CR22DK-S18G Breakthrough: Revealing a novel antimicrobial peptide with rifampicin and isoniazid-like potency against *Mycobacterium tuberculosis* - a comprehensive exploration from synthesis to immune modulation and selective action

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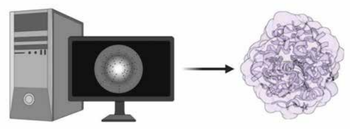
Introduction

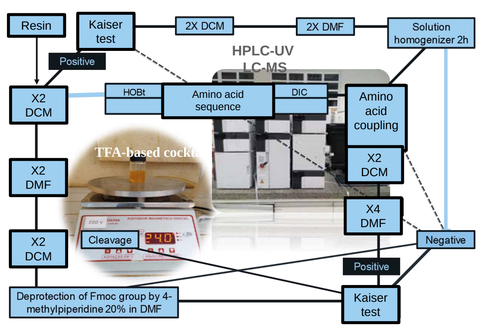
According to WHO, 1.6 million people died from Tuberculosis (TB) in 2021, making TB the second-leading infectious killer after COVID-19.[1,2,3] In recent years, antimicrobial peptides (AMPs) with promising anti-TB activity have been identified. B1CTcu5 is a peptide amide, isolated in 2014 from the skin secretion of the Indian toad *Clinotarsus curtipes*, and showing initial MIC values of 12.5 µg/mL against *Mycobacterium tuberculosis* (MTB).[4] The objective of the present work was to generate a library of B1CTcu5 analogues by replacing Cys[15] and Cys[21] with Ala, Ser and/or Lys, and to evaluate their activity against MTB and cytotoxicity.

Materials and Methods

1. In silico prediction and molecular docking

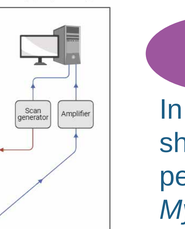


2. Solid Phase Peptide Synthesis (SPSS)



3. Antimicrobial activity and cytotoxicity

Mycobacterium tuberculosis



Fluorescence Microplate Reader

4. AMES test

Salmonella enterica subsp. *enterica* serovar Typhimurium

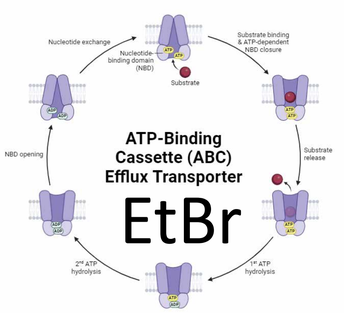
TA100
TA98
TA102
TA97a

5. Morphological structure interaction

Scanning Electron Microscopy (SEM)

6. Intracellular AMP transport

7. Efflux pumps inhibition



8. Galleria mellonella Infection model

Toxicity

Infection/Treatment

Histology

Conclusions

In conclusion, B1CTcu5 analogs show promise as a potential new peptide-based therapy against *Mycobacterium tuberculosis*, specially CRDK-s18G.

Results and Discussions

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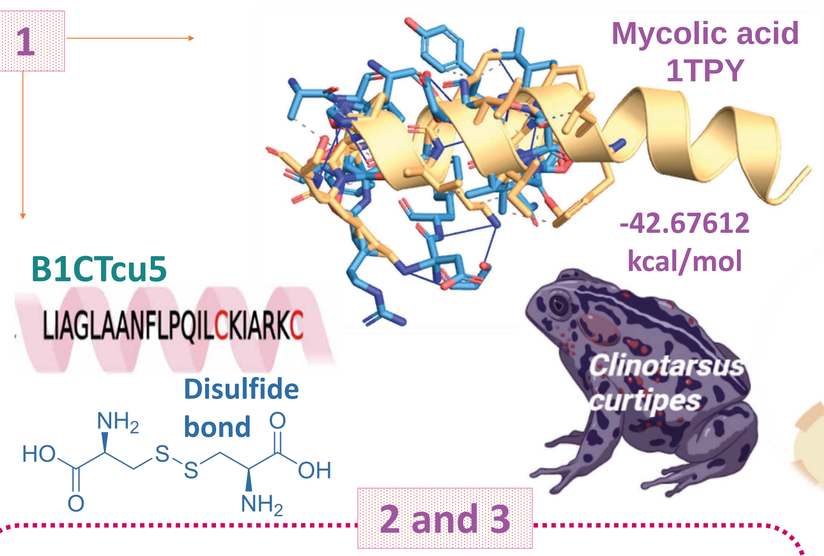


2 and 3

Table 1. Cysteine replacement - Minimal inhibitory concentration of B1CTcu5 peptide analogs against *Mycobacterium tuberculosis* strains and cytotoxic activity on murine macrophages. Peptide analogs was evaluated at concentrations between 2 to 250 µg/mL.

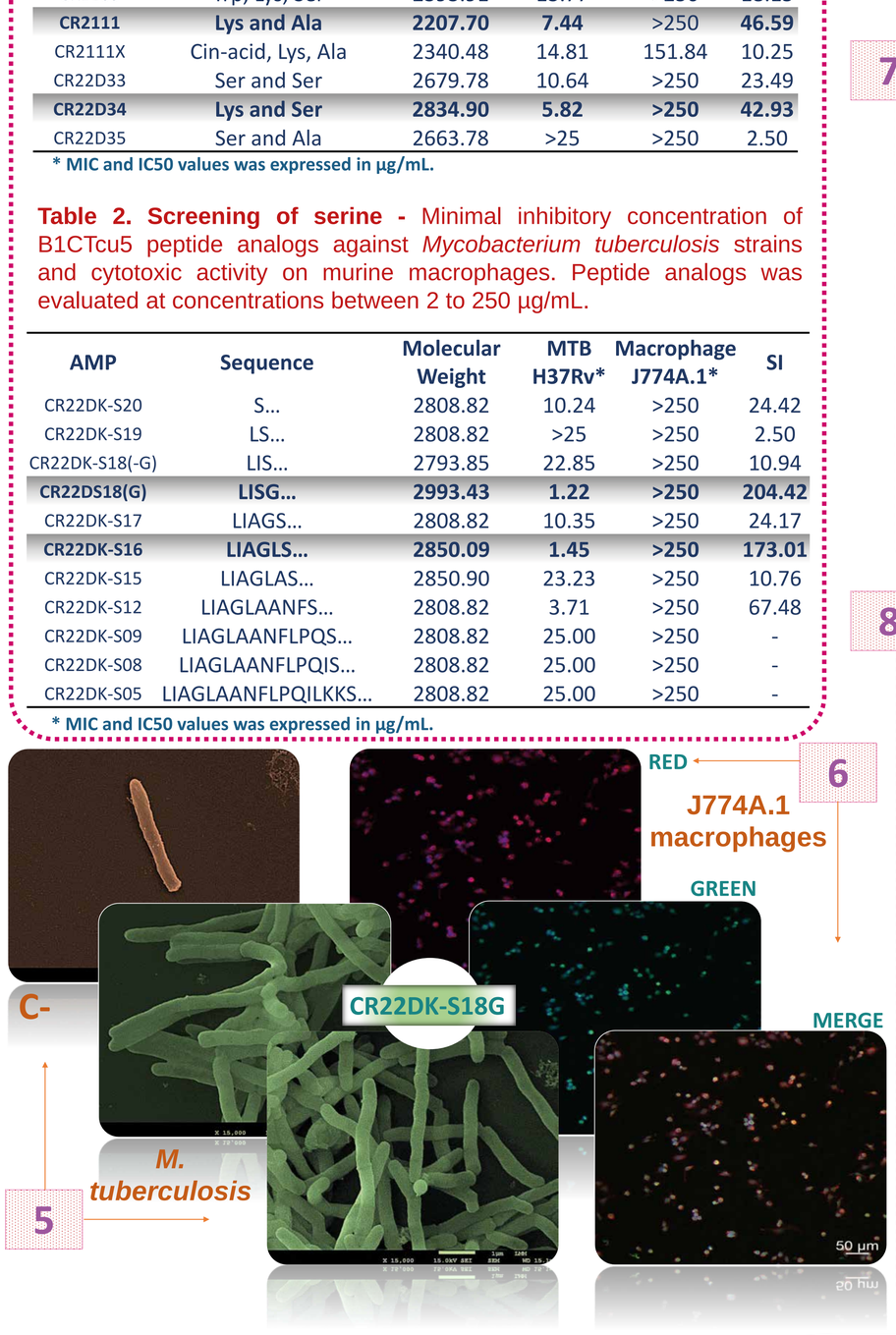
AMP	Sequence	Molecular Weight	MTB H37Rv	Macrophage J774A.1	SI
B1CTcu5	**linear**	**2711.92**	**12.27**	**>250**	**20.38**
W-B1CTcu5	W-linear	2898.13	3.24	>250	77.18
cB1CTcu5	cyclic	2709.90	13.31	>250	18.79
cW-B1CTcu5	Trp cyclic	2896.11	4.68	125	26.70
CR2101	Lys	2305.89	14.02	124.34	8.87
CR2102	Ala	2197.70	31.87	178.31	5.60
CR2103	Lys and Ala	2248.80	13.31	>250	18.78
CR2104	**Trp, Ser, Ser**	**2409.91**	**7.42**	**>250**	**33.69**
CR2105	Trp, Ala	2396.49	10.73	21.36	1.99
CR2106	Trp, Ser	2296.76	22.31	>250	11.21
CR2107	**Trp, Lys, Ser**	**2451.01**	**7.21**	**>250**	**34.67**
CR2108	Trp, Ser, Lys	2451.01	17.73	>250	14.10
CR2109	Trp, Lys, Ser	2393.91	13.77	>250	18.15
CR2111	**Lys and Ala**	**2207.70**	**7.44**	>250	**46.59**
CR2111X	Cin-acid, Lys, Ala	2340.48	14.81	151.84	10.25
CR22D33	Ser and Ser	2679.78	10.64	>250	23.49
CR22D34	**Lys and Ser**	**2834.90**	**5.82**	**>250**	**42.93**
CR22D35	Ser and Ala	2663.78	>25	>250	2.50

* MIC and IC50 values was expressed in µg/mL.

Table 2. Screening of serine - Minimal inhibitory concentration of B1CTcu5 peptide analogs against *Mycobacterium tuberculosis* strains and cytotoxic activity on murine macrophages. Peptide analogs was evaluated at concentrations between 2 to 250 µg/mL.

AMP	Sequence	Molecular Weight	MTB H37Rv*	Macrophage J774A.1*	SI
CR22DK-S20	S...	2808.82	10.24	>250	24.42
CR22DK-S19	LS...	2808.82	>25	>250	2.50
CR22DK-S18(-G)	LIS...	2793.85	22.85	>250	10.94
CR22DS18(G)	**LISG...**	**2993.43**	**1.22**	**>250**	**204.42**
CR22DK-S17	LIAGS...	2808.82	10.35	>250	24.17
CR22DK-S16	**LIAGLS...**	**2850.09**	**1.45**	**>250**	**173.01**
CR22DK-S15	LIAGLAS...	2850.90	23.23	>250	10.76
CR22DK-S12	LIAGLAANFS...	2808.82	3.71	>250	67.48
CR22DK-S09	LIAGLAANFLPQS...	2808.82	25.00	>250	-
CR22DK-S08	LIAGLAANFLPQIS...	2808.82	25.00	>250	-
CR22DK-S05	LIAGLAANFLPQILKKS...	2808.82	25.00	>250	-

* MIC and IC50 values was expressed in µg/mL.

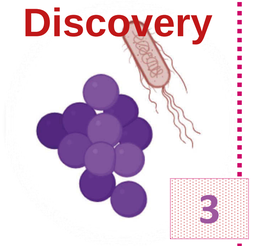


Multi-Drug Resistant MTB

3

AMP	MDR-MTB CF110 (ug/mL)	Fibroblast IC50 (ug/mL)
B1CTcu5	> 25	408.83
W-B1CTcu5	4.25	30.05
cB1CTcu5	8.63	203.72
cW-B1CTcu5	8.19	20.55
CR22D33	11.24	204.61
CR22D34	**3.74**	**233.17**
CR22D35	**1.17**	**662.94**
CR22DS20	> 25	875.30
CR22DS19	> 25	> 1000
CR22DS18(-G)	> 25	195.40
CR22DS18(G)	**1.20**	**593.74**
CR22DS17	22.72	> 1000
CR22DS16	21.48	466.08

Selective Drug Discovery



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AMP	E. coli MIC (µg/mL)	S. aureus MIC (µg/mL)
B1CTcu5	64	64
W-B1CTcu5	128	128
cB1CTcu5	128	64
cW-B1CTcu5	128	128
CR22D33	128	128
CR22D34	128	128
CR22D35	128	128
CR22DS20	128	128
CR22DS19	128	128
CR22DS18 (-G)	64	128
CR22DS18(G)	128	128
CR22DS17	128	128
CR22DS16	128	128
CR22DS15	128	128
CR22DS12	128	128
CR22DS09	128	128
CR22DS08	128	128
CR22DS05	128	128

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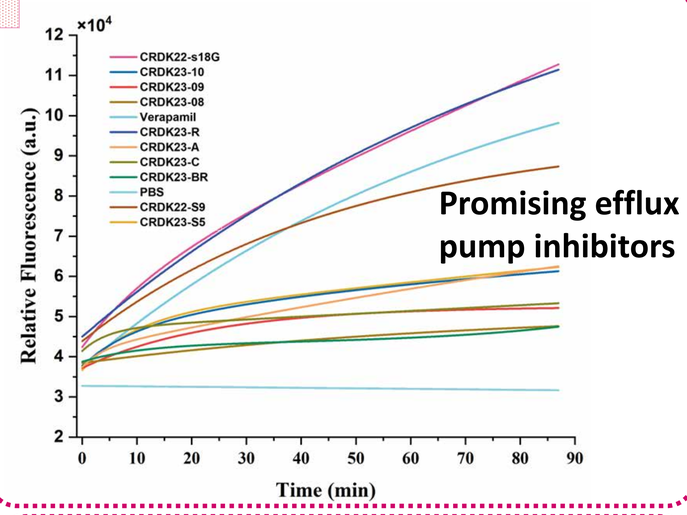

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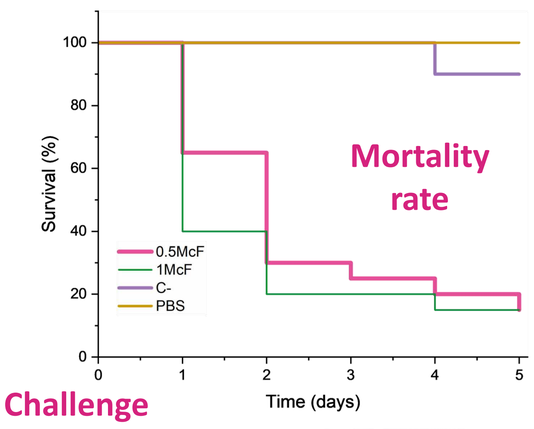
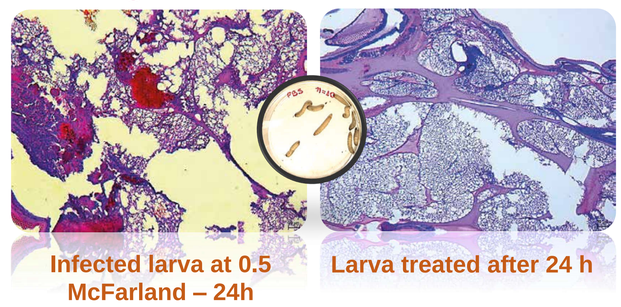


Challenge



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Acknowledgments

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