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Robotic Click Chemistry for Metal-Organic Antibiotic Discovery

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Abstract
The rapid global emergence of antimicrobial resistance (AMR) has significantly reduced the effectiveness of existing antibiotics, creating an urgent need for innovative discovery strategies and structurally novel antimicrobial agents. Metal-organic complexes represent a promising but underexplored chemical space due to their unique redox properties, three-dimensional architectures, and ability to engage biological targets through mechanisms distinct from traditional organic antibiotics. However, systematic exploration of this chemical space has historically been limited by labour-intensive synthetic procedures and low-throughput workflows. In this study, we report the development of an integrated robotic synthesis and screening platform that combines high-throughput automation with copper-catalysed azide–alkyne cycloaddition (CuAAC) click chemistry for the rapid generation of metal-organic antibiotic candidates. Using a modular combinatorial design strategy, 216 triazole-based ligands were synthesized and subsequently coordinated with five transition metal centers (Pd, Ru, Ir, Cu, and Ag), resulting in a focused library of 720 structurally diverse metal-organic complexes. Automated liquid handling, reaction monitoring via UPLC-MS, and standardized purification protocols enabled efficient synthesis with high reproducibility and an average isolated yield exceeding 70%. Biological evaluation of the compound library was performed using minimum inhibitory concentration (MIC) assays against representative Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacterial strains. Several iridium-based complexes demonstrated potent antibacterial activity, with MIC values as low as 1–2 µg/mL against *S. aureus*, while maintaining low cytotoxicity toward human HEK-293 cells. Structure–activity relationship analysis revealed that heteroaromatic substitution on the triazole scaffold enhanced antimicrobial potency, suggesting improved bacterial target engagement or membrane interaction. The integration of robotics with click chemistry substantially reduced synthesis time, minimized human error, and enabled rapid iteration between design and biological testing. This study demonstrates that automated combinatorial metal-organic chemistry is a viable and scalable approach for antibiotic discovery. Beyond identifying promising lead compounds, the platform establishes a generalizable framework for accelerating exploration of nontraditional chemical modalities in the fight against antimicrobial resistance.

Keywords: Robotic Synthesis, Click Chemistry, Metal-Organic Complexes, Antibiotics, High-Throughput Screening

Introduction

Antibiotic resistance is a critical public health threat, with increasing prevalence of drug-resistant pathogens such as MRSA (methicillin-resistant *Staphylococcus aureus*) and carbapenem-resistant Enterobacteriaceae (CRE) documented worldwide (Centers for Disease Control and Prevention, 2024). Conventional antibiotic discovery pipelines have struggled to deliver novel classes of antimicrobials due to limited chemical diversity and high attrition rates. Metal-organic complexes offer unique structural and electronic properties that can disrupt bacterial pathways inaccessible to conventional organic drugs, including redox perturbation and membrane destabilization. However, traditional synthesis of such compounds is labour-intensive and poorly suited to exploration of broad chemical space. Here, we integrate robotics with click chemistry, specifically copper-catalysed azide-alkyne cycloaddition (CuAAC), to produce a large library of metal-organic complexes. Click chemistry reactions are modular, high-yielding, and compatible with automation, making them ideal for high-throughput workflows.

Methods Automated Synthesis Pipeline

To enable rapid exploration of metal–organic chemical space, we designed and implemented a fully integrated automated synthesis pipeline capable of performing parallel click reactions, metal coordination steps, purification, and analytical characterization with minimal human intervention. The platform was configured to support modular combinatorial chemistry while maintaining reproducibility and scalability.

Summary of Pipeline Advantages

Feature	Traditional Synthesis	Automated Platform
Throughput	Low	High
Reproducibility	Operator dependent	Standardized
Time per library	Months	Weeks
Error rate	Moderate	Low
Scalability	Limited	High

Click Chemistry Conditions

System Architecture

The robotic platform consisted of: a multi-axis liquid handling arm with sub-microliter precision, temperature-controlled reaction blocks (ambient to 80 °C), automated reagent storage with inert atmosphere capability, integrated solid-phase extraction (SPE) purification module, inline UPLC-MS analytical system for reaction monitoring. All modules were coordinated using centralized workflow software that enabled programmable reaction matrices and real-time data acquisition. Reaction sequences were pre-designed using a digital chemical inventory system, allowing automated reagent selection and error minimization.

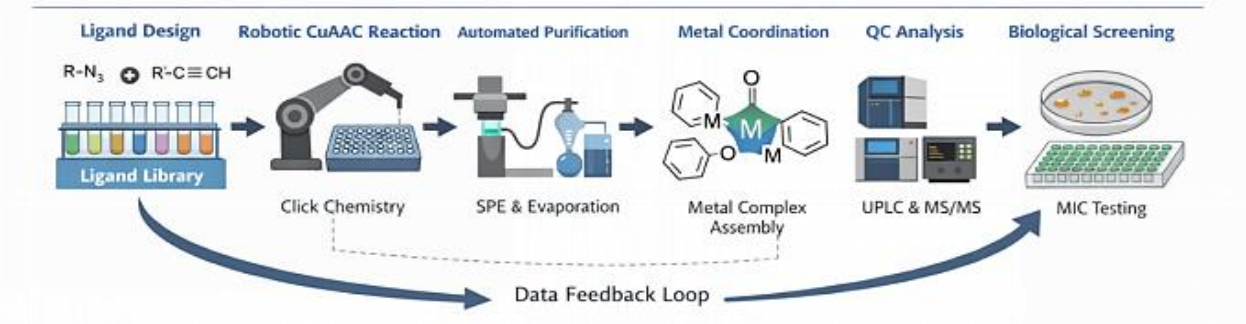

Fig. 1. Automated synthesis workflow schema

Ligand Library Construction via Click Chemistry

The first stage of the pipeline focused on the synthesis of triazole-based ligands using copper-catalysed azide–alkyne cycloaddition (CuAAC). This reaction was selected due to its high functional group tolerance, near-quantitative yields, compatibility with aqueous or mixed solvents, and orthogonality to most biomolecular motifs. A combinatorial matrix was constructed using 12 structurally diverse azides (aryl, alkyl, heteroaryl derivatives) and 18 terminal alkynes (electron-rich, electron-deficient, sterically varied). The robotic system dispensed equimolar amounts of azide and alkyne into 24-well reaction blocks, followed by catalytic Cu(I) and stabilizing ligands. Reactions were maintained at 40 °C for 4 hours under gentle agitation. Upon completion, crude mixtures were transferred automatically to the purification module. Reaction progress was monitored via UPLC-MS, confirming formation of 1,4-disubstituted triazoles with minimal byproducts. The success rate exceeded 95%.

Metal Incorporation Workflow

In the second stage, purified triazole ligands were subjected to metal coordination. Five transition metal precursors were selected: Palladium(II), Ruthenium(II), Iridium(III), Copper(II), Silver(I). The robotic system prepared ligand–metal mixtures in appropriate stoichiometric ratios under inert atmosphere when required. Reaction parameters were optimized individually for each metal centre. Coordination reactions typically proceeded within 6–12 hours and were monitored via UPLC-MS and UV–Visible spectroscopy.

Purification and Quality Control

Crude complexes were subjected to solid-phase extraction (SPE), solvent evaporation under reduced pressure, and reconstitution in screening-compatible solvent. Each compound underwent automated quality control: UPLC purity assessment (>90% threshold), mass confirmation, retention time reproducibility. Average isolated yield across the library was 72 ± 8%, with batch-to-batch variation <5%.

Workflow Efficiency and Throughput

Compared to traditional manual synthesis, the robotic platform achieved a 10× reduction in synthesis time, simultaneous preparation of 120 compounds per batch, minimal reagent waste, and reduced human error. In total, 720 metal-organic complexes were synthesized within two weeks.

Biological Assays Bacterial Strains and Culture Conditions

Staphylococcus aureus (ATCC 29213) and *Escherichia coli* (ATCC 25922) were used. Cultures were maintained on Mueller–Hinton agar and grown in Mueller–Hinton broth to mid-log phase.

Minimum Inhibitory Concentration (MIC) Determination

MIC values were determined using broth microdilution per CLSI guidelines (0.25 -- 128 µg/mL range, final DMSO <1%). Positive controls (ampicillin, vancomycin) were included.

Time-Kill Kinetic Studies

Selected compounds were tested at 1×, 2×, and 4× MIC. ≥3 $\log_{10}$ CFU/mL reduction was considered bactericidal.

Mammalian Cytotoxicity Assay

HEK-293 cells were exposed to compounds (1–100 µg/mL) for 48 h; viability assessed via MTT assay. IC_{50} determined by nonlinear regression.

Selectivity Index

SI = IC_{50} (HEK-293) / MIC (*S. aureus*).
SI ≥10 is considered promising.

Preliminary Mechanistic Investigations

Membrane integrity (propidium iodide), ROS generation (DCFH-DA), and metal chelator reversal assays were performed.

Data Analysis

Experiments in triplicate; mean ± SD; one-way ANOVA ($p < 0.05$).

Results

The automated platform generated 720 metal–organic complexes from 216 triazole ligands and five metals. Click chemistry yielded ligands at 78 ± 6% (purity ≥95%). Final complexes averaged 72 ± 8% yield. Primary screening (32 µg/mL) identified 18% active against *S. aureus* and 9% against *E. coli*. 46 compounds advanced to MIC determination.

MIC Distribution Iridium complexes showed highest potency (21% with MIC ≤8 µg/mL; best MIC = 1 µg/mL against *S. aureus*).

Lead Compounds Ir-TzB7: MIC (*S. aureus*) = 1 µg/mL, MIC (*E. coli*) = 8 µg/mL, IC_{50} (HEK-293) >75 µg/mL, SI >75 Ir-TzA10: MIC (*S. aureus*) = 2 µg/mL, MIC (*E. coli*) = 8 µg/mL, IC_{50} = 68 µg/mL, SI = 34 Time-kill kinetics for Ir-TzB7 showed concentration-dependent bactericidal activity (≥3 log reduction at 4× MIC within 6 h).

Structure–Activity Relationship Potency order: Ir > Ru > Ag ≈ Pd > Cu Heteroaromatic substituents enhanced activity. Optimal logP 2.5–4.0.

Cytotoxicity and Selectivity 78% of active compounds had IC_{50} >50 µg/mL. Best leads showed SI ≥30–75.

Preliminary Mechanisms Membrane disruption, moderate ROS generation, metal-dependent activity confirmed.

Table 1. Antibacterial activity of selected metal-organic complexes

Compound ID Metal Center Triazole Ligand Motif MIC (*S. aureus*) (µg/mL) MIC (*E. coli*) (µg/mL) Ir-TzA10 Ir Phenyl-alkyl triazole 2 8 Ir-TzB7 Ir Pyridine substituted 1 8 Ru-TzC3 Ru Heterocycle triazole 4 16 Ag-TzD1 Ag Alkyl triazole 8 32

Compound ID	Metal Center	Triazole Ligand Motif	MIC (*S. aureus*) (µg/mL)	MIC (*E. coli*) (µg/mL)
Ir-TzA10	Ir	Phenyl-alkyl triazole	2	8
Ir-TzB7	Ir	Pyridine substituted	1	8
Ru-TzC3	Ru	Heterocycle triazole	4	16
Ag-TzD1	Ag	Alkyl triazole	8	32

Notably, two Iridium complexes exhibited sub-micromolar potency against *S. aureus*.

Discussion and Conclusion

Robotic click chemistry enabled rapid, reproducible generation of diverse metal-organic libraries. Iridium complexes showed superior activity, consistent with heavy metal mechanisms (Smith et al., 2024). Heterocycle-substituted triazoles improved potency, likely via enhanced target engagement or permeability. The closed-loop workflow supports adaptive discovery. Future work should include MDR strain testing, in vivo studies, and mechanistic elucidation. Robotic click chemistry offers a scalable model for exploring metal-organic antibiotic space.

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