SUPPLEMENTARY INFORMATION

Repurposing Approved Drugs as Fluoroquinolone Potentiators to Overcome Efflux Pump Resistance in *Staphylococcus aureus*

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RUNNING TITLE

Repurposing drugs for resistant *S. aureus*

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SUPPLEMENTARY INFORMATION (LEGENDS)

Table S1 Binding energy (kcal/mol) of the lead compounds to the homology model of NorA efflux pump.

Table S2 MIC of ciprofloxacin, norfloxacin in combination with compounds for *S. aureus* SA1199 (Wild-type strain).

Table S3 MIC of tetracycline and moxifloxacin for *S. aureus* XU212 and *S. aureus* SA-1199B respectively in combination with different drugs.

Table S4 MIC of moxifloxacin for *S. aureus* clinical strains (MRSA) in combination with different drugs.

Fig. S1 Growth kinetics of *Staphylococcus aureus* SA-1199B in CA-MHB under sub-inhibitory concentrations (1/4×MICs) of the drugs. OD600 nm values are the means of three independent experiments. The control (untreated) set was included to monitor normal bacterial growth.

Fig. S2 Effect of sub-inhibitory concentrations (1/4 × MICs) of raloxifene, ezetimibe, propafenone, nefazodone, chlorprothixene, pyrvinium on the efflux inhibition of EtBr against *S. aureus* SA-1199B (*norA* over-expressed) in the presence and absence of 0.4% glucose; the positive control reserpine was included for comparison. The results presented correspond to the average of three independent assays ± SD.

Fig. S3 Effect of sub-inhibitory concentrations (1/4 × MICs) of raloxifene, ezetimibe, propafenone, nefazodone, chlorprothixene, pyrvinium on the efflux inhibition of EtBr against *S. aureus* K1758 (*norA* deletion) in the presence and absence of 0.4% glucose. The results presented correspond to the average of three independent assays ± SD.

Table S1 Binding energy (kcal/mol) of the lead compounds to the homology model of NorA efflux pump.

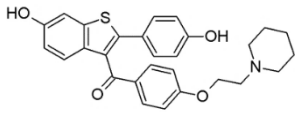
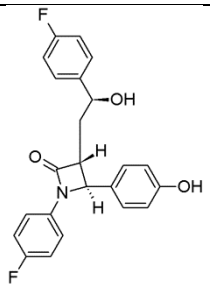
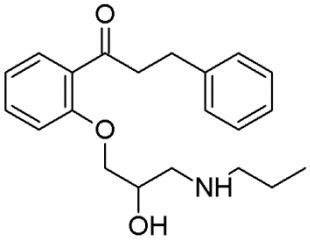
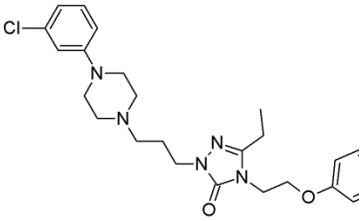
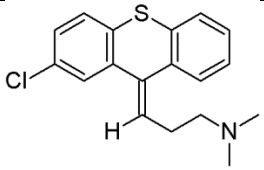
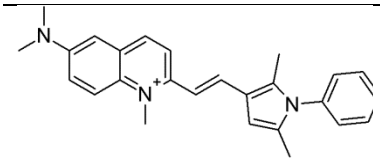
Compounds	Docking Score (kcal/mol)	Residues Implicated in the Interaction	MMGBSA Score (kcal/mol)
 Raloxifene	-9.064	Hydrophobic: Ile19, Phe303, Leu218, Tyr225, Hydrogen bonding : Asn340, Phe16 Pi-Pi stacking: Phe140 Polar: Gln51, Ser337, Thr336 Charged (Negative): Glu222	-70.08
 Ezetimibe	-7.807	Hydrogen bonding: Gly106, Gln51, Thr336, Asn340 Hydrophobic: Val108, Phe16, Ile19, Phe140, Ala215 Polar: Gln51, Ser337, Thr336 Charged (Positive): Arg310	-51.65
 Propafenone	-7.848	Hydrophobic: Ile19, Val108, Leu218, Phe16, Met109, Ala215 Hydrogen bonding : Asn340, Thr336 Polar: Gln51, Ser337, Thr336 Charged (Positive): Arg310	-48.41
 Nefazodone	-7.756	Hydrophobic: Ile19, Phe303, Leu218, Phe140, Hydrogen bonding : Asn340 Polar: Gln51, Ser337, Thr336 Charged (Positive): Arg310	-60.78
 Chlorprothixene	-3.757	Hydrophobic: Val44, Ile19, Phe47, Ala105, Met109 Polar: Asn340, Ser337, Thr336, Gln51 Charged (Positive): Arg310	-50.18
 Pyrvinium	-4.217	Hydrophobic: Ile136, Phe16, Leu218, Met132, Tyr225, Met109 Pi-Pi stacking: Phe140 Polar: Asn340, Ser337, Thr336 Charged (Positive): Arg310, Lys238 Charged (Negative): Glu222	-45.35

Table S2 The MIC of ciprofloxacin, norfloxacin in combination with compounds for *S. aureus* SA1199 (Wild-type strain).

	Intrinsic MIC (μM)	Concentration used (μM)	MIC in the presence of compounds	
			Ciprofloxacin	Norfloxacin
			0.5	1
Raloxifene	>200	50	0.125	0.25
		25	0.25	0.5
		12.5	0.5	1
Ezetimibe	>200	50	0.25	0.25
		25	0.25	1
		12.5	0.5	1
Propafenone	>200	50	0.25	0.5
		25	0.5	0.5
		12.5	0.5	1
Nefazodone	>200	50	0.25	0.5
		25	0.5	1
		12.5	0.5	1
Chlorprothixene	>200	50	0.25	0.5
		25	0.25	0.5
		12.5	0.5	1
Pyrvinium	3.125	0.78	0.125	0.25
		0.39	0.25	0.5
		0.195	0.5	1
Reserpine	210	52	0.25	0.5
		26	0.25	0.5
		13	0.5	1

Table S3 MIC of tetracycline, and moxifloxacin for *S. aureus* XU212, and *S. aureus* SA-1199B respectively in combination with different drugs.

Compounds	*S. aureus* XU212			*S. aureus* SA-1199B		
	Intrinsic MIC (μM)	Conc. used (μM)	MIC[a] (μg/mL)	Intrinsic MIC (μM)	Conc. used (μM)	MIC[b] (μg/mL)
	-	-	256	-	-	0.25
Raloxifene	200	50	256	200	50	0.25
Ezetimibe	≥100	50	256	200	50	0.25
Propafenone	≥200	50	256	800	50	0.25
Nefazodone	≥200	50	256	200	50	0.25
Chlorprothixene	100	12.5	256	200	50	0.25
Pyrvinium	3.125	0.78	256	1.56	0.39	0.25

[a] MIC of tetracycline in the presence of compounds; [b] MIC of moxifloxacin in the presence of compounds.

Table S4 MIC of moxifloxacin for *S. aureus* clinical strains (MRSA) in combination with different drugs.

Compounds	Concentration used (μM)	MIC of moxifloxacin in the presence of compounds					
		MRSA 1	MRSA2	MRSA3	MRSA4	GMCH 831	GMCH 839
-	-	2	1	2	4	1	1
Raloxifene	50	2	1	2	4	1	1
Ezetimibe	50	2	1	2	4	1	1
Propafenone	50	2	1	2	4	1	1
Nefazodone	50	2	1	2	4	1	1
Chlorprothixene	50	2	1	2	4	1	1
Pyrvinium	0.195	2	1	2	4	1	1

Supplementary figures

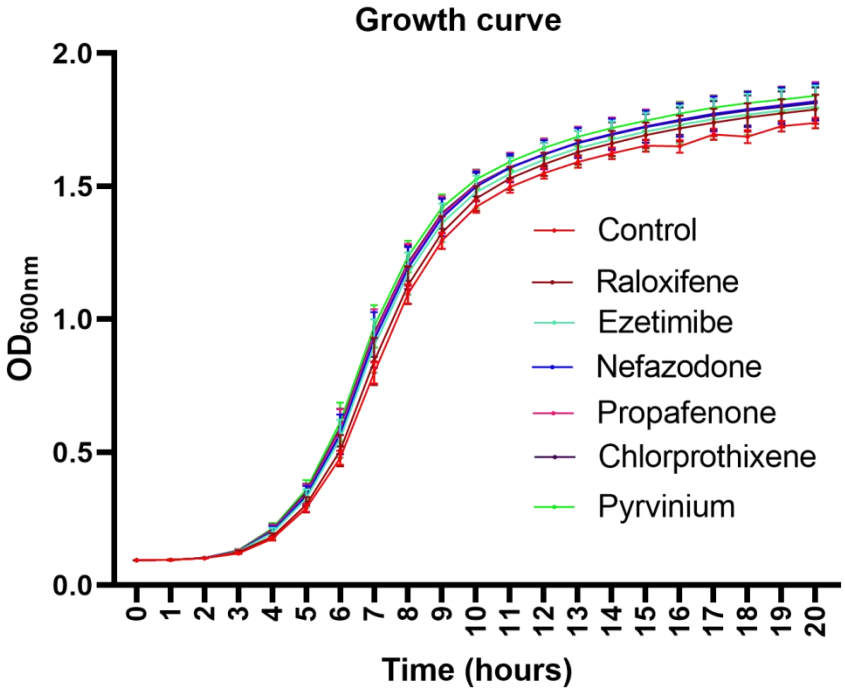


Fig. S1 Growth kinetics of *Staphylococcus aureus* SA-1199B in CA-MHB under sub-inhibitory concentrations (1/4×MICs) of the drugs. OD_{600nm} values are the means of three independent experiments. The control (untreated) set was included to monitor normal bacterial growth.

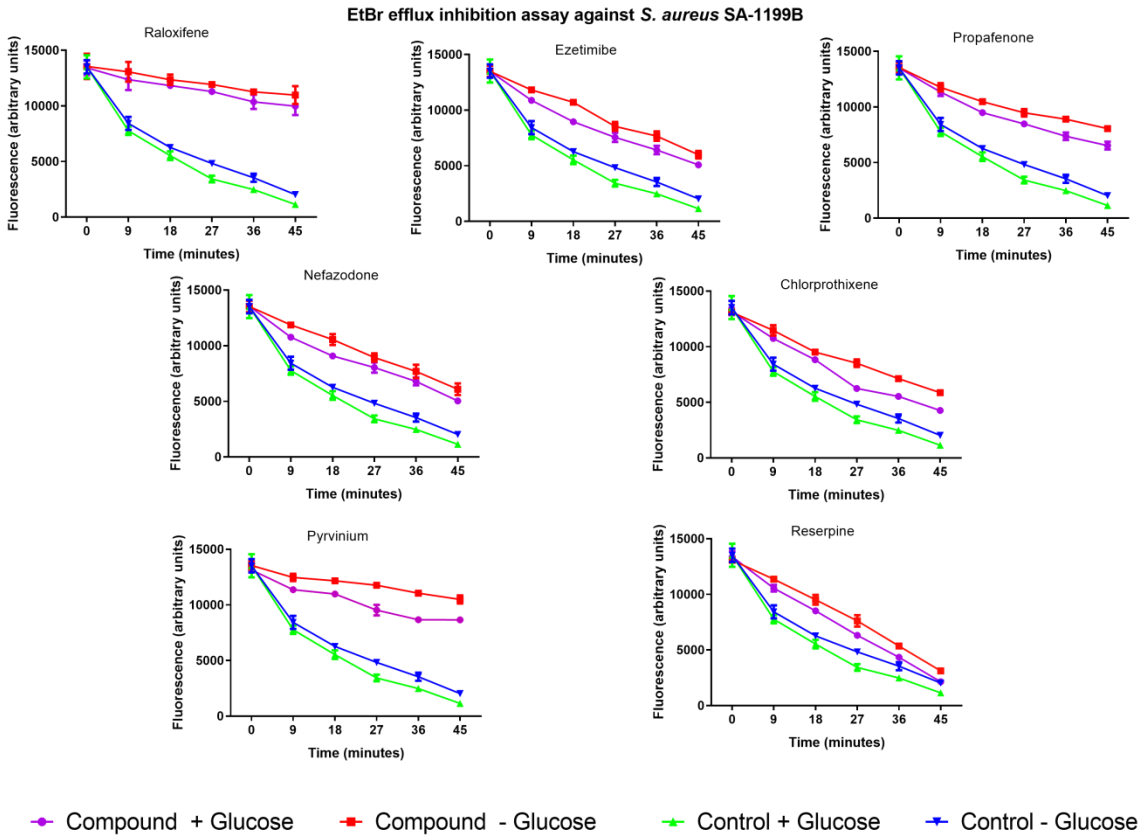
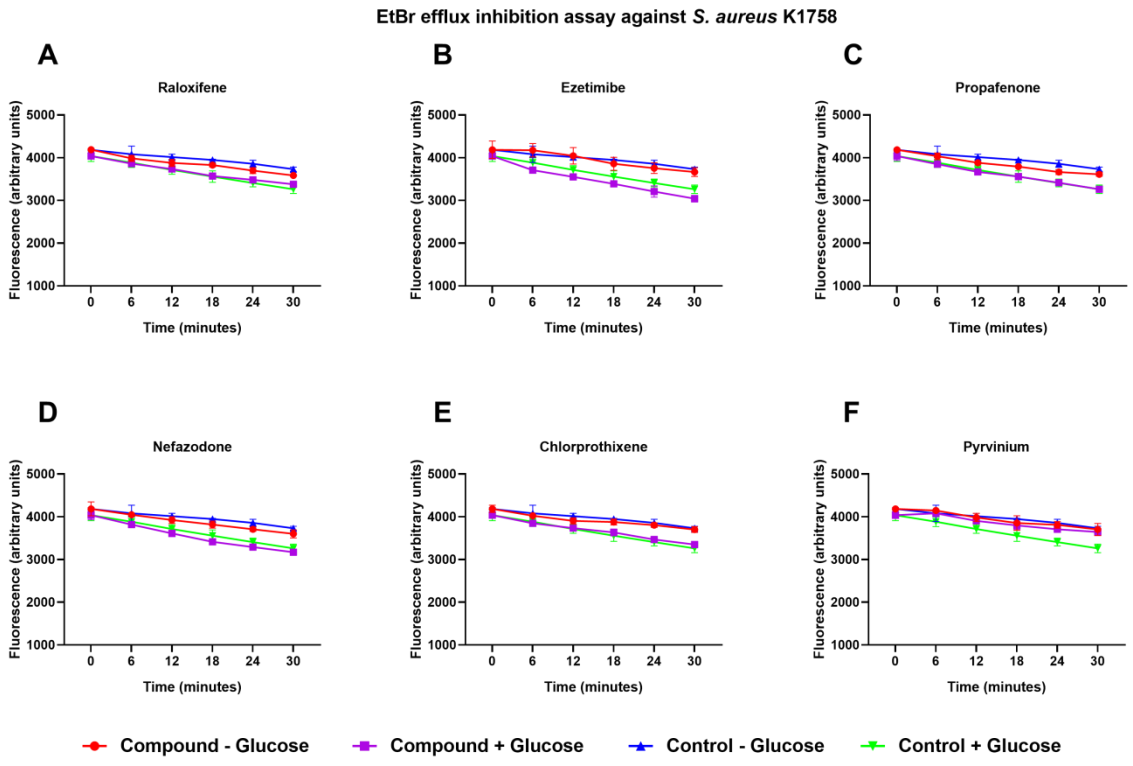


Fig. S2 Effect of sub-inhibitory concentrations (1/4 × MICs) of raloxifene, ezetimibe, propafenone, nefazodone, chlorprothixene, pyrvinium on the efflux inhibition of EtBr against *S. aureus* SA-1199B (*norA* over-expressed) in the presence and absence of 0.4% glucose; the positive control reserpine was included for comparison. The results presented correspond to the average of three independent assays ± SD.



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