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New Fluoroquinolone-phenothiazine Hybrids and their Antimicrobial Activity

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BACKGROUND

Fluoroquinolones are classified by WHO as "Watch" – group with **higher resistance potential**.

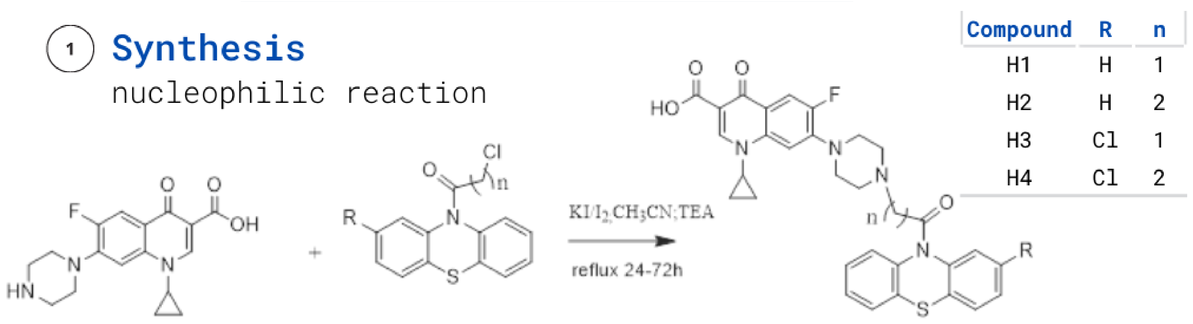
Efflux pumps display an **important role** and have been associated with multidrug resistance. **Efflux pump inhibitors (EPI)** can be an approach to **restore the antimicrobial activity**.

Phenothiazines have **EPI properties** and inhibit biofilm formation [1].

Hybridization of **ciprofloxacin (CIP)** and **phenothiazines:** display antibacterial and efflux pump inhibitory activities?

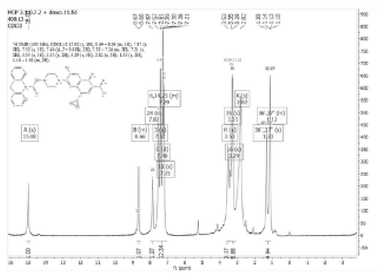
METHODS

1 Synthesis
nucleophilic reaction



Compound	R	n
H1	H	1
H2	H	2
H3	Cl	1
H4	Cl	2

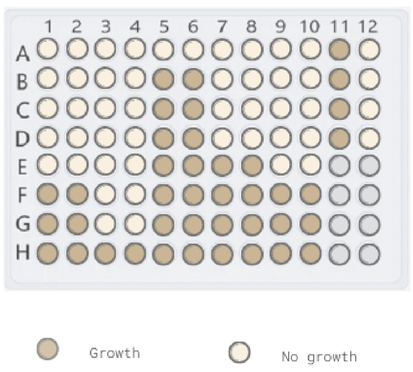
Structure confirmation
nuclear magnetic resonance and infrared spectroscopy



2

3 Molecular Dynamics
Docking NorA (homology - Alphafold Swiss Model. Access: P0A0J7)

4 MIC - Minimum inhibitory concentration



5 Plate the no growth wells in compound-free agar


Growth


No growth

→ **MBC** - Minimum bactericidal concentration

RESULTS

TABLE 1. MIC and MBC results for *S. aureus* wild-type (SA1199) and NorA overexpressing (SA1199B) strains. Data are the result of at least three independent assays.

	S. aureus SA1199		*S. aureus* SA1199B (NorA ++)	
	MIC (μM)	**MBC** (μM)	**MIC** (μM)	**MBC** (μM)
CIP	3.125	12.5	12.5	25
H1	6.25	12.5	12.5	100
H2	**3.125**	**12.5**	**6.25**	**12.5**
H3	6.25	100	200	200
H4	3.125	25	25	50

H2 and H4 kept the antimicrobial activity against the wild-type isolate and H2 **potentiate** antimicrobial activity against *S. aureus* SA1199B.

TABLE 2. Molecular docking results of H2, ciprofloxacin, and chlorpromazine (CHP)

Compounds	Residues implicated in the interaction	Docking score (kcal/mol)
CIP	H bond: ARG98; GLU222; SER219. Hydrophobic: ILE19; **IL23**; LEU43; PHE47; ALA48; PHE 140;LEU218; ARG310; ASN340. π-σ: VAL44.	-7.66
CHP	π-stacking: PHE140. H bond: ASN137. π-anion: ASP307. Hydrophobic: ILE141; ILE136; LEU218; PRO311; ARG310; GLN255; ALA252; GLY248; PRO144, GLU222; ILE136.	-6.48
H2	H bond: ARG98; GLU222; SER337. Hydrophobic:PHE16; ILE19; VAL22; **ILE23**; ILE26; PHE140; THR223; ASN340. π-σ:VAL44. π-lone pair: GLN51. π-π: **PHE47** .	**-8.46**

H2 interacts with NorA **groove binding site** [2] (residues in bold).

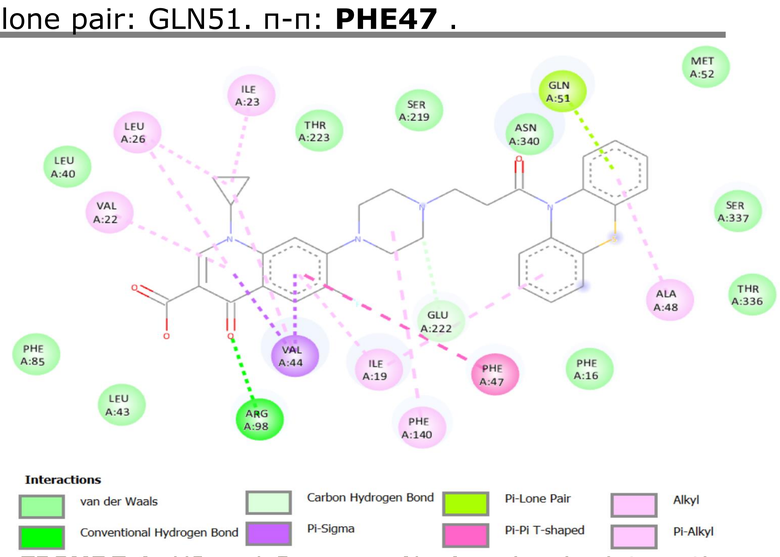


FIGURE 1. H2 and *S. aureus* NorA molecular interactions.

CONCLUSIONS

- Hybridization of CIP and phenothiazines was able to **maintain CIP activity** for *S. aureus* wild-type (SA1199) while it was able to potentiate CIP activity against the overexpressing NorA strain (SA1199B).
- The activity of this new molecule (H2) may be the result of a synergetic antibacterial and efflux pump inhibitory activity.

Acknowledgements

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References

1. Grimsey, E.M.; Piddock, L.J. V Do Phenothiazines Possess Antimicrobial and Efflux Inhibitory Properties? *FEMS Microbiol. Rev.* **2019**, *43*, 577–590, doi:10.1093/femsre/fuz017.
2. Zárate, S.G.; Morales, P.; Świderek, K.; Bolanos-Garcia, V.M.; Bastida, A. A Molecular Modeling Approach to Identify Novel Inhibitors of the Major Facilitator Superfamily of Efflux Pump Transporters. *Antibiot. (Basel, Switzerland)* **2019**, *8*, doi:10.3390/antibiotics8010025.

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