Mistry et al., 2015)

![Synthesis and Characterisation](image)

The procedure used to prepare L2 was followed with 30 mL of ethanol and 18.6 mmol of 1-H-indole-2-carboxylic acid. The pure compound L3 was obtained as a white powder (4.1 g, 91%). The synthesis and characterisation are in accordance with the literature. (Kuoljala et al., 2011)

![Cytoxicity Studies](image)

![Bacterial Culture](image)

![Synthesis and Characterisation](image)

![Materials and Methods](image)

1H NMR (DMSO-d6, 400 MHz): δ = 11.86 (1H, s, H1), 7.65 (1H, d, 3J1H= 8.0 Hz, H4), 7.45 (1H, d, 3J1H= 8.4 Hz, H7), 7.25 (1H, d, 3J1H= 7.2 and 8.4 Hz, H6), 7.14 (1H, m, H3), 7.07 (1H, dd, 3J1H= 7.2 and 8.0 Hz, H5), 4.34 (2H, q, JCH= 7.1 Hz, H11), 1.34 ppm (3H, t, JCH= 7.0 Hz, H12).

13C NMR (DMSO-d6, 101 MHz): δ = 161.3 (C10), 137.4 (C2), 127.3 (C8 or C9), 126.7 (C8 or C9), 124.7 (C6), 122.1 (C4), 120.2 (C5), 112.6 (C7), 107.8 (C3), 51.8 ppm (C11).

HRMS (ESI+): m/z calc. for C16H16NO2 [M + H]+ 276.0667; found 276.0702.

[Cyclohexyl indole-2-carboxylate] (L4). The procedure used to prepare L3 was followed with 10 mL of cyclohexanol and 18.6 mmol of 1-H-indole-2-carboxylic acid. The pure compound L4 was obtained as a light-brown powder (4 g, 91%).

![Materials and Methods](image)

1H NMR (DMSO-d6, 400 MHz): δ = 11.72 (1H, s, H1), 7.55 (1H, d, 3J1H= 8.0 Hz, H4), 7.37 (1H, d, 3J1H= 8.4 Hz, H7), 7.16 (1H, d, 3J1H= 7.2 and 7.8 Hz, H6), 7.05 (1H, m, H3), 6.98 (1H, dd, 3J1H= 7.0 and 7.6 Hz, H5), 4.86 (1H, m, H11), 1.86 – 1.15 ppm (10H, br, Hcyclohexane).

13C NMR (DMSO-d6, 101 MHz): δ = 160.8 (C10), 137.4 (C2), 127.7 (C8 or C9), 126.8 (C8 or C9), 124.6 (C6), 122.1 (C4), 120.1 (C5), 112.6 (C7), 107.7 (C3), 60.4 (C11), 14.3 ppm (C12).

HRMS (ESI+): m/z calc. for C16H16NO2 [M + H]+ 190.0823; found 190.08564.

![Bacterial Culture](image)

![Synthesis and Characterisation](image)

[1Methyl indole-2-carboxylate] (L2). 1-H-indole-2-carboxylic acid (3.0 g, 18.6 mmol) was added to a round-bottom flask (100 mL) and dissolved in methanol (30 mL). Concentrated sulphuric acid (1.5 mL, 28.0 mmol) was added to the mixture and subsequently heated to reflux for 2 h. Then, the solvent was removed under reduced pressure, and the crude was dissolved in ethyl acetate (6 mL), and subsequently extracted with a saturated solution of NaHCO3 (3×30 mL). The organic phases were combined and dried over MgSO4 and filtered. The filtrate was evaporated under vacuum to afford a light-brown powder (4.3 g, 93%).
crude solution was quenched with a saturated aqueous solution of NaHCO₃ and extracted with H₂O (3×30 mL). The organic phases were combined and dried over MgSO₄ and filtered. The filtrate was evaporated under vacuum to afford a white powder (2.8 g, 37%). The synthesis and characterisation are in accordance with the literature. (Guo et al., 2016)

\[\text{[Cyclohexyl indole-2-thionoester] (L8). The procedure used to prepare L6 was followed with 250 mg (1.0 mmol) of L4 and Lawesson's Reagent (499 mg, 1.23 mmol) and refluxed for 4 days. The pure compound L8 was obtained as a bright yellow solid (190 mg, 71%).} \]

1H NMR (DMSO-\(d_6\), 400 MHz): \(\delta = 11.62\) (1H, s, H1), 7.67 (1H, d, \(J_{3H-H} = 8.0\) Hz, H4), 7.49 (1H, d, \(J_{3H-H} = 8.4\) Hz, H7), 7.28 (2H, m, H3 and H6), 7.07 (1H, dd, \(J_{3H-H} = 7.6\) and 8.0 Hz, H5), 4.24 ppm (3H, s, \(J_{1H-H} = 7.59\) (1H, d, \(J_{3H-H} = 7.6\) Hz, H2, H6)).

13C (H) NMR (DMSO-\(d_6\), 101 MHz): \(\delta = 184.4\) (C10), 154.1 (C12), 126.0 (C8 or C9), 122.3 (C4), 122.0 (C2 and C16), 120.5 (C5), 112.7 (C7), 109.5 ppm (C3).

HRMS (ESI\(^{+}\)): \(m/z\) calc. for C\(_{15}\)H\(_{12}\)N\(_2\)O\(_2\) [M + H\(^+\)] 238.0823; found 238.0852.

[Methyl indole-2-thionoester] (L6). L2 (250 mg 1.43 mmol), and Lawesson’s reagent (693 mg, 1.7 mmol) were added to a round-bottom flask and dissolved in toluene (35 mL). The mixture was refluxed for 6 days. The solution was cooled down and toluene was removed under reduced pressure. The crude was dissolved in ethyl acetate and extracted with an aqueous saturated solution of NaHCO₃, and then with brine, and dried over MgSO₄ and filtered. The combined organic phases were brought to dryness, leaving a bright yellow oil, which was purified by column chromatography (hexane/ethyl acetate, 80:20 \(v/v\)). The solvent was removed under vacuum to give a bright yellow solid (203 mg, 74%).

1H NMR (DMSO-\(d_6\), 400 MHz): \(\delta = 11.75\) (1H, s, H1), 7.67 (1H, d, \(J_{3H-H} = 8.4\) Hz, H4), 7.48 (1H, d, \(J_{3H-H} = 8.4\) Hz, H7), 7.28 (2H, m, H3 and H6), 7.07 (1H, dd, \(J_{3H-H} = 7.6\) and 8.0 Hz, H5), 4.24 ppm (3H, s, H11).

13C (H) NMR (DMSO-\(d_6\), 101 MHz): \(\delta = 184.4\) (C10), 154.1 (C12), 126.0 (C8 or C9), 122.3 (C4), 122.0 (C2 and C16), 120.5 (C5), 112.7 (C7), 109.5 ppm (C3).

HRMS (ESI\(^{+}\)): \(m/z\) calc. for C\(_{15}\)H\(_{12}\)N\(_2\)O\(_2\) [M + H\(^+\)] 238.0823; found 238.0852.

[Ethyl indole-2-thionoester] (L7). The procedure used to prepare L6 was followed with 250 mg (1.3 mmol) of L3 and Lawesson’s reagent (625 mg, 1.57 mmol) and refluxed for 6 days. The pure compound L7 was obtained as a bright yellow solid (220 mg, 81%).

1H NMR (DMSO-\(d_6\), 400 MHz): \(\delta = 11.69\) (1H, s, H1), 7.67 (1H, d, \(J_{3H-H} = 8.4\) Hz, H4), 7.49 (1H, d, \(J_{3H-H} = 8.4\) Hz, H7), 7.28 (2H, m, H3 and H6), 7.07 (1H, dd, \(J_{3H-H} = 7.6\) and 8.0 Hz, H5), 1.47 ppm (3H, t, \(J_{1H-H} = 7.1\) Hz, H11).

13C (H) NMR (DMSO-\(d_6\), 101 MHz): \(\delta = 201.0\) (C10), 138.3 (C2), 136.6 (C8 or C9), 126.9 (C8 or C9), 126.5 (C6), 122.6 (C4), 120.6 (C5), 119.2 (C7), 107.2 (C3), 58.4 ppm (C11).

HRMS (ESI\(^{+}\)): \(m/z\) calc. for C\(_{15}\)H\(_{12}\)N\(_2\)O \([\text{M} + \text{H}]^+\) 294.1048; found 294.1045.

[Ruthenium dimer \([\text{p-cym}]\text{RuCl(methyl indole-2-thionoester)}\)] (1). Ruthenium dimer \([\text{p-cym}]\text{RuCl(methyl indole-2-thionoester)}\) (70 mg, 0.11 mmol) and L6 (46 mg, 0.24 mmol) were placed in a 50 mL round-bottom flask and dissolved in 15 mL of dry dichloromethane. Once dissolved, 62 μL of dry triethylamine (0.46 mmol) were added to the mixture. The bright orange mixture was stirred under nitrogen overnight at 25 °C, until a brown solution was obtained. The crude was extracted with an aqueous solution of 0.1 M HCl (3×10 mL) and the combined organic phases were dried over MgSO₄ and filtered. The product was purified by column chromatography (ethyl acetate/hexane 80:20 \(v/v\)) and crystallised in dichloromethane/hexane to obtain a bright red solid (30 mg, 58%).
7.56 (1H, d, \( J_{HH} = 8.4 \) Hz, H16), 7.22 (1H, dd, \( J_{HH} = 6.8 \) and 8.0 Hz, H15), 7.07 (1H, s, H12), 6.96 (1H, dd, \( J_{HH} = 6.8 \) and 8.0 Hz, H14), 5.83 (2H, q, \( J_{HH} = 6.2 \) Hz, H3 and H4), 5.72 (1H, d, \( J_{HH} = 5.8 \) Hz, H6), 5.58 (1H, d, \( J_{HH} = 6.0 \) Hz, H5), 4.37 (3H, s, H2O), 2.53 (1H, sept, \( J_{HH} = 6.9 \) Hz, H8), 2.25 (3H, s, H1), 1.07 (3H, d, \( J_{HH} = 6.9 \) Hz, H10), 0.92 ppm (3H, d, \( J_{HH} = 6.9 \) Hz, H9).

\[^{13}C\] NMR (CDCl\(_3\), 101 MHz): \( \delta = 206.6 \) (C19), 152.0 (C11), 144.4 (C17 or C18), 130.9 (C17 or C18), 125.5 (C15), 124.5 (C13), 120.0 (C14), 116.8 (C16), 108.2 (C12), 103.0 (C7), 102.3 (C2), 85.9 (C6), 83.7 (C3 or C4), 83.2 (C3 or C4), 82.0 (C5), 60.1 (C20), 31.1 (C8), 23.0 (C10), 21.8 (C9), 19.0 ppm (C1).

HRMS (ESI\(^{+}\)) m/z calc. for \( C_{29}H_{39}NO \) Ru S [M - Cl\(^{-}\)]\(^{+}\) 426.0466; found 426.0452.

[p-cym]RuCl(phenyl indole-2-thionoester) (2).

Complex 2 was synthesised following the procedure of complex 1 with L9 (61 mg, 0.24 mmol). The pure compound 2 was obtained as a bright red solid (30 mg, 56%).

\[^{1}H\] NMR (CDCl\(_3\), 400 MHz): \( \delta = 7.58 \) (2H, m, H13, H16), 7.21 (1H, dd, \( J_{HH} = 6.8 \) and 8.0 Hz, H15), 7.08 (1H, s, H12), 6.96 (1H, dd, \( J_{HH} = 6.8 \) and 7.6 Hz, H14), 5.82 (2H, q, \( J_{HH} = 6.0 \) Hz, H3, H4), 5.71 (1H, d, \( J_{HH} = 5.9 \) Hz, H6), 5.56 (1H, d, \( J_{HH} = 5.9 \) Hz, H5), 4.74 (2H, q, \( J_{HH} = 7.1 \) Hz, H2O), 2.53 (1H, sept, \( J_{HH} = 6.9 \) Hz, H8), 2.25 (3H, s, H1), 1.55 (3H, t, \( J_{HH} = 7.0 \) Hz, H21), 1.07 (3H, d, \( J_{HH} = 7.2 \) Hz, H10), 0.92 ppm (3H, d, \( J_{HH} = 6.9 \) Hz, H9).

\[^{13}C\] NMR (CDCl\(_3\), 101 MHz): \( \delta = 205.8 \) (C19), 151.9 (C11), 144.5 (C17 or C18), 130.9 (C17 or C18), 125.3 (C15), 124.5 (C13), 119.9 (C14), 116.8 (C16), 108.0 (C12), 103.0 (C7), 102.1 (C2), 85.9 (C6), 83.7 (C3 or C4), 83.2 (C3 or C4), 82.0 (C5), 69.8 (C20), 31.1 (C8), 23.0 (C10), 21.8 (C9), 19.1 (C1), 14.4 ppm (C21).

HRMS (ESI\(^{+}\)) m/z calc. for \( C_{29}H_{39}NORuS [M - Cl\(^{-}\)]\(^{+}\) 440.0622; found 440.0614.

[p-cym]RuCl(cyclohexyl indole-2-thionoester) (3).

Complex 3 was synthesised following the procedure of complex 1 with L8 (62 mg, 0.24 mmol). The pure compound 3 was obtained as a bright red solid (39 mg, 65%).

\[^{1}H\] NMR (CDCl\(_3\), 400 MHz): \( \delta = 7.58 \) (2H, m, H13, H16), 7.21 (1H, dd, \( J_{HH} = 6.8 \) and 8.0 Hz, H15), 7.07 (1H, s, H12), 6.95 (1H, dd, \( J_{HH} = 6.8 \) and 7.6 Hz, H14), 5.80 (2H, m, H3, H4), 5.70 (1H, d, \( J_{HH} = 6.0 \) Hz, H6), 5.55 (1H, d, \( J_{HH} = 5.9 \) Hz, H5), 5.37 (1H, m, H1, H20), 2.52 (1H, sept, \( J_{HH} = 6.9 \) Hz, H8), 2.25 (3H, s, H1), 2.20 – 1.24 (10H, br. H, cyclohexane), 1.07 (3H, d, \( J_{HH} = 7.0 \) Hz, H21), 0.93 ppm (3H, d, \( J_{HH} = 7.0 \) Hz, H9).

\[^{13}C\] NMR (CDCl\(_3\), 101 MHz): \( \delta = 204.8 \) (C19), 151.8 (C11), 145.0 (C17 or C18), 130.8 (C17 or C18), 125.2 (C15), 124.4 (C13), 119.8 (C14), 116.8 (C16), 107.9 (C12), 103.0 (C7), 101.9 (C2), 85.9 (C6), 83.8 (C3 or C4), 83.3 (C3 or C4), 82.9 (C20), 82.0 (C5), 31.5 (CH\(_2\)cyclohexane), 31.1 (C8), 25.4 (CH\(_2\)cyclohexane), 23.7 (CH\(_2\)cyclohexane), 23.6 (CH\(_2\)cyclohexane), 22.9 (C10), 21.8 (C9), 19.0 ppm (C1).

HRMS (ESI\(^{+}\)) m/z calc. for \( C_{29}H_{39}NORuS [M - Cl\(^{-}\)]\(^{+}\) 494.1092; found 494.1088.

[p-cym]RuCl(phenyl indole-2-thionoester) (4).

Complex 4 was synthesised following the procedure of complex 1 with L9 (61 mg, 0.24 mmol). The pure compound 4 was obtained as a bright red solid (31 mg, 51%).
each well and incubated for 2 h at 37 °C and 5% CO₂ humidified atmosphere. All solutions were then removed and 100 µL of DMSO was added to each well in order to dissolve the purple formazan crystals. A Thermo Scientific Multiskan EX microplate photometer was used to measure the absorbance in each well at 570 nm.

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Supplementary materials

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