Design and antimicrobial activity of short and novel tripeptides

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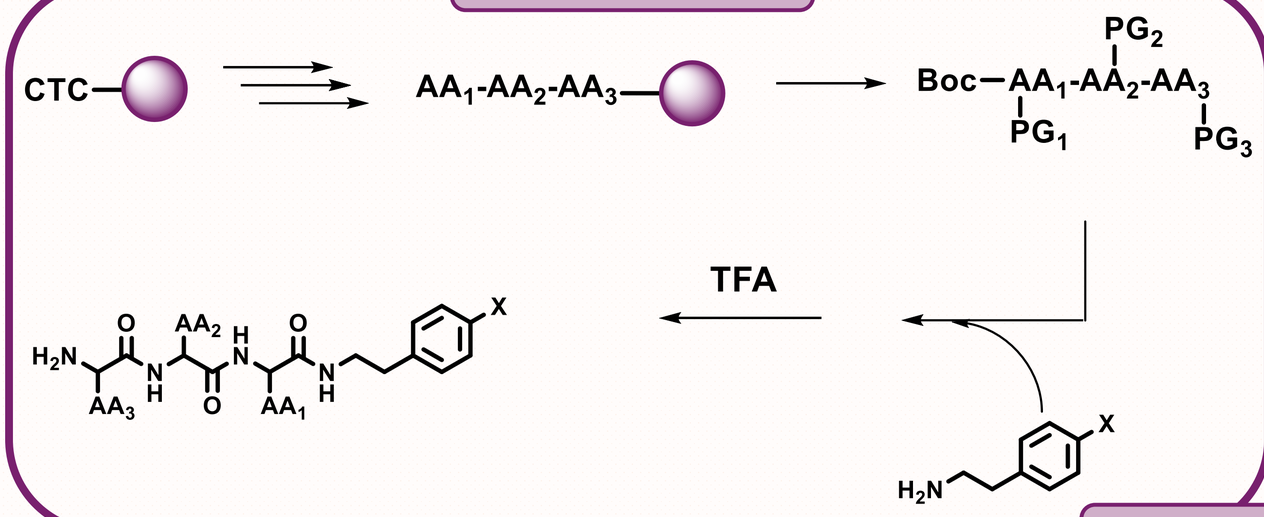
Introduction

Antimicrobial peptides (AMPs) are promising antibacterial agents that could avoid the problematic development of drug resistance. The main advantage of using AMPs is related to their peculiar mechanism of action involving the lytic disruption of the bacterial cellular membrane of both Gram-positive and Gram-negative bacteria. Among the AMPs, Lytixar (LTX-109) is an interesting short tripeptide containing a bulky hydrophobic moiety (Tbt) and cationic amino acids (Arg). In this work we explored structural amphiphilic alternatives to LTX-109 and investigated, through molecular modelling, their interaction with the bacterial membrane, disclosing the critical moieties involved in the lytic mechanism of action. Thus, structural changes were synthetically introduced to evaluate the effect on the antimicrobial activity and the metabolic stability of these new molecules. SAR studies were used to rationalize the combined effect of lipophilicity, steric hindrance and charges.

Synthesis

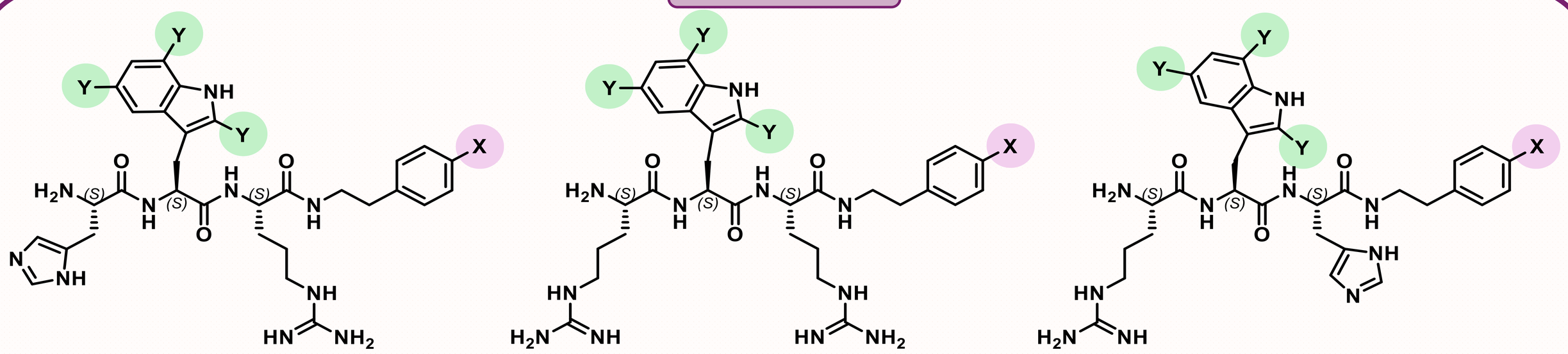


SAR studies

From SAR studies lipophilicity was evaluated, and eventually used to rationalize antibiotic activity. LogKow showed how *para* substitution influenced lipophilicity and, according to biological data, to higher values corresponded better activities. Introduction of 2,5,7-Tris-tert-butyl-tryptophan (tBt) lead to a significant increase of lipophilicity as it did for activity. Interestingly, hystidine substitution corresponded to an increase of LogKow values and, in some cases, to a better activity; especially in the sequences containing tBt instead of Trp.

	LogKow
RTR-H	5.50
RTR-I	6.67
RTR-F	5.70
RTR-CF_3	6.46
RTR-tBu	7.41
RWR-H	-0.23
RTH-H	6.28
HTR-H	6.28

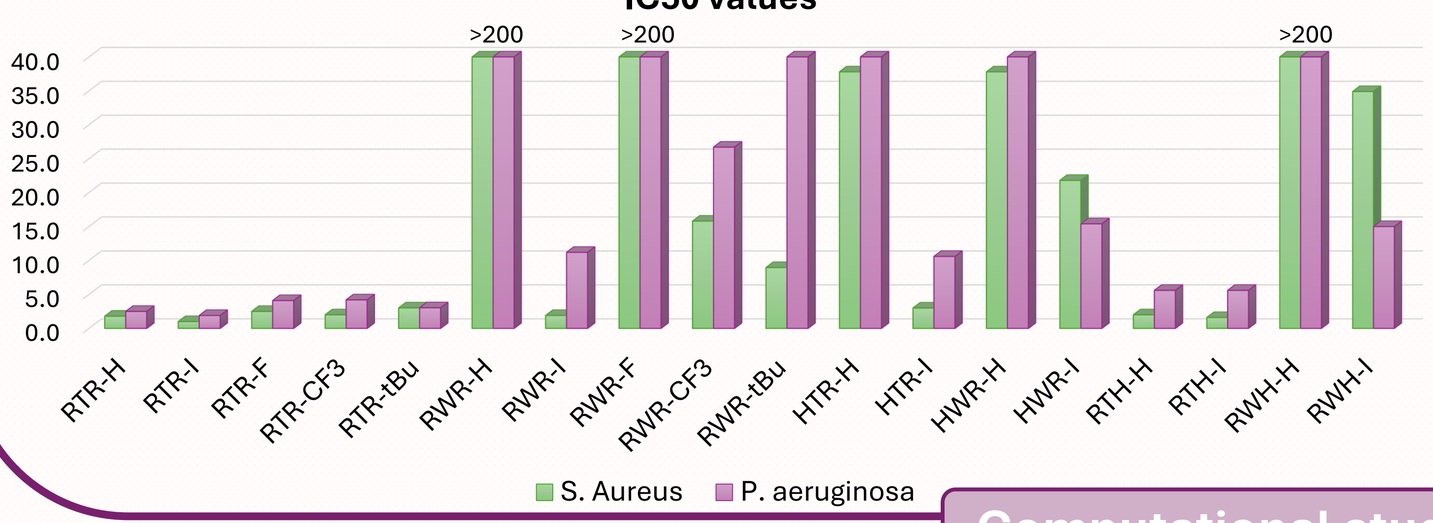
Target Library



X = -H, -I, -F, -CF_3, -tBu Y = -H, -tBu

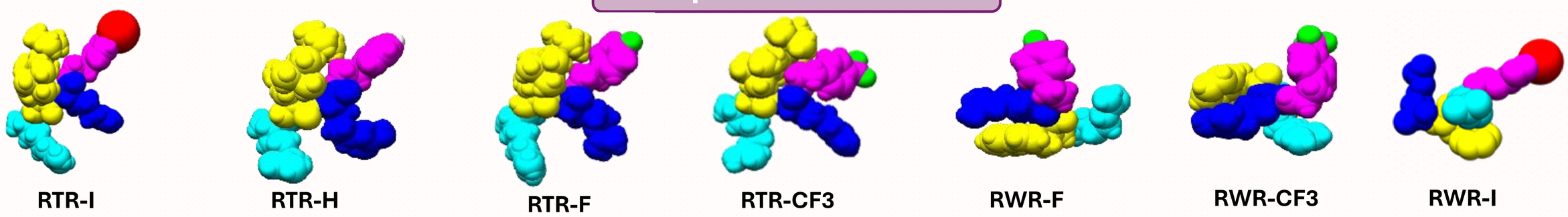
Biological results

According to IC50 values good results were obtained for peptides containing the bulky, unnatural tri-tert-butyl-triptophan residue (T) and little variation of activity was reported by the aromatic ring para-substituted compounds. The influence of these substitutions was tested on the W series tripeptides. Iodine derivatives demonstrated better activity than the -F and -CF_3 substituted, indicating that sterical effects might be more significant on biological activity than electronic ones. According to MHC and CC50 values none of the tested molecules demonstrated hemolytic activity (at MIC concentrations) nor relevant cytotoxicity. For what concerns selectivity (SI), all peptides of the T series showed good results compared to the original LTX.



	CC50 (μg/mL)	Selectivity index (CC50/IC50)		MHC
	Hel299 cells	S. aureus	P. aeruginosa	
RTR-H	47.4	26.3	19.0	200.0
RTR-I	24.1	24.1	12.7	50.0
RTR-F	50.6	20.2	12.3	50
RTR-CF_3	28.9	14.4	6.9	25
RTR-tBu	19.39	6.4	6.4	6.25
RWR-H	200.0	n.d.	n.d.	>200
RWR-I	123.2	64.8	11.0	200.0
RWR-F	86.4	n.d.	n.d.	>200
RWR-CF_3	>200	>12.6	>7.5	200
RWR-tBu	200	n.d.	n.d.	200
HTR-H	54.0	18.0	5.1	100
HTR-I	179.6	6.8	1.9	>200
HWR-H	200.0	5.2	2.4	>200
HWR-I	>200	>9.2	>13.0	>200
RTH-H	29.5	14.8	5.3	50
RTH-I	23.7	14.8	4.2	25
RWH-H	200.0	n.d.	n.d.	>200
RWH-I	93.5	2.7	6.2	>200

Computational studies



MD studies were performed to evaluate the most populated conformations in water. These results showed a characteristic “X” shape where the hydrophobic moieties of the tbt and Ph(X) are close in contact, pushing the arginines to the opposite side. Docking with Gram+ e Gram- bacterial membrane showed that X conformation is crucial for correct lytic mechanism of action influencing the orientation of interaction with the membrane as antibiotic activity. In fact, molecules containing Trp instead of tBt are not able to adopt this conformation and this behavior is reflected in their scarce antibiotic activity.

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