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Integrated Lead Profiling of SJ-7: Bactericidal Kinetics, MRSA Biofilm Activity, Cytotoxic Selectivity and Membrane Perturbation

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Abstract

A low minimum inhibitory concentration does not by itself establish that an antibacterial hit is suitable for progression. This study integrates bactericidal, kinetic, biofilm, mammalian-cell and membrane-oriented data for SJ-7, the leading member of a ten-compound thiazole-Schiff base hybrid series. SJ-7 showed MIC values of 2 µg/mL against methicillin-resistant *Staphylococcus aureus* (MRSA), 4 µg/mL against vancomycin-resistant *Enterococcus faecium* (VRE), 16 µg/mL against multidrug-resistant *Escherichia coli* and KPC-producing *Klebsiella pneumoniae*, and 64 µg/mL against multidrug-resistant Pseudomonas aeruginosa. Its MBC was two-fold above the MIC for all five organisms. At 24 h, viable counts against MRSA declined by 2.10, 4.00 and 5.20 log10 CFU/mL at 1×, 2× and 4× MIC, respectively; corresponding VRE reductions were 1.78, 3.40 and 4.50 log10 CFU/mL. In an MRSA biofilm assay, SJ-7 produced 67.5% biomass inhibition at 2× MIC, exceeding SJ-4 and SJ-9. HEK-293 testing gave a CC50 of 164 µg/mL and preliminary selectivity indices of 82 against MRSA and 41 against VRE. Membrane-oriented measurements increased with concentration, reaching 360 RFU permeability and 80% depolarisation at 4× MIC. Collectively, the data support SJ-7 as the strongest progression candidate in the series and are consistent with a membrane-perturbation component to its phenotype.

Keywords: SJ-7, MRSA, time-kill kinetics, biofilm, cytotoxicity, selectivity index, membrane permeability, antibacterial lead

1. Introduction

Antibacterial discovery requires more than identifying the compound with the lowest minimum inhibitory concentration (MIC). MIC is a static growth-inhibition endpoint: it does not show how quickly bacteria are killed, whether killing is sustained, whether activity is retained in a biofilm-associated state, or whether the active concentrations are separated adequately from mammalian-cell toxicity. These distinctions are particularly important for resistant pathogens, where reduced drug exposure, target modification, efflux, physiological tolerance and biofilm formation can create very different responses to the same chemical series (Blair *et al*., 2015; Darby *et al*., 2023) [2, 4].

Time-kill analysis adds a dynamic dimension by following viable counts during exposure. Two compounds can share the same MIC yet differ substantially in the rate, depth and durability of killing. MBC measurements provide another complementary endpoint by assessing viable recovery after exposure. Biofilm assays address a distinct biological state in which matrix material, nutrient gradients and slow-growing subpopulations can reduce responsiveness even without additional genetic resistance (Flemming *et al*., 2016; Hall & Mah, 2017) [5, 6]. For lead selection, these assays should be interpreted together rather than treated as interchangeable measures of activity.

Host-cell selectivity provides a second major filter. A compound that disrupts bacterial membranes may appear potent while also damaging mammalian cells at nearby concentrations. MTT-type assays offer an early estimate of the concentration range affecting cellular metabolic activity and can be compared with antibacterial potency using a preliminary selectivity index (Mosmann, 1983) [8]. Such

indices are useful for ranking analogues, but they do not establish *in-vivo* safety or tolerability.

SJ-7 emerged as the leading member of a ten-compound thiazole-Schiff base hybrid series on the basis of MIC and MBC data. The present paper focuses specifically on the deeper profile of this lead. It integrates time-kill data against MRSA, VRE and MDR E. *coli*; MRSA biofilm measurements; HEK-293 cytotoxicity; and membrane-permeability/depolarisation observations. The aim is to determine whether SJ-7 remains the preferred progression candidate when potency is evaluated alongside pharmacodynamic, selectivity and mechanism-oriented evidence.

2. Materials and Methods

2.1 Lead selection and antibacterial endpoints

SJ-7 was selected for advanced profiling because it had the lowest MIC in the parent series against all five test organisms: MRSA ATCC 43300, vancomycin-resistant *Enterococcus faecium* 700221, MDR *Escherichia coli* EC-17, KPC-producing Klebsiella pneumoniae KPC-3 and MDR Pseudomonas aeruginosa PA-21. MIC determination used serial two-fold broth microdilution in accordance with the study records and standard dilution-testing principles (Wiegand *et al*., 2008) [13]. MBC values were obtained by subculture from non-growing wells onto drug-free agar. The MBC/MIC relationship was used descriptively rather than as a substitute for time-kill evidence.

2.2 Time-kill analysis

Time-kill measurements were available for SJ-7 against MRSA, VRE and MDR E. *coli* at 1×, 2× and 4× MIC. The source dataset records viable counts over the exposure period in triplicate. For the present analysis, the principal comparison was the 24-h viable count and the corresponding reduction from the starting bacterial burden. The kinetic pattern was interpreted in terms of concentration dependence and organism-specific depth of killing. Because the study provides an *in-vitro* pharmacodynamic profile, no clinical dosing inference was made.

2.3 MRSA biofilm assay

Biofilm data compared SJ-4, SJ-7 and SJ-9 at 0.25×, 0.5×, 1× and 2× their respective MICs. The recorded endpoints were biomass inhibition (%) and viable log10 CFU/mL, each with three replicate observations. Biomass inhibition and viable burden were treated as complementary but distinct endpoints: reduced crystal-violet-type biomass does not necessarily imply equivalent killing of biofilm-associated cells. The available dataset did not include a complete untreated viable-count baseline for calculation of an absolute biofilm kill reduction, so viable counts are reported directly.

2.4 Cytotoxicity and selectivity

Cytotoxicity was assessed in HEK-293 cells after a 24-h MTT-type exposure. The source dataset provides CC50 values for all ten analogues. A preliminary selectivity index (SI) was calculated as CC50 divided by the MIC for the relevant organism. The analysis focuses on MRSA and VRE because these were the organisms against which SJ-7 showed the strongest antibacterial potency. The MTT endpoint was interpreted as an early cell-viability screen rather than a definitive toxicology measure.

2.5 Membrane-oriented measurements and reporting scope

Mechanism-oriented observations were available for SJ-4, SJ-7 and SJ-9 at 0.5×, 1×, 2× and 4× MIC. The dataset reports a permeability signal in relative fluorescence units (RFU) and membrane depolarisation (%), each as mean ± SD from three observations. These measurements were used to test whether the leading analogues showed a concentration-dependent membrane perturbation phenotype. Docking scores were also present in the workbook, but because those scores varied with the experimental concentration multiple, they were not treated as a conventional dose-response measure of ligand binding.

The numerical workbook used for manuscript preparation was supplied as the student's final dataset, but its internal README describes the records as synthetic demonstration data. That provenance conflict cannot be resolved from the manuscript alone. The source also contains unresolved compound-formula/substituent discrepancies. Accordingly, the values are analysed exactly as supplied and the conclusions are restricted to comparative lead profiling. Original laboratory notebooks, instrument files and the provenance statement should be reconciled before submission as primary experimental evidence.

3. Results

3.1 MIC, MBC and time-kill profile of SJ-7

SJ-7 retained the strongest antibacterial profile in the parent series, with MIC values of 2 µg/mL against MRSA, 4 µg/mL against VRE, 16 µg/mL against MDR E. *coli* and K. *pneumoniae*, and 64 µg/mL against P. aeruginosa. The MBC was two-fold above the MIC for each organism, giving MBC values of 4, 8, 32, 32 and 128 µg/mL, respectively.

The time-kill data showed a clear concentration-response relationship. Against MRSA, 24-h viable counts were 4.10, 2.20 and 1.00 log10 CFU/mL at 1×, 2× and 4× MIC, corresponding to reductions of 2.10, 4.00 and 5.20 log10 CFU/mL. Against VRE, the respective 24-h reductions were 1.78, 3.40 and 4.50 log10 CFU/mL. MDR E. *coli* showed the shallowest response, with reductions of 1.16, 2.20 and 2.91 log10 CFU/mL at the same concentration multiples. Thus, the kinetic advantage of higher exposure was evident in all three organisms, but the depth of killing remained organism dependent.

Table 1: SJ-7 MIC, MBC and 24-h time-kill summary

Organism	MIC (µg/mL)	MBC (µg/mL)	24-h CFU/mL at 1× / 2× / 4× MIC	24-h log10 reduction at 1× / 2× / 4× MIC
MRSA	2	4	4.10 / 2.20 / 1.00	2.10 / 4.00 / 5.20
VRE	4	8	4.42 / 2.80 / 1.70	1.78 / 3.40 / 4.50
MDR E. *coli*	16	32	5.04 / 4.00 / 3.29	1.16 / 2.20 / 2.91

3.2 MRSA biofilm response

SJ-7 also led the selected-comparator set in the MRSA biofilm assay. At 1× MIC, mean biomass inhibition was 47.0% for SJ-7, compared with 34.77% for SJ-4 and 41.77% for SJ-9. At 2× MIC, inhibition increased to 67.5% for SJ-7,

while SJ-4 and SJ-9 reached 48.1% and 59.2%, respectively. Viable counts followed the same rank order. At 2× MIC, the mean viable burden was 3.40 log10 CFU/mL for SJ-7, compared with 4.31 for SJ-4 and 3.79 for SJ-9. These observations indicate that the potency advantage of SJ-7 was retained under the biofilm-related conditions represented in the dataset.

Table 2: MRSA biofilm response at 1× and 2× MIC (mean ± SD; n = 3)

Compound	Concentration	Biomass inhibition (%)	Viable log10 CFU/mL
SJ-4	1× MIC	34.77 ± 1.35	5.16 ± 0.03
SJ-4	2× MIC	48.10 ± 1.40	4.31 ± 0.03
SJ-7	1× MIC	47.00 ± 1.40	4.70 ± 0.03
SJ-7	2× MIC	67.50 ± 1.40	3.40 ± 0.03
SJ-9	1× MIC	41.77 ± 1.35	4.89 ± 0.03
SJ-9	2× MIC	59.20 ± 1.40	3.79 ± 0.03

3.3 Cytotoxicity and preliminary selectivity

The HEK-293 dataset materially changed the interpretation of antibacterial potency. SJ-7 had a CC50 of 164 µg/mL. When related to its MIC, this gave an SI of 82 against MRSA and 41 against VRE; the SI was 10.25 against both MDR E. *coli* and K. *pneumoniae* and 2.56 against P. aeruginosa. SJ-6 showed a lower MRSA SI of 37, whereas SJ-9 combined a strong MRSA MIC of 4 µg/mL with a lower CC50 of 72 µg/mL, yielding an MRSA SI of 18. The comparison demonstrates that potency alone would over-rank SJ-9 relative to SJ-7.

Table 3: HEK-293 cytotoxicity and preliminary MRSA selectivity of selected analogues

Compound	CC50 (µg/mL)	MRSA MIC (µg/mL)	MRSA SI	Lead interpretation
SJ-4	118	8	14.75	Comparator
SJ-6	148	4	37.00	Secondary lead
SJ-7	164	2	82.00	Primary lead
SJ-9	72	4	18.00	Potent but cytotoxicity-limited

3.4 Membrane perturbation phenotype

Permeability and depolarisation increased with concentration for all three compounds tested, but SJ-7 generated the largest responses. At 4× MIC, permeability was 360.0 ± 3.0 RFU and depolarisation was 80.0 ± 0.8% for SJ-7. Corresponding values were 269.0 ± 3.0 RFU and 54.8 ± 0.8% for SJ-4, and 334.0 ± 3.0 RFU and 72.8 ± 0.8% for SJ-9. The same ordering was present at 1× and 2× MIC. This pattern is consistent with a concentration-dependent membrane-perturbation component to the phenotype, although it does not identify a molecular target or establish whether membrane changes are primary or downstream events.

Table 4: Membrane-oriented measurements at 4× MIC (mean ± SD; n = 3)

Compound	Permeability (RFU)	Depolarisation (%)	Docking score as supplied
SJ-4	269.0 ± 3.0	54.8 ± 0.8	-9.06
SJ-7	360.0 ± 3.0	80.0 ± 0.8	-10.60
SJ-9	334.0 ± 3.0	72.8 ± 0.8	-10.16

4. Discussion

The integrated dataset strengthens the case for SJ-7 because its lead status is supported by several independent dimensions rather than by MIC alone. The compound combined the lowest MICs in the parent series with a two-fold MBC/MIC relationship, concentration-dependent time-kill activity, the strongest MRSA biofilm response among the selected analogues, a comparatively wide HEK-293 selectivity window and the largest membrane-oriented signals. This convergence is the main reason SJ-7 is a more defensible progression candidate than an analogue chosen only for a favourable single endpoint.

The time-kill profile is particularly informative. Against MRSA, increasing exposure from 1× to 4× MIC deepened the 24-h reduction from 2.10 to 5.20 log10 CFU/mL. VRE showed the same qualitative response, reaching a 4.50-log reduction at 4× MIC. These results indicate that the inhibitory endpoint translated into substantial loss of viable bacteria at higher concentrations. By contrast, the maximum 24-h reduction against MDR E. *coli* was 2.91 log10 CFU/mL. The weaker Gram-negative kinetic response is consistent with the spectrum seen in the MIC matrix and may reflect lower intracellular exposure, greater efflux or different target access, although the present experiments cannot distinguish those possibilities (Nikaido, 2003; Li *et al*., 2015) [9, 7].

The biofilm findings provide an additional test of lead robustness. Biofilms are not simply planktonic cultures at higher density; matrix structure, nutrient limitation and heterogeneous metabolic states can alter antimicrobial responsiveness (Flemming *et al*., 2016; Hall & Mah, 2017) [5, 6]. SJ-7 retained the strongest rank at both 1× and 2× MIC, and the difference was evident in both biomass inhibition and viable burden. This is encouraging because it suggests that the lead advantage is not confined to a conventional planktonic endpoint. Nevertheless, the assay should not be described as biofilm eradication. The source data do not provide a complete untreated viable-count baseline, and biomass reduction cannot be equated automatically with killing of established biofilm cells.

Cytotoxicity is essential to interpreting the apparent potency of membrane-active or amphipathic chemical matter. SJ-9 illustrates why this matters: its MRSA MIC of 4 µg/mL is attractive, but a CC50 of 72 µg/mL narrows the preliminary MRSA SI to 18. SJ-7, by contrast, combines greater antibacterial potency with a higher CC50, producing an SI of 82. The result does not demonstrate clinical safety. HEK-293 MTT is an early screening assay, and metabolic conversion can be influenced by compound colour, redox behaviour or other assay interference (Mosmann, 1983) [8]. Even so, the wider separation between antibacterial and cell-viability endpoints is a meaningful lead-selection advantage.

The membrane-oriented measurements are consistent with, but do not prove, a membrane-related contribution to the antibacterial phenotype. SJ-7 produced the strongest permeability and depolarisation signals at each comparable concentration, and the magnitude of both responses increased with exposure. The ordering SJ-7 > SJ-9 > SJ-4 broadly parallels antibacterial and biofilm performance. Such concordance supports a testable hypothesis that membrane perturbation contributes to activity. It does not establish that the membrane is the primary molecular target,

because permeability changes and depolarisation can occur as downstream consequences of lethal cellular damage.

The docking values require even greater restraint. In the source workbook, the docking score changes as the experimental concentration multiple changes. Conventional docking scores are normally associated with a ligand-target pose and scoring protocol rather than with an experimental concentration-response series. The values may reflect separately entered computations or a data-organisation issue, but they cannot be treated as evidence that binding affinity becomes stronger simply because the tested concentration increases. Before any target-level mechanism is claimed, the target identity, docking software, scoring function, input structures and original output files should be checked.

The next experimental stage should therefore be designed around confirmation and discrimination. First, SJ-7 should be re-tested using independently prepared and fully verified material, with comparator antibiotics assayed in parallel. Second, time-kill experiments should be repeated with sufficient independent biological replicates to estimate variability and examine regrowth. Third, the Gram-negative spectrum should be investigated using accumulation, outer-membrane permeability and efflux-modulation experiments. Finally, membrane findings should be supported by orthogonal methods such as membrane-integrity controls, microscopy, macromolecular-synthesis assays, resistant-mutant analysis or target-specific biochemical work.

The series also provides an optimisation framework. SJ-6 and SJ-9 remain useful secondary analogues because they preserve strong Gram-positive activity while differing in selectivity and membrane response. Future chemistry should aim not merely to lower MIC, but to preserve the selectivity advantage of SJ-7 while improving Gram-negative exposure and avoiding the narrower cytotoxicity window observed for SJ-9. This multi-parameter strategy is consistent with modern antibacterial discovery, where potency, selectivity, bacterial exposure and resistance resilience must be improved together (Silver, 2011; Tommasi *et al*., 2015; Brown & Wright, 2016) [11, 12, 3].

Taken as a whole, the data demonstrate the value of an integrated lead-profiling sequence. MIC identifies inhibition; MBC and time-kill describe viable recovery and kinetics; biofilm assays challenge the lead in a more recalcitrant bacterial state; cytotoxicity provides an early host-cell filter; and membrane assays generate a mechanistic hypothesis. When those endpoints converge, lead selection becomes more robust. In the supplied dataset, the convergence consistently favours SJ-7.

The MBC and time-kill results should be read together. A low MBC/MIC ratio can suggest a stronger killing relationship, but the ratio alone does not describe the speed of killing or the possibility of late regrowth. In this dataset, the kinetic curves add the information that matters for progression: the depth of viable-count reduction increased markedly as the exposure rose from 1× to 4× MIC, and the magnitude of that effect differed by organism. This distinction is important for future pharmacodynamic work because the same nominal MBC/MIC relationship can conceal very different time-dependent responses. Confirmatory experiments should therefore preserve the full time course rather than rely only on a 24-h endpoint.

The biofilm data also point to a useful experimental refinement. The current endpoint is best described as inhibition of MRSA biofilm biomass accompanied by lower viable burden under the tested conditions. A journal-level antibiofilm claim would be stronger if experiments separated prevention of initial biofilm formation from treatment of an established mature biofilm, because those are biologically different questions. Including untreated viable-count baselines, a recognised antibiofilm comparator and an orthogonal metabolic or microscopy endpoint would help determine whether SJ-7 primarily limits biomass accumulation, kills biofilm-associated cells, or does both. Such work would also clarify whether the membrane phenotype observed in planktonic assays remains relevant within the biofilm state.

Selectivity should likewise be expanded beyond a single cell line before translational conclusions are drawn. HEK-293 data provide a useful first filter, but a broader panel could reveal cell-type-specific toxicity or a narrower window than the initial SI suggests. Haemolysis testing would be especially informative if membrane perturbation remains the leading mechanistic hypothesis, because red-cell membrane damage can expose non-specific amphipathic activity that is not evident in one metabolic cell-viability assay. These follow-up studies would not replace the present ranking; rather, they would test whether the favourable SJ-7 profile is robust when the biological system becomes more demanding.

Independent replication should be a priority in the next phase. The present triplicate summaries support an internal ranking, but journal-level inference would be stronger if the key time-kill, biofilm, cytotoxicity and membrane experiments were repeated with independently prepared SJ-7 batches and separate biological runs. That design would allow uncertainty to be estimated more meaningfully and would show whether the apparent advantage of SJ-7 is stable across experimental occasions rather than confined to one dataset.

5. Conclusion

SJ-7 remained the preferred lead when its low MIC was tested against broader progression criteria. It produced concentration-dependent killing of MRSA and VRE, retained the strongest activity in the MRSA biofilm comparison, showed the most favourable preliminary selectivity among the most active analogues, and generated the largest membrane-permeability and depolarisation responses. The evidence supports prioritising SJ-7 for repeat confirmation, complete structural verification, Gram-negative exposure studies and deeper mechanism work. The membrane data are hypothesis-generating rather than target-defining, and the HEK-293 result is an early safety screen rather than a toxicology conclusion. Most importantly, the unresolved provenance statement and structural-record discrepancies in the source workbook must be reconciled before the numerical findings are submitted as independently validated primary experimental evidence.

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