Development of membrane targeting peptidomimetics against resistant bacteria

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Introduction

Steady increase in antimicrobial resistance (AMR) have become a critical global healthcare problem. In 2019, AMR caused 1.27 direct death and 4.95 indirect deaths, and the annual death was predicted to be 10 million by 2050 (Fig. 1). The lack of new therapeutic strategies to fight the alarming rise of resistant strains has lead to an "antibiotic crisis". Antimicrobial peptidomimetics targeting bacterial membrane is promising to tackle the issue of AMR because the structure of bacterial membrane is evolutionarily conserved with less mutations. However, poor understanding of the action mechanism and the lack of design principles have impeded their development. We previously developed a fragment based pharmacophore model for rational design of peptidomimetics targeting bacterial membranes. By fine-tuning the structure of each fragment, we obtained a series of membrane targeting peptidomimetics with excellent antimicrobial activity against Gram positive bacteria. We also show the development of combinational therapy against colistin resistant bacteria mcr-1. The pharmacophore model and the combinational therapy provide a useful framework for the development of membrane targeting antimicrobials against resistant bacterial infections.

Methods

To design a molecule that significantly perturbs the bacterial membrane, we first decompose the bacterial inner membrane into three regions: two anionic head group regions and one lipid tail region. To maximize the interactions with the three regions of bacterial membrane, we developed a pharmacophore model (Fig. 2) consisting two cationic fragments and one hydrophobic fragment. A series of peptidomimetics showed excellent antimicrobial activity against Gram positive bacteria.

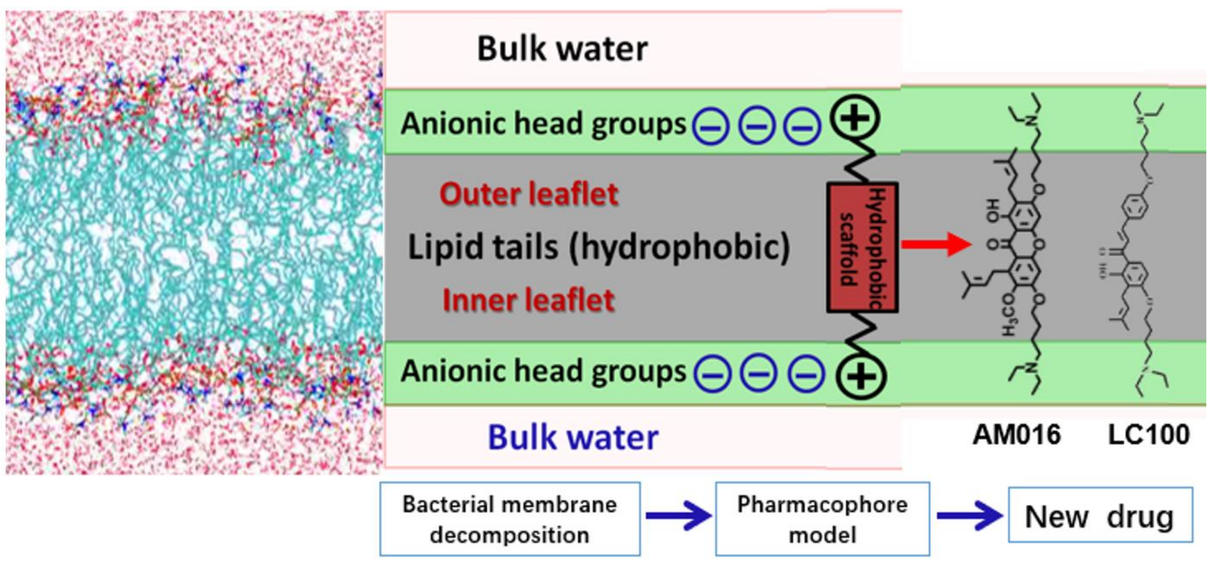


Figure 2. Schematic view of the pharmacophore model. The two compounds AM016 and LC100 are two derivatives of the pharmacophore model based two natural products mangostin and isobavachalcone.

To further target Gram negative bacteria, we developed a strategy of combining one peptidomimetic and one outer membrane permeabilizing peptides. The peptide permeabilize the outer membrane, which enables the diffusion of peptidomimetic across the outer membrane and disrupt the inner membrane. The antimicrobial combination can target both outer and inner membrane of Gram negative bacteria, therefore is unlikely to induce antimicrobial resistance.

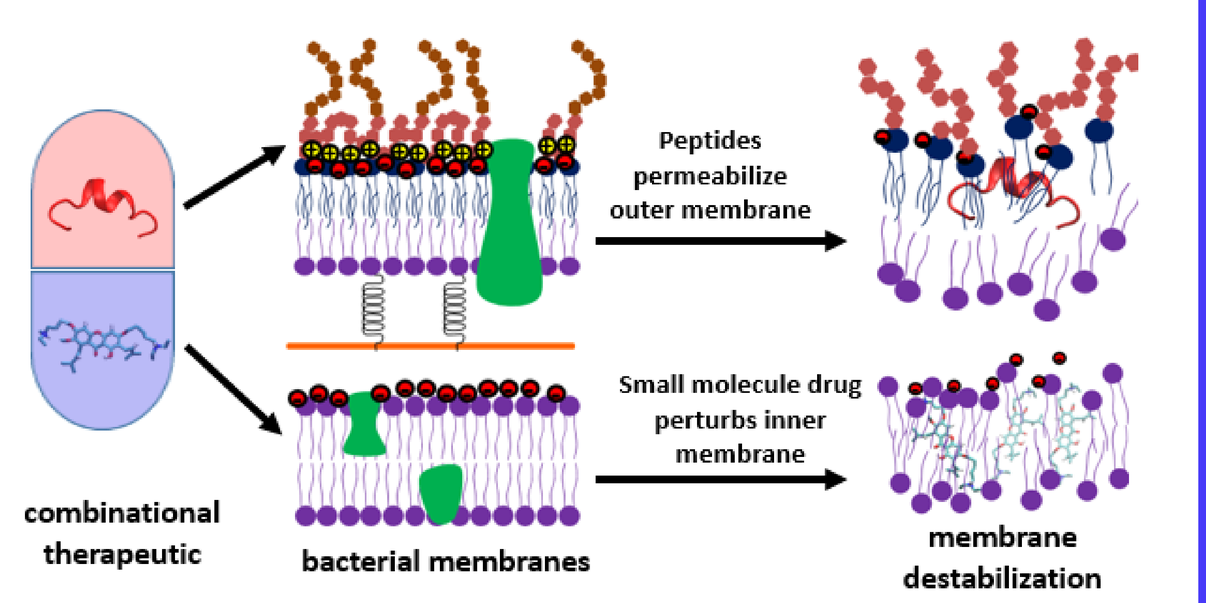


Figure 3. Schematic of the mechanism of action of the combinational therapy. The antibiotic combination consists of one peptide based outer membrane permeabilizer and one small molecule targeting the bacterial inner membrane.

Results and Discussions

Peptidomimetics targeting Gram positives

Selecting different hydrophobic scaffold and cationic fragments of the pharmacophore model enabled us to obtain a series of membrane targeting peptidomimetics that are active against Gram positive bacteria, including MRSA. Calcein-leakage experiments confirms the inner membrane targeting mechanism of the peptidomimetics.

Table 1. Minimum inhibiting concentration (MIC) of a series of pharmacophore model derived peptidomimetics against a panel of Gram positive bacteria

	Natural product	Cationic group	Gram positive		
			MRSA 9808R	SA DM 4001R	MRSA 21455
LC100	Isobavachalcone	Diethylamine	3.125	6.25	3.125
LC101	Isobavachalcone	Arginine	1.56	1.56	1.56
LC102	Xanthohumol	Diethylamine	6.25	3.125	6.25
LC103	Xanthohumol	Arginine	6.25	12.5	6.25
LC105	Glabridin	Diethylamine	25	12.5	6.25
LC106	Glabridin	Arginine	25	12.5	6.25
LC107	Isoliquiritigenin	Diethylamine	50	25	50
LC108	Isoliquiritigenin	Arginine	50	50	50
LC402	Licochalcone A	Diethylamine	6.25	6.25	6.25
LC501	Licochalcone A	Arginine	12.5	12.5	12.5

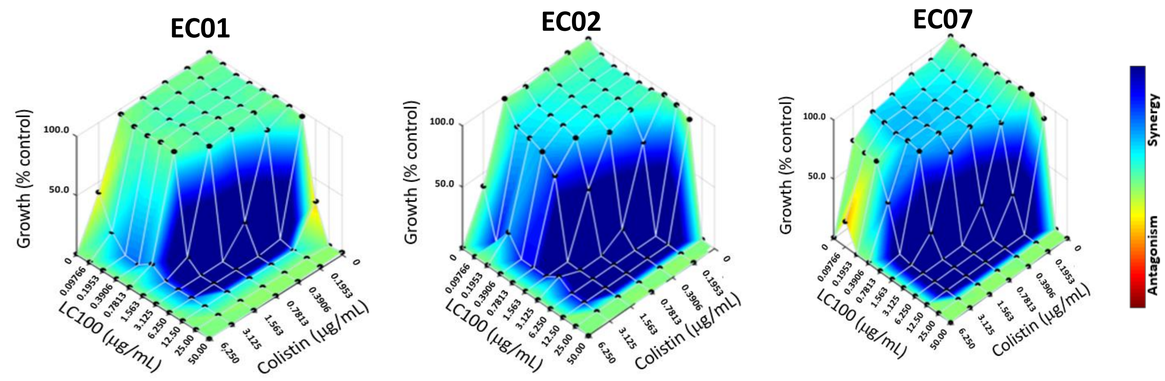
Antimicrobial combination against Gram negatives

- Target bacteria: mobile colistin resistant strains mcr-1
- Antimicrobial combination consists of one peptide based outer membrane permeabilizer (e.g., colistin) and one peptidomimetics targeting the inner membrane.
- In the presence of peptidomimetics e.g., LC100, the mcr-1 strains become sensitive to colistin, with colistin MIC decreased more than 10 folds.
- Fluorescence experiments using mcr-1 bacteria confirms the synergistic mechanism of the antimicrobial combination.

Table 2. The synergy of colistin with a series of LC compounds.

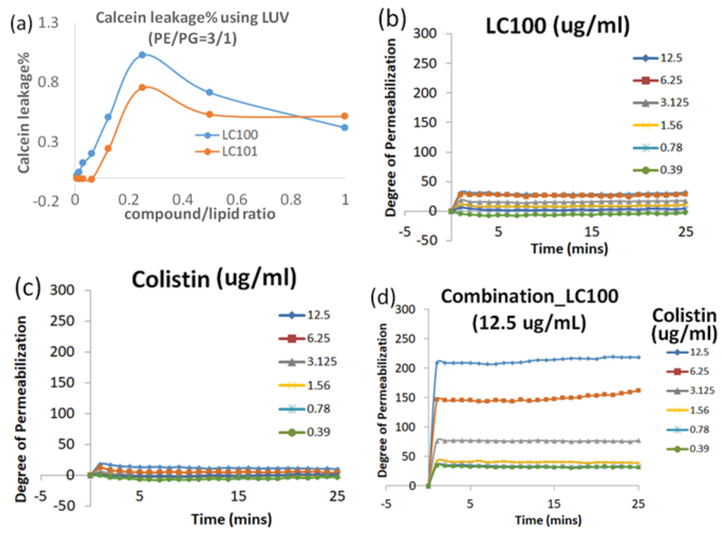
Clinical isolates of mcr1 strains	colistin	LC014	colistin with 3.125 ug/ml LC014	LC100	colistin with 2ug/ml LC100	LC101	colistin with 7 ug/ml LC101	LC137	colistin with 5 ug/ml LC137
6083655967	6.25	6.25	0.39	100	<0.195	25	<0.125	12.5	<0.195
7023444207	6.25	6.25	0.39	100	<0.195	25	<0.125	12.5	<0.195
6123520276	6.25	6.25	0.78	100	<0.195	25	<0.125	12.5	<0.195
6075066346	6.25	6.25	0.78	100	<0.195	25	<0.125	12.5	<0.195
6103645107	6.25	12.5	0.78	100	<0.195	25	<0.125	12.5	<0.195
7023436560	6.25	6.25	0.39	100	<0.195	25	<0.125	12.5	<0.195
7013177695	6.25	6.25	0.39	100	<0.195	25	<0.125	12.5	<0.195

Figure 3. Synergy of LC100 and colistin against E. Coli with mcr-1 mutations



Validation of action mechanism

Figure 4. Fluorescence experiments to characterize the action mechanism of action of the antibiotic combination. (a). Calcein leakage experiments of LC100 and LC101 using large laminar vesicle. (b)-(d) are the fluorescence assay using sytox green and live bacteria.



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