**Screening Report COMPOUND PLATFORM**

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**Report Initials:** AD

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**Type: Primary Screen & Secondary Screen**

Antimicrobial activity against health-care associated pathogens

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# [1] Preliminary Information

[1.1] Introduction/Status

All of your compounds are routinely screened for antimicrobial activity by our cooperation partner in Australia, the “Community for Open Antimicrobial Drug Discovery (CO-ADD)”. Here, compounds are tested against seven key pathogens for antimicrobial activity.

Amongst the 13 tested compounds, one compound, namely **X5247**, showed activity against Gram-positive bacterium *Staphylococcus aureus* in the primary screening. However, this compound was also found to be toxic.

## [1.2] Information about the Screen

For antimicrobial activity screenings, the compounds are screened in cooperation with the Community for Open Antimicrobial Drug Discovery (CO-ADD) in Brisbane, Australia. Therefore, seven different microorganisms are used as a basis for an experiment-based selection of attractive model systems for the development of new antimicrobials. These microorganisms include different so-called ‘ESKAPE’ pathogens, which are responsible for two-thirds of all hospital acquired infections. These pathogens are the Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii.* Furthermore, theGram-positive multi-resistant *Staphylococcus aureus* (MRSA) and the yeasts *Cryptococcus neoformans* and *Candida albicans* are being tested*.*

During primary screenings, the compounds are tested at a single concentration, namely 32 µM. Compounds are considered active in the primary screening process when the inhibition of bacterial growth is 80% or higher. Inactive compounds showed inhibition values below 50% and are not further tested (n.t.) for toxic activity.

All active compounds are then tested during hit confirmation, where the lowest concentration at which inhibition of bacterial growth occurs, the so-called minimal inhibitory concentration (MIC), is determined. In parallel, cytotoxicity against human HEK293 cells and haemolytic activity against human red blood cells are evaluated to exclude toxic effects of the compounds. Compounds are declared as a hit in the secondary screening process when the MIC is less or equal 10µM (16 µg/mL).

For toxicity, compounds with a CC50/HC10 (concentration at 50% cytotoxicity/ concentration of 10% haemolytic activity) of 20µM or lower are considered active or toxic respectively. A therapeutic index cannot be calculated since the maximum tested concentration is the same for toxicity and antimicrobial activity. Hence, all samples with toxic activity are sorted out.

## [1.3] Model system

Gram-negative bacteria: *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii*

Gram-positive bacterium: *Staphylococcus aureus*

Fungi: *Candida albicans, Cryptococcus neoformans*

# [2] Compounds and Results

***Table 1.*** Antimicrobial and toxic activity

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | **Antimicrobial activity**  **Primary Screening (PS): Maximum Inhibition [%]**  **Hit Confirmation (HC): Minimal inhibitory concentration *MIC* [µM]** | | | | | | |  | |
| **Gram-negative bacteria** | | | | **Gram-positive bacteria** | **Fungi** | |
| **X number** | **Structure** | ***E. coli*** | ***K. pneumoniae*** | ***A. baumannii*** | ***P. aeruginosa*** | ***S. aureus*** | ***C. albicans*** | ***C. neoformans*** | ***Cytotoxic activity***  ***CC50*** | ***Haemolytic activity***  ***HC10*** |
| X5247 |  | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS  (101.0%  101.2%) | PS (<50%) | PS (<50%) | TOXIC  5.40 µM  5.67 µM | > 20 µM |
| HC  10 µM |
| X5248 |  | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | n.t. | n.t. |
| X5249 |  | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | n.t. | n.t. |
| X6751 |  | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | n.t. | n.t. |
| X6752 |  | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | n.t. | n.t. |
| X6753 |  | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | n.t. | n.t. |
| X6754 |  | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | n.t. | n.t. |
| X6755 |  | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | n.t. | n.t. |
| X6756 |  | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | n.t. | n.t. |
| X7095 |  | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | n.t. | n.t. |
| X7096 |  | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | n.t. | n.t. |
| X7097 |  | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | n.t. | n.t. |
| X7098 |  | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | PS (<50%) | n.t. | n.t. |

# [3] Explanation of Results

One of the compounds, namely **X5247**, showed activity against the Gram-positive bacterium *Staphylococcus aureus* in the Primary Screening at a single concentration of 32 µM. In the subsequent Secondary Screening/ Hit Confirmation, the compound X5247 was able to inhibit the growth of *Staphylococcus aureus* at a minimal concentration of 10 µM. However, it proved to be toxic against HEK293 cells below concentrations of approximately 5 µM, which makes it unsuitable for further development as a therapeutic agent.

The compounds that did not show any activity in the Primary Screening, were not further tested with regard to toxicity (n.t.).