RnpA inhibitors as potential antimicrobials to fight antibiotic-resistance: Computational design, synthesis, and biological evaluation

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RnpA: STATE OF THE ART

Besides of the currently pandemic situation, due to the COVID-19, antibiotic resistant remains one of the most worrying health emergency. [1] The unneeded and incorrect use of antibiotics is leading to a pre-antibiotic era, in which simple infections can kill again.

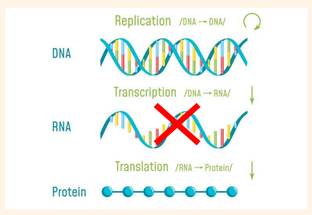
A possible strategy to avoid this outcome is to develop molecules able to interact with novel and unexploited molecular targets. Among them, RNA processing is a crucial physiological system that has been only recently recognized as exploitable for this purpose[2].

RnpA is a 14 KDa protein of *Staphylococcus aureus*, known to be crucial for, at least, **two important RNA-related bacterial processes: mRNA degradation and ptRNA maturation**. [3]

For these reasons, RnpA arose as a potential target for antibiotic therapy.

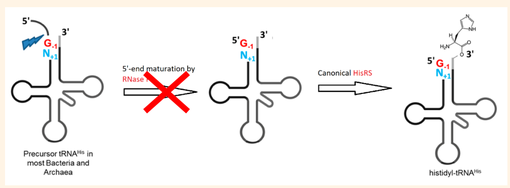
mRNA degradation

RnpA itself is able to catalyse mRNA degradation, thus controlling the expression of crucial proteins based on the different growth phases.



ptRNA maturation

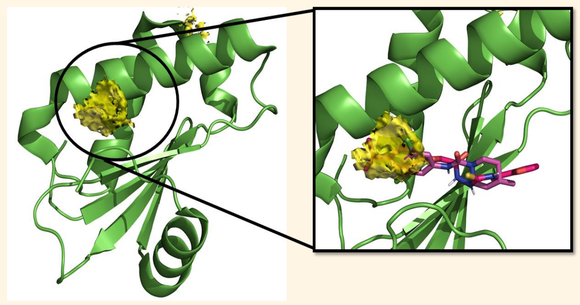
RnpA can associate forming a riboproteic complex which catalyse the removal of the 5' leader sequence of ptRNA, promoting tRNA maturation.



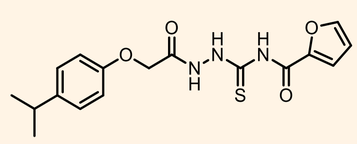
COMPUTATIONAL DESIGN OF NOVEL DERIVATIVES

Starting from known RnpA-inhibitors, we aimed at understanding which part of the protein could be **the interaction site** of our inhibitors.

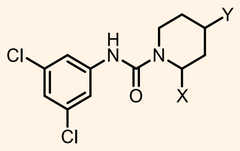
Using *S. aureus* RnpA protein, we performed a detailed **Hotspots Maps calculation**, and, as yellow-highlighted in the figure below, one main hotspot area was identified.



We validated our hypothesize by docking **RNPA2000** [4,5] and **JC1/2** [6], known RnpA inhibitors reported here below, and we understood the main interactions achieved by these compounds. Having this information in hands, we designed a set of **12 novel derivatives** (depicted here on the right), to start exploring the SAR of these classes. [7]

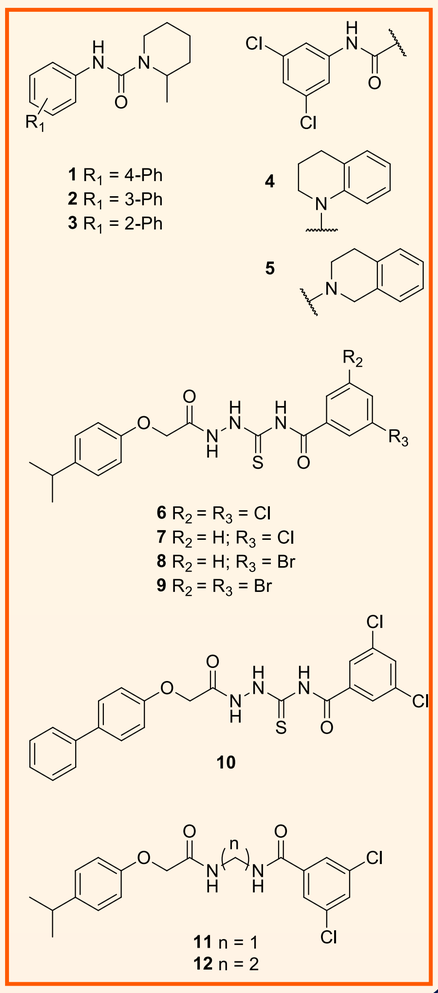


RNPA2000

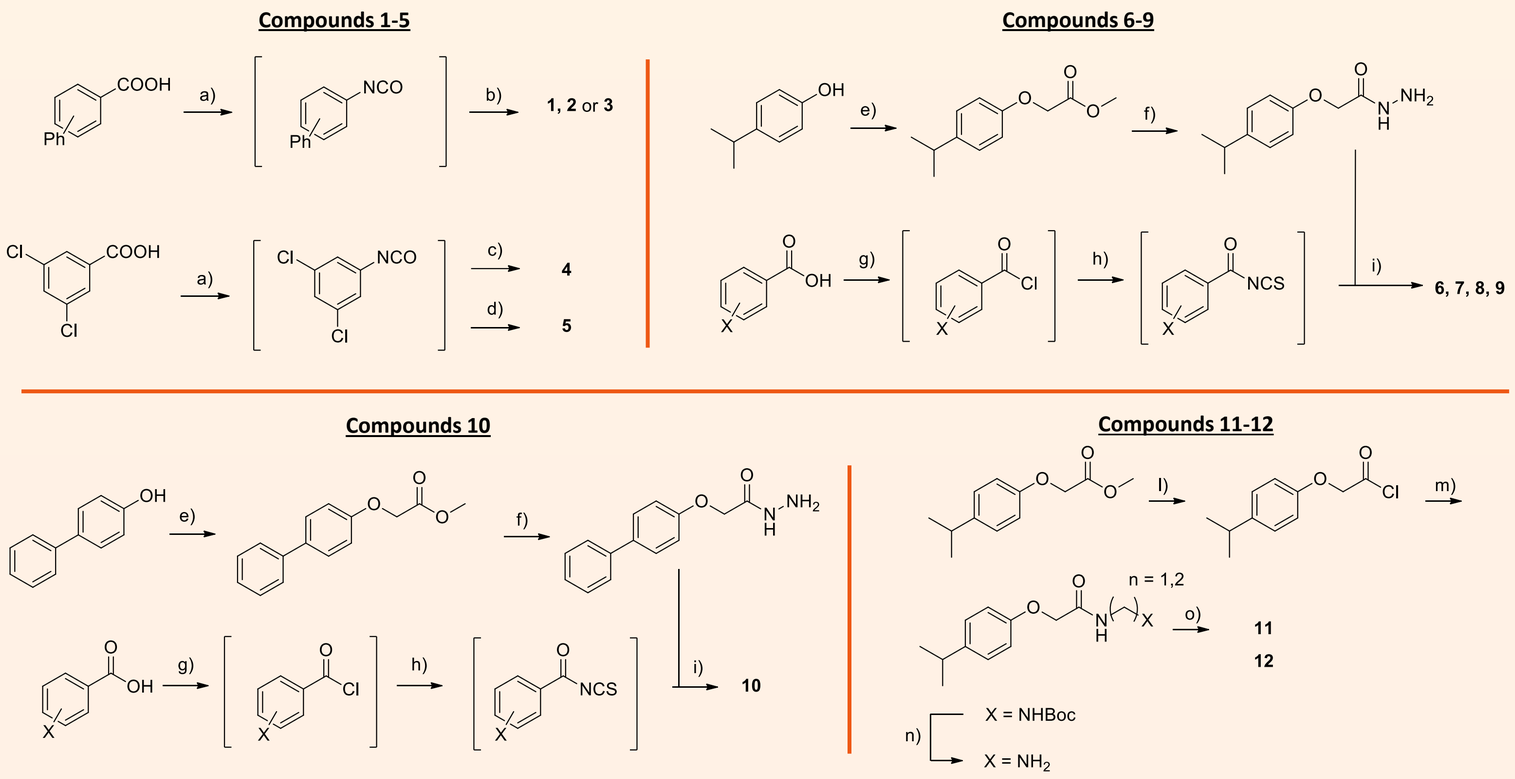


JC1 X = H; Y = Me

JC2 X = Me; Y = H



CHEMISTRY



Reagents and solvents: a) DPPA, TEA, toluene, 95°C; b) 2-methylpiperidine, toluene, 95°C; c) 1,2,3,4-tetrahydroquinoline, toluene, 95°C; d) 1,2,3,4-tetrahydroisoquinoline, toluene, 95°C; e) Methyl 2-chloroacetate, K_2CO_3, DMF, 50 ° C; f) Hydrazine hydrate, Methanol, Reflux; g) $SOCl_2$, Reflux; h) Potassium thiocyanate, Acetonitrile, room temperature (RT); i) Acetonitrile, RT, ; l) 1 - 2.5 N aqueous NaOH, MeOH, RT; 2- $SOCl_2$, reflux; m) *N*-Boc corresponding amine, TEA, DCM, RT; n) 10% aqueous HCl, MeOH, reflux; o) 3,5-dichlorobenzoyl chloride, TEA, DCM, RT.

BIOLOGICAL EVALUATION

Compounds **1-12** were **evaluated as antimicrobials**, calculating their MICs toward 3-different *S. aureus* strains: one Methicillin-resistant (MRSA) and two Methicillin-sensible (MSSA). Moreover, their capability of inhibit RnpA was tested using peculiar ***in vitro* assays**.

Compound	MIC			*In vitro* Assays	
	MSSA (μM)	MRSA (μM)	UAMS-1 (μM)	$_DIC_{50}$ (μM)	$_PIC_{50}$ (μM)
RNPA2000	-	-	44	275	140
1	>500	>500	>500	72.5	36
2	>500	>500	>500	233	37
3	>500	>500	>500	324	>500
4	311	311	250	66	50
5	>500	>500	>500	>500	75
6	21.1	21.1	64	53	59
7	24.7	24.7	31.25	77	28
8	22.2	22.2	62.5	49	76
9	>500	18.9	*On going*	-	-
10	21.1	21.1	1.96	188	33
11	>500	>500	>500	31	153
12	>500	>500	>500	165	423

$_DIC_{50}$ = *in vitro* mRNA degradation IC_{50};
$_PIC_{50}$ = *in vitro* ptRNA processing IC_{50}.

CONCLUSIONS

This preliminary set of data allowed us to:

- **Predict** the key interactions of RNPA2000 and JC1/2 with their molecular target RnpA.
- **Understand** which structural changes are productive or detrimental for the activity. Knowing which moieties are crucial for the inhibitory activity let us to expand the structure-activity relationship (SAR).
- **Guide** future investigations in order to obtain more potent and promising antibiotics.

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