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Tetrazolium iridium complexes as potential antibacterial agents

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In this work, three iridium(III) tetrazolato complexes have been used in antibacterial, biofilm removal and for other bioactivities for the first time. Notably, these iridium(III) tetrazolato complexes with high antibacterial, especially, Ir-CF_3TAZ showed the best antimicrobial activity and the most effective hemolytic performance, which may pave the way to explore the value of the complexes for clinical applications in the future.

Introduction

Antimicrobial resistance has become one of the most significant challenges to global public health in the 21st century.[1] The World Health Organization (WHO) has identified that bacterial drug resistance can result in difficulties in treatment, prolonged duration of illness, increased mortality rates, and increased healthcare costs, which collectively impose a substantial economic burden on the global population.[2,3] Therefore, the shortage of antibacterial drug stockpiles highlights the urgent need to search for new antibacterial drugs.

In recent years, there has been a notable increase in research activity surrounding metal coordination compounds, largely due to their distinctive antimicrobial mechanisms and the vast range of activities they exhibit. The distinctive characteristics of transition metals have prompted a growing interest in the design and synthesis of complexes for therapeutic applications.[4] For example, cisplatin-based metal compounds have been widely employed in clinical tumour chemotherapy;[5] lithium has been demonstrated to be efficacious in the treatment of acute mania and bipolar disorder;[6] ruthenium complexes showed excellent antibacterial bioactive against *Staphylococcus aureus*;[7] and a number of bismuth compounds have been shown to be effective in combination therapy for *Helicobacter pylori* infections.[8] While metallic iridium(III) complexes have been identified as promising novel drug candidates. They have demonstrated antitumor and antiviral activities.[9–11] Some of these complexes have been demonstrated to exhibit antibacterial capacity.[12–14]

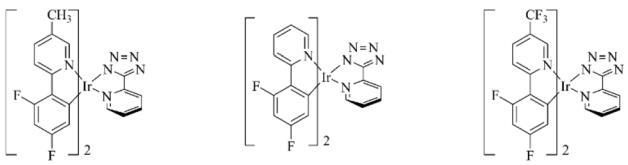

Fig. 1 Structures of the complexes.

In our previous work, we synthesized tetrazolium iridium complexes (Fig. 1).[15] This study systematically explored the antimicrobial properties of iridium(III) tetrazolato complexes for the first time. We hope that the findings presented in this paper will provide a theoretical foundation for the development of novel antibacterial agents and contribute to addressing the issue of bacterial drug resistance.

Results and discussions

Solid-phase antibacterial

Fig. 2 and Table 1 illustrate the performance and the sizes of the inhibition zones for the complexes at various concentrations. Compared to the positive control kanamycin, the antibacterial activity of Ir-CF_3TAZ was the most potent. In contrast, the antimicrobial effect of Ir-CH_3TAZ was minimal and showed no significant differences across different concentrations. Further results indicated that bacterial inhibition was dose-dependent.

Liquid-phase antibacterial

The inhibitory effects of the three complexes on bacterial growth diminished over the four-hour study period. Initially,

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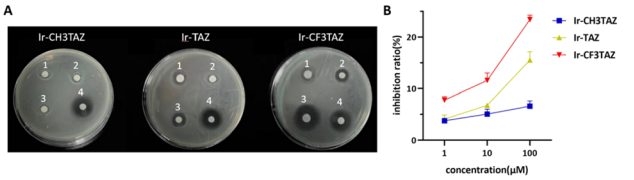

Fig. 2 The inhibitory effect of iridium(III) tetrazolato complexes on bacterial growth in a solid medium. (A) Inhibition ring performance. (1) 1 μM, (2) 10 μM, (3) 100 μM, (4) kanamycin (control) (B) bacteriostatic effect of media with complexes. The inhibition rate was treated with a blank group as control.

Table 1 The results of inhibition ring tests (diameter/mm, $X \pm s$, $n = 3$)

	Concentrations		
Complexes	1 μM	10 μM	100 μM
Ir-CH_3TAZ	5.1 ± 0.30	5.7 ± 0.18	5.4 ± 0.33
Ir-TAZ	—	5.4 ± 0.21	8.4 ± 0.35
Ir-CF_3TAZ	5.2 ± 0.15	10.5 ± 0.50	19.5 ± 0.48
Kanamycin	—	20.5 ± 0.66	—

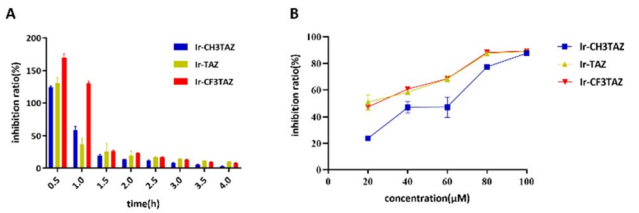

Fig. 3 The inhibitory effect of iridium(III) tetrazolato complexes on bacterial growth in a liquid medium. (A) The inhibition rate $= \frac{A_B - A_S}{A_B} \times 100\%$, A_B was the absorbance value of blank control bacterial solution, A_S was the absorbance value of sample bacterial solution, (B) the removal of iridium(III) tetrazolato complexes from biofilms.

within the first hour, each complex showed a significant inhibitory impact, with bacterial growth rates significantly lower than the bacteriostasis rates, resulting in inhibition rates exceeding 100%. Notably, Ir-CF_3TAZ exhibited the highest inhibition rate (Fig. 3A). However, during the later stages of the logarithmic growth phase, the inhibition rates for all complexes decreased considerably.

Bacterial biofilms are communities of bacteria that adhere to surfaces and produce a film-like substance, comprising polysaccharides, proteins, and lipids, which encapsulate the bacteria. Biofilm bacteria exhibit resistance to various antimicrobials, including traditional antibiotics. The ability to eradicate biofilms is a critical measure of antibacterial efficacy. Among the three complexes, Ir-CF_3TAZ and Ir-TAZ showed stronger bacteriostatic effects at low concentrations compared to Ir-CH_3TAZ. At high concentrations, however, the bacteriostatic effects of the three compounds were similar. At a concentration of 80 μM, the biofilm removal rate peaked at approximately 90% (Fig. 3B and Table 2).

Some metal(loid)-based antimicrobials interfere with essential metal uptake and substitute essential metals in metalloproteins,[16,17] as seen with the iridium center in Ir-CF_3TAZ. This complex might interact with amino acid residues on bacterial proteins, leading to function losing.[17] Additionally, as we all know, the presence of a trifluoromethyl group not only modifies the electron distribution on the pyridine ring and enhances the nitrogen atom's coordination ability, but also possibly promotes binding to bacterial proteins through electrostatic interactions, thereby amplifying the antibacterial effect.

The destruction of the bacterial cell membrane can lead to cell death.[18] The lipophilicity of 2-(2,4-fluorophenyl)pyridine allows Iridium(III) tetrazolato complexes to interact with the phospholipid bilayer of the bacterial cell membrane,[19–21] potentially inducing structural changes and increased permeability, which can compromise the membrane's integrity. The electron-withdrawing nature of the trifluoromethyl group may render the complex partially positively charged, facilitating its binding to negatively charged cell membrane molecules through electrostatic interactions[22] and further enhancing the antibacterial activity.[23–25]

Haemolysis

In vitro haemolysis tests were conducted to evaluate the potential toxicity of iridium(III) tetrazolato complexes on erythrocytes. As shown in Fig. 4, lower values indicate a reduced likelihood of causing haemolysis. The results indicated that these complexes had minimal haemolytic effects, as evidenced by low haemolysis values. However, it's important to note that these findings are confined to *in vitro* conditions, and further *in vivo* studies and clinical trials are necessary to fully assess the safety profile of these complexes.

Overall, considering the inhibition zone, inhibition rate calculations and biofilm eradication analysis, these three iridium(III) tetrazolato complexes with their excellent

Table 2 The results of inhibition ratio for three complexes at different concentrations

	Concentrations				
Complexes	20 μM	40 μM	60 μM	80 μM	100 μM
Ir-CH_3TAZ	23.8 ± 0.45	49.4 ± 0.06	51.5 ± 0.47	77.8 ± 0.26	88.3 ± 0.19
Ir-TAZ	47.6 ± 0.12	58.8 ± 0.01	69.8 ± 0.05	87.5 ± 0.55	89.2 ± 0.3
Ir-CF_3TAZ	47.7 ± 0.06	61.16 ± 0.03	70.1 ± 0.05	88.6 ± 0.09	89.5 ± 0.29

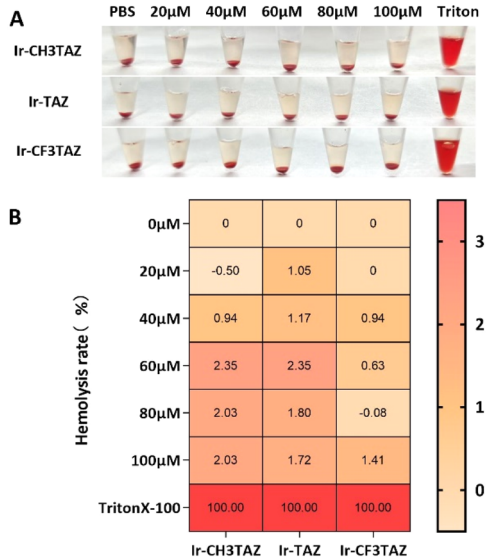

Fig. 4 Effect of iridium(III) tetrazolato complexes on *in vitro* haemolysis. (A) Haemolysis assay for Ir-CH_3TAZ, Ir-TAZ and Ir-CF_3TAZ. (B) Haemolysis was treated with 1% Triton X-100 as a positive control.

antimicrobial properties inevitably, show significant potential for future applications as antibacterial agents.

Conclusions

This preliminary study highlights the potential of iridium(III) tetrazolato complexes as antibacterial agents. Their antimicrobial activity, haemolytic potential and other biological properties were evaluated through inhibition zone analysis, inhibition rate calculations and haemolysis assessments. The development of metallic iridium-based drugs could offer a novel approach to combat bacterial infections, positioning them as a promising alternative to conventional antibiotics in future therapeutic strategies.

Experimental section

Inhibition ring tests

The bacteria used in this study were *Pseudomonas aeruginosa*. The experimental method was based on the filter paper slice method.[26] Briefly, these complexes were serially diluted tenfold from 100 μM, then added to each filter sheet discs which was then air-dried and be placed on the plate with the bacterial medium. The treated plates were placed in an incubator at 37 °C atmosphere for 24 h.

The inhibition of bacterial colony number

Different concentration of complexes was to be applied to the solid medium. Then bacteria in the logarithmic growth phase were diluted and spread on the medium plate after the complex solution had dried. After drying, the plates were placed in an incubator set at 37 °C overnight. The number of colonies was observed and determined.

Liquid antibacterial

Mixed one complex and bacterial solution each. Then incubated them in a shaker at 37 °C with constant shaking for 4 hours, following which the absorbance was measured at 600 nm using a spectrophotometer every 30 minutes.

Biofilm eradication analysis

Bacterial solutions were allowed to form biofilms in culture in a 24-well flat-bottom plate by incubation for 72 hours at 37 °C. Then they would be removed completely, and cultured with LB for 48 hours. Then different concentrations of the complex were added. And plate was shaken slowly on a shaker for 40 minutes and incubated for 24 hours at 37 °C. The non-adherent bacteria were removed by sterile ddH_2O and stained with crystal violet for the biofilm inhibition assay. The absorbance was measured at 562 nm.[27–30]

Haemolysis

The serum was removed by centrifuging the whole blood of mice, after which the obtained blood cells were washed three times with PBS and be diluted. Incubated complex solution with blood cells at 37 °C for one hour. After that, centrifuged samples and collected the supernatant. Then the absorption value at 562 nm was determined using an enzyme marker.

Erythrocytes treated with 1% Triton X-100 were applied as a positive control, with the release of haemoglobin in this group set to 100%. The haemolysis rate of each of the other samples was calculated based on the positive control.[31,32]

Ethical statement

All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Nanjing Medical University and approved by the Animal Ethics Committee of Nanjing Medical University.

Data availability

Data will be made available on request.

Author contributions

Yifei Lu: writing – original draft, data curation, formal analysis; Xiujuan Zhang: writing – original draft; Minmin Song: writing – review and editing; Hua Xie: investigation; Shuhua Chen: investigation; Yuyang Zhou: investigation, writing – review and editing; Junli Jia: conceptualization, writing – review and editing; Huamin Tang: writing – review, revision and editing, and funding acquisition.

Conflicts of interest

There are no conflicts to declare.

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