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Nanoformulation With a Cationic Copolymer Enhanced the Antibacterial Activity of a Weakly Active Pyrazole

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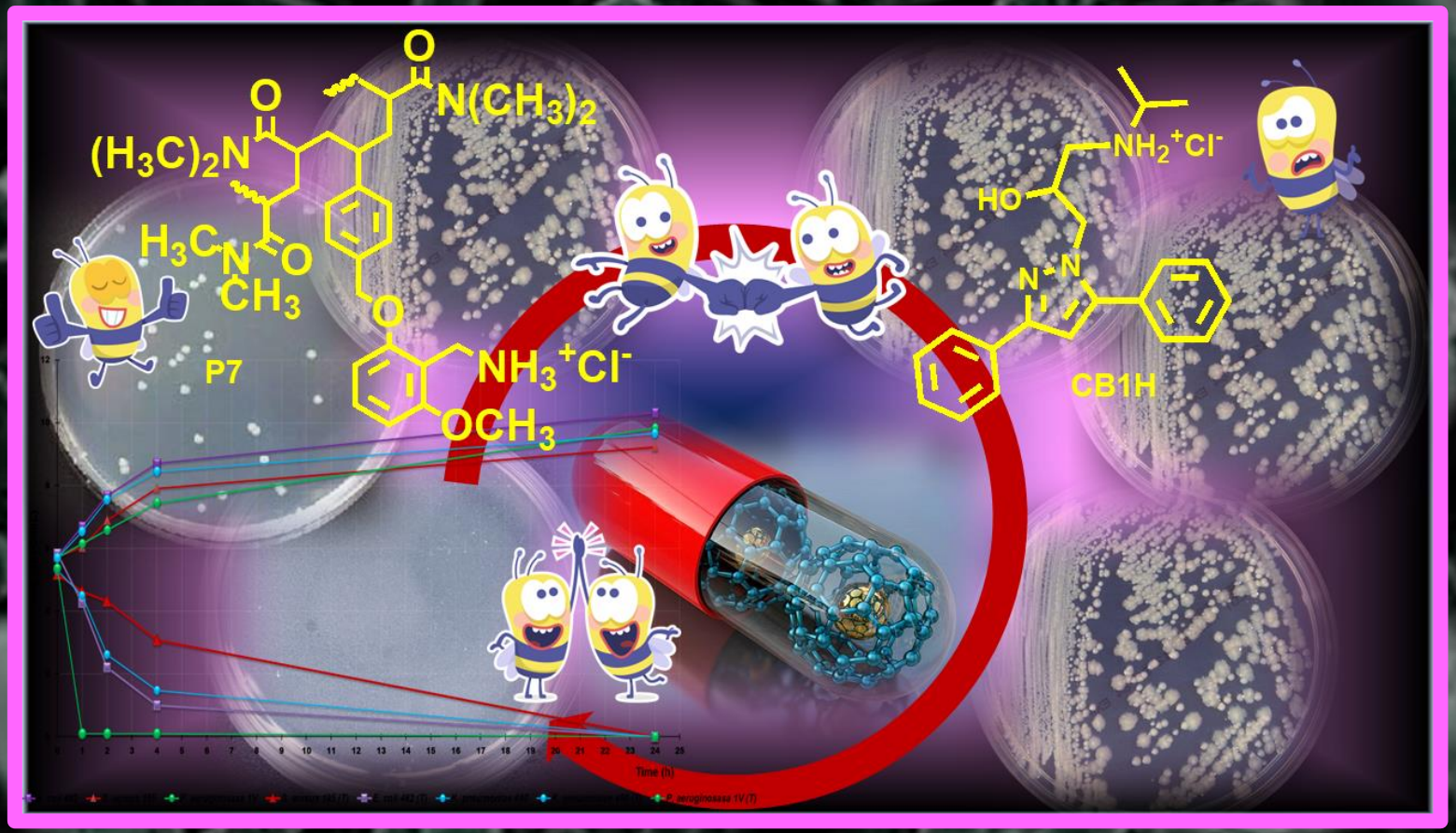
Nanoformulation With a Cationic Copolymer Enhanced the Antibacterial Activity of a Weakly Active Pyrazole



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Abstract: Molecules containing the pyrazole nucleus are promising candidates for the development of new antimicrobial compounds against multidrug resistant (MDR) bacteria no longer inhibited by available antibiotics. Recently, aiming at improving the excessive minimum inhibitory concentrations (MICs) of a pyrazole hydrochloride (CB1H), we prepared CB1H-loaded nanoparticles (CB1H-P7 NPs) using a potent cationic bactericidal macromolecule (P7) as polymer matrix. Here, CB1H-P7 NPs have been tested on 36 MDR clinical isolates of Gram-positive and Gram-negative species, finding MICs even lower than those of P7 (0.6-4.8 µM vs. 1.2-9.3 µM). Additionally, upon complexation, the antibacterial effects of pristine CB1H were improved by 2-16.4-fold, as desired. Furthermore, 24 hours time-killing experiments have established that CB1H-P7 NPs possess rapid bactericidal effects against representative strains of both Gram-positive and Gram-negative species, such as methicillin-resistant *Staphylococcus aureus* (MRSA), MDR *Pseudomonas aeruginosa*, including a colistin-resistant isolate, and carbapenemases-producing *Escherichia coli* and *Klebsiella pneumoniae*. Selectivity indices up to 2.4 were determined by cytotoxicity experiments on human keratinocytes (HaCaT), thus suggesting that CB1H-P7 NPs could be promising candidates for therapeutic uses in the treatment of infections sustained by most dangerous MDR isolates tested in this study.

Keywords: CB1H-loaded copolymer NPs; cytotoxicity to human cells; Gram-positive and Gram-negative MDR isolates; MICs and MBCs; pyrazole compounds; selectivity index; time-kill experiments.

Introduction

Drug resistance is a multifactorial phenomenon that limits the action of antibiotics

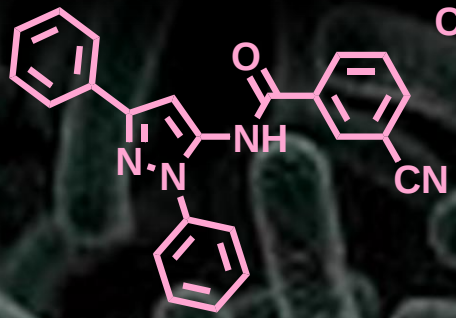
The pyrazole ring is known to have numerous biological activities, including antimicrobial effects

Infections caused by the MDR, XDR, or even PDR bacteria are a global concern because they are nearly untreatable

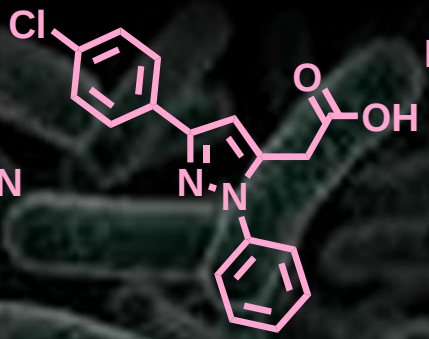
Conventional antibiotics are no longer effective and need to be replaced with new antibacterial agents suitable for clinical application.

Commercial Drugs Containing Pyrazoles

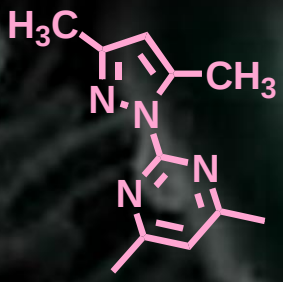
Celecoxib
Anti-inflammatory



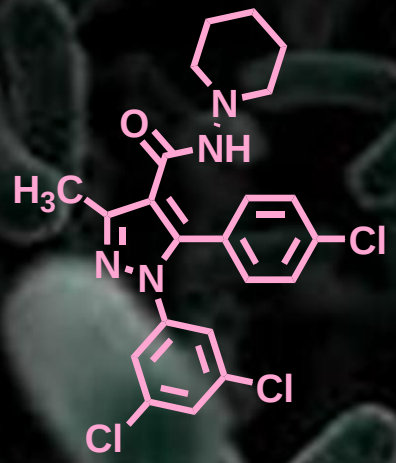
CDPPB
Anti-psychotic



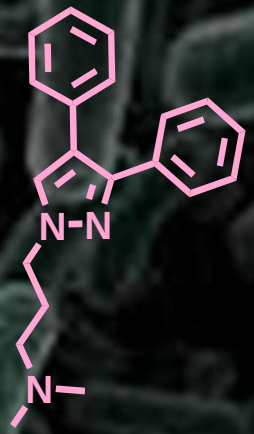
Lonazolac
Anti-inflammatory



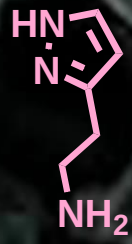
Mepirizole
Anti-Inflammatory



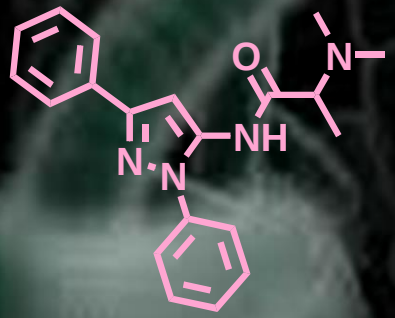
Rimodabant
Anti-obesity



Fezolamine
Anti-depressant



Betazole
H2-receptor agonist



Difenamizole
Analgesic

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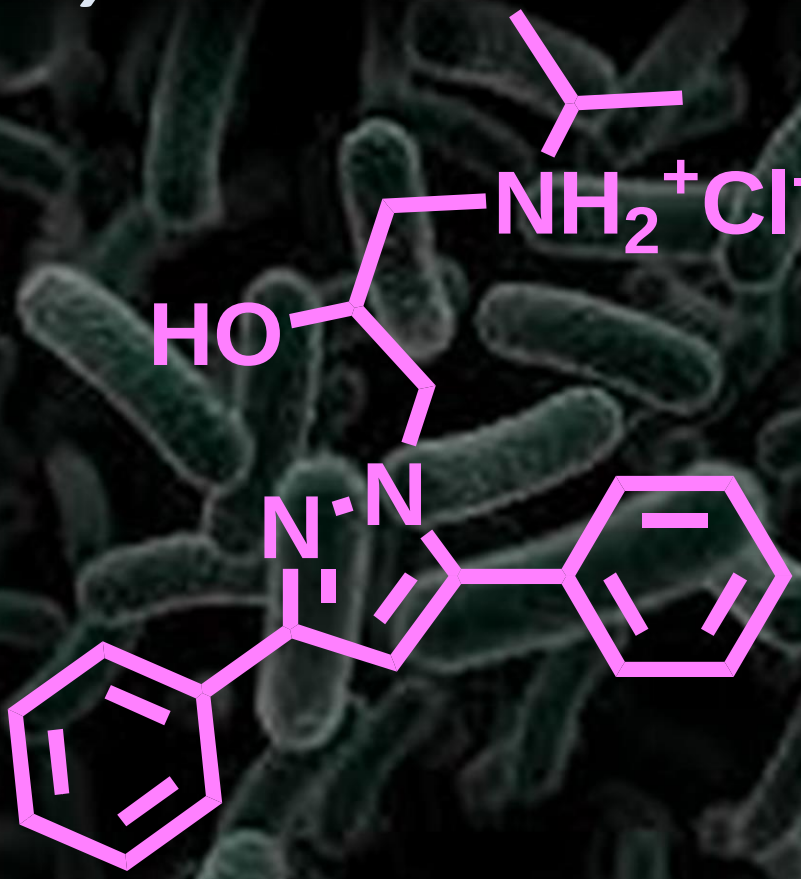
Pyrazole Used in This Study



[3-(3,5-Diphenyl-pyrazol-1-yl)-2-hydroxy-propyl]-isopropyl-ammonium; chloride

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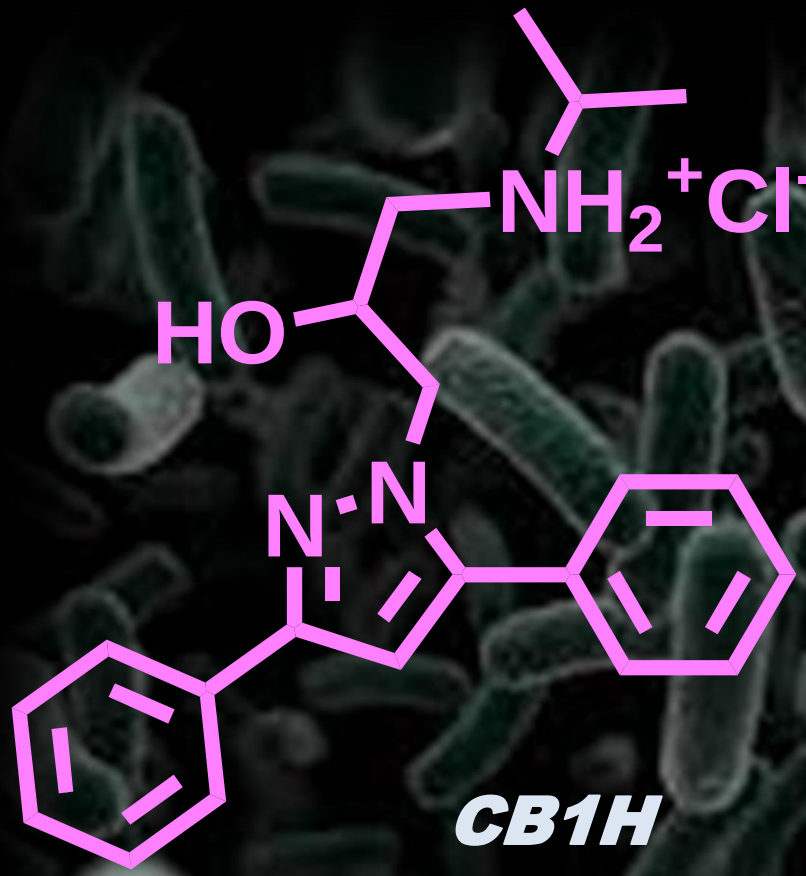
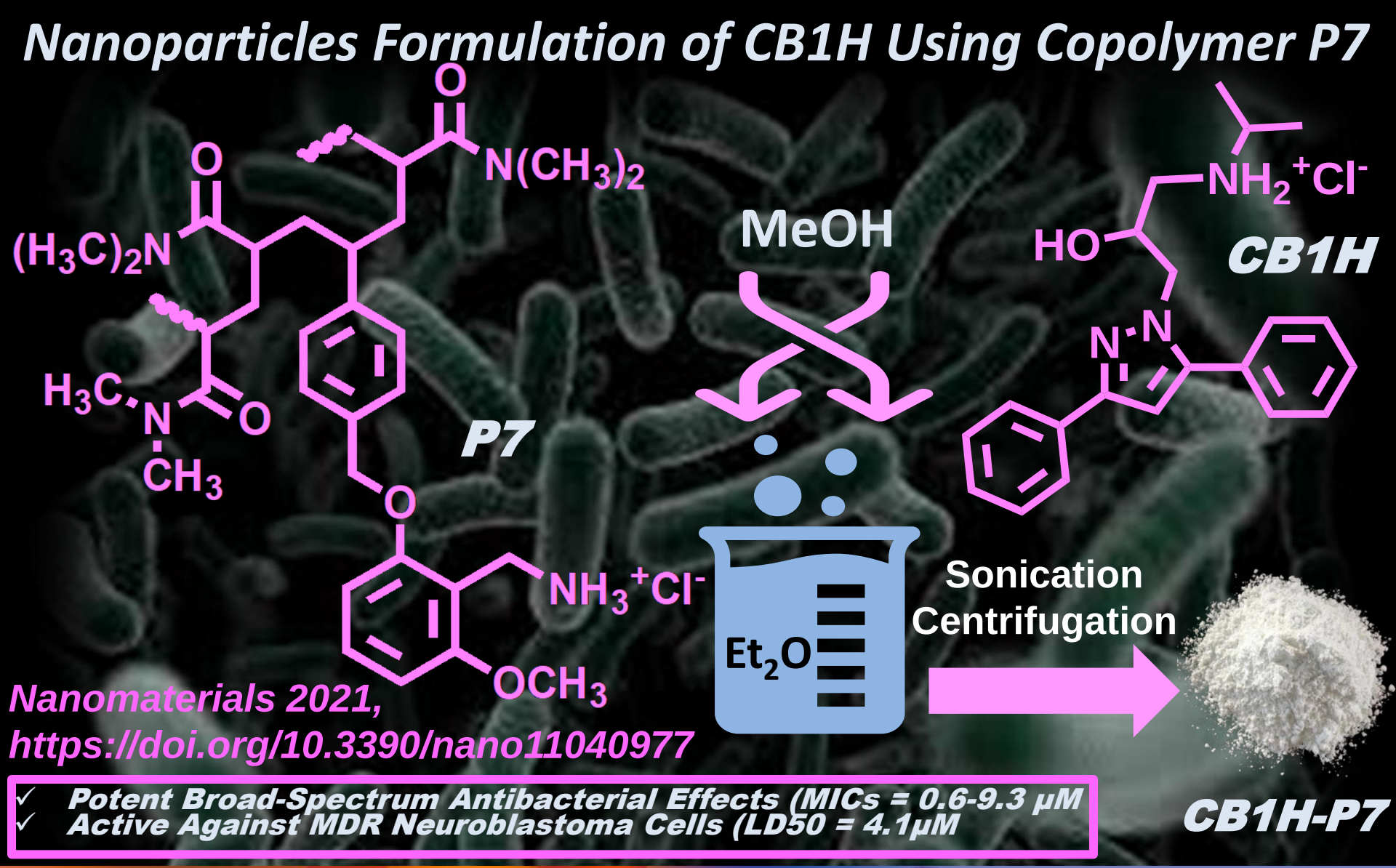


CB1H

- ✓ ***Well Water-Soluble***
- ✓ ***Weak Antibacterial Effects on Gram-positives***
  (MICs = 128-256 µg/mL)
- ✓ ***No Antibacterial Activity on Gram-negatives***

Nanomaterials 2022, *https://doi.org/10.3390/nano12071215*

Nanoparticles Formulation of CB1H Using Copolymer P7



Nanomaterials 2021,
https://doi.org/10.3390/nano11040977

- ✓ *Potent Broad-Spectrum Antibacterial Effects (MICs = 0.6-9.3 μM*
- ✓ *Active Against MDR Neuroblastoma Cells (LD50 = 4.1μM*

In this study, CB1H-P7 NPs have been tested on several clinical isolates of Gram-positive and Gram-negative species, including MDR strains, observing high improvements in the antibacterial properties of both CB1H and P7 by a complex-based cooperation, and strong bactericidal effects already after 1 h of exposure. Particularly, very low MICs were observed for CB1H-P7 NPs, thus establishing that when complexed, P7 was 2-fold more potent that P7 alone against all bacteria tested, while CB1H was 2–16-fold and 4–33-fold more potent than pristine CB1H against Gram-positive and Gram-negative bacteria respectively.

Conjecturing a possible clinical use of CB1H-P7 NPs, cytotoxicity experiments on human keratinocytes (HaCaT) were performed with P7, CB1H and CB1H-P7 NPs. Even if both P7 and CB1H-P7 NPs manifested values of LD_{50} lower than that of pristine CB1H, the selectivity indices (SIs) determined for all bacteria for both compounds were significantly higher than those of CB1H and SIs of CB1H-P7 NPs were higher than those of P7 for the most part of bacteria tested in this study.

Results and discussion

Antibacterial Effects on Gram-positives

Strains	Original P7 (13,719) [1] MIC μM (μg/mL)	Original CB1H (371.9)[2] MIC μM (μg/mL)	CB1H-P7 NPs (26624)[1] MIC μM (μg/mL)	Complexed P7 (13,719) [1] MIC μM (μg/mL)	Complexed CB1H (371.9)[2] MIC μM (μg/mL)
			Enterococcus genus		
E. faecalis 1*,#	**2.3 (32)**	**344.2 (128)**	1.2 (32)	**1.2 (16.4)**	**41.9 (15.6)**
E. faecalis 365*	2.3 (32)	**344.2 (128)**	2.4 (64)	2.4 (32.9)	**83.6 (31.1)**
E. faecalis 450*	2.3 (32)	**344.2 (128)**	2.4 (64)	2.4 (32.9)	**83.6 (31.1)**
E. faecium 21*	1.2 (16)	**344.2 (128)**	1.2 (32)	1.2 (16.4)	**41.9 (15.6)**
E. faecium 325*	**1.2 (16)**	**344.2 (128)**	0.6 (16)	**0.6 (8.2)**	**21.0 (7.8)**
E. faecium 341*,#	**1.2 (16)**	**344.2 (128)**	0.6 (16)	**0.6 (8.2)**	**21.0 (7.8)**
			Staphylococcus genus		
S. aureus 18**	**4.6 (64)**	**344.2 (128)**	2.4 (64)	**2.4 (32.9)**	**83.6 (31.1)**
S. aureus 187**	4.6 (64)	**344.2 (128)**	4.8 (128)	4.8 (65.8)	**167.2 (62.2)**
S. aureus 195**	**4.6 (64)**	**344.2 (128)**	2.4 (64)	**2.4 (32.9)**	**83.6 (31.1)**
S. epidermidis 22**	**1.2 (16)**	**344.2 (128)**	0.6 (16)	**0.6 (8.2)**	**21.0 (7.8)**
S. epidermidis 180***	1.2 (16)	**344.2 (128)**	1.2 (32)	1.2 (16.4)	**41.9 (15.6)**
S. epidermidis 181***	**1.2 (16)**	**344.2 (128)**	0.6 (16)	**0.6 (8.2)**	**21.0 (7.8)**

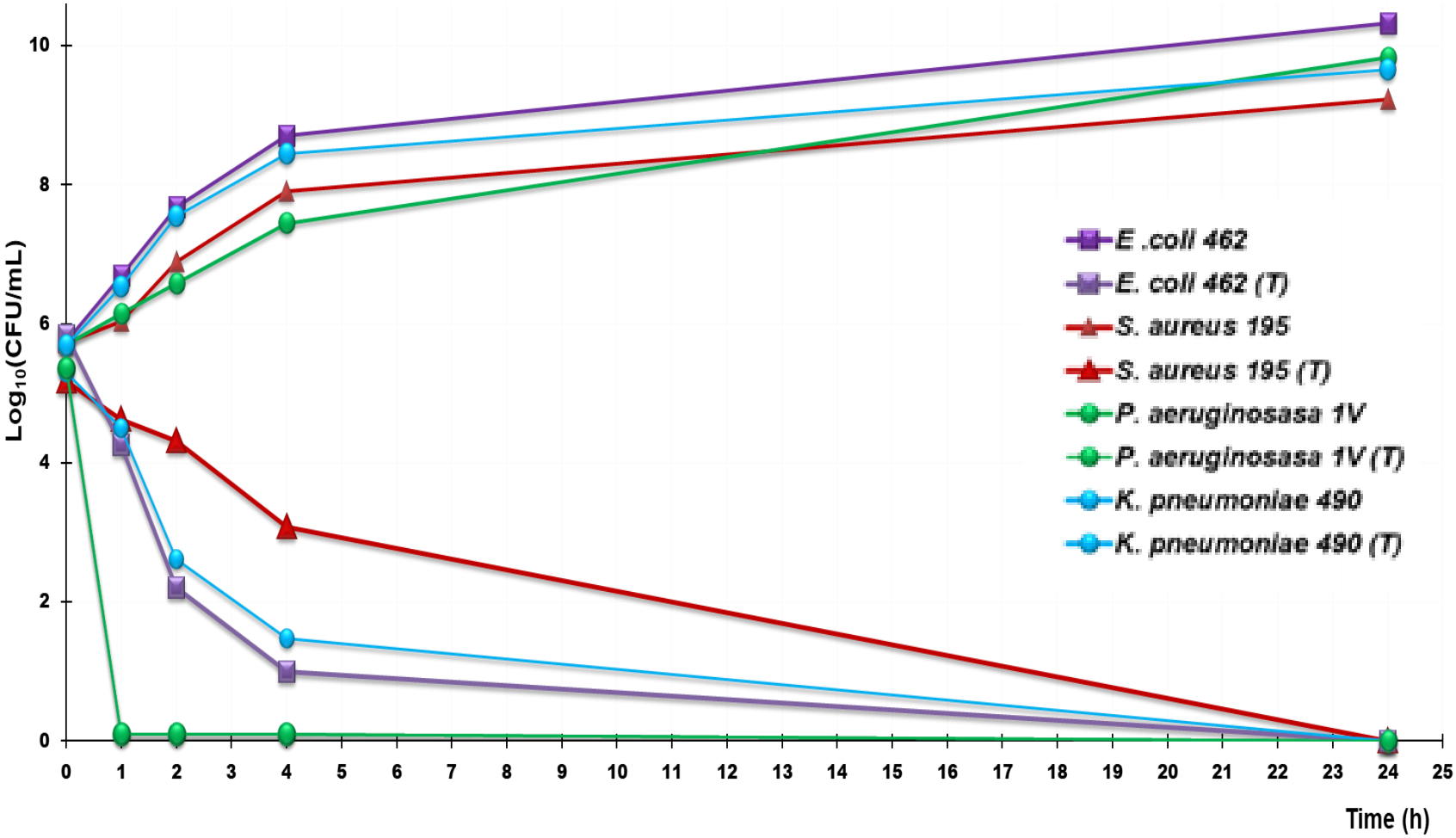
[1] Average molecular mass (Mn); [2] MW; [3] refers to CB1H-P7 NPs; * denotes vancomycin resistanCE (VRE), *,#VRE resistant also to teicoplanin; ** denotes methicillin resistanCE; *** denotes resistance toward methicillin and linezolid.

Antibacterial Effects on Gram-negatives

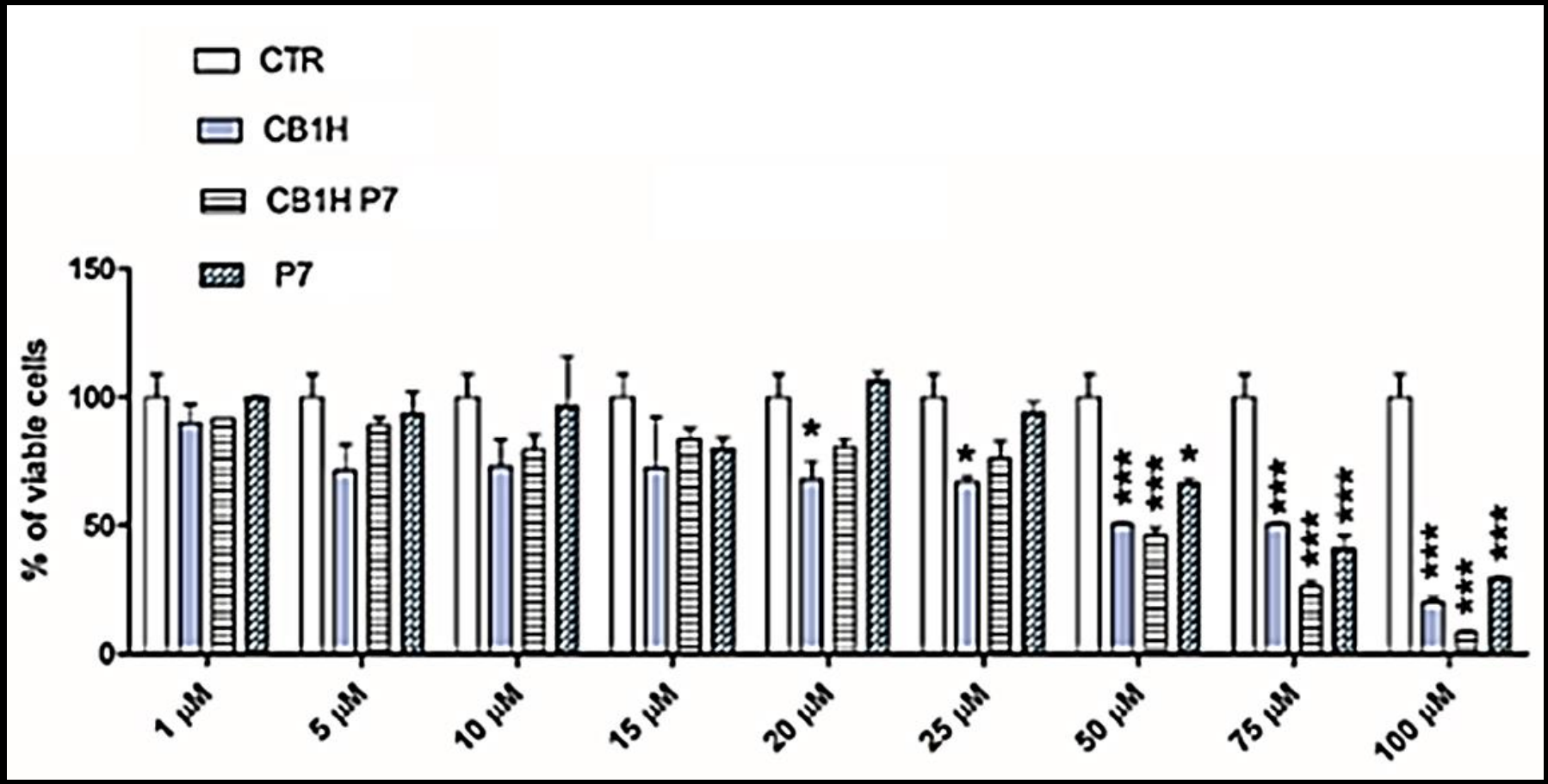
Strains	Original P7 (13,719) [1] MIC μM (μg/mL)	Original CB1H (371.9)[2] MIC (μg/mL)	CB1H-P7 NPs (26624)[1] MIC μM (μg/mL)	Complexed P7 (13,719) [1] MIC μM (μg/mL)	Complexed CB1H (371.9)[2] MIC μM (μg/mL)[4]
			Enterobacteriaceae family		
E. coli 238 #	**4.6 (64)**	**>688.4 (>256)**	1.2 (32)	**1.2 (16.5)**	**41.9 (15.6)**
E. coli 477#	**2.3 (32)**	**>688.4 (>256)**	1.2 (32)	**1.2 (16.5)**	**41.9 (15.6)**
E. coli 462§	**2.3 (32)**	**>688.4 (>256)**	1.2 (32)	**1.2 (16.5)**	**41.9 (15.6)**
K. pneumoniae 375#	4.6 (64)	**>688.4 (>256)**	4.8 (128)	4.8 (65.8)	**167.2 (62.2)**
K. pneumoniae 376#	9.3 (128)	**>688.4 (>256)**	9.6 (256)	9.1 (124.4)	**35.4 (131.6)**
K. pneumoniae 377#	**9.3 (128)**	**>688.4 (>256)**	4.8 (128)	**4.8 (65.8)**	**167.2 (62.2)**
K. pneumoniae 490 CR,#	**4.6 (64)**	**>688.4 (>256)**	2.4 (64)	**2.3 (32.9)**	**83.6 (31.1)**
E. aerogenes 484##	**4.6 (64)**	**>688.4 (>256)**	1.2 (32)	**1.2 (16.5)**	**41.9 (15.6)**
			Non-fermenting species		
A. baumannii 257	**2.3 (32)**	**>688.4 (>256)**	1.2 (32)	**1.2 (16.5)**	**41.9 (15.6)**
A. baumannii 279	**2.3 (32)**	**>688.4 (>256)**	1.2 (32)	**1.2 (16.5)**	**41.9 (15.6)**
A. baumannii 245 COR	**9.3 (128)**	**>688.4 (>256)**	1.2 (32)	**1.2 (16.5)**	**41.9 (15.6)**
P. aeruginosa 1V##	**2.3 (32)**	**>688.4 (>256)**	1.2 (32)	**1.2 (16.5)**	**41.9 (15.6)**
P. aeruginosa 5V##	**4.6 (64)**	**>688.4 (>256)**	2.4 (64)	**2.3 (32.9)**	**83.6 (31.1)**
P. aeruginosa 6V##	**4.6 (64)**	**>688.4 (>256)**	2.4 (64)	**2.3 (32.9)**	**83.6 (31.1)**
P. aeruginosa 7G##	**4.6 (64)**	**>688.4 (>256)**	2.4 (64)	**2.3 (32.9)**	**83.6 (31.1)**
P. aeruginosa 265 CR,##	**≥18.6 (≥256)**	**>688.4 (>256)**	1.2 (32)	**1.2 (16.5)**	**41.9 (15.6)**
P. aeruginosa 432 PY,##	1.2 (16)	**>688.4 (>256)**	1.2 (32)	1.2 (16.5)	**41.9 (15.6)**
P. aeruginosa 447 PY,##	**4.6 (64)**	**>688.4 (>256)**	1.2 (32)	**1.2 (16.5)**	**41.9 (15.6)**
P. aeruginosa 244 PY,##	**4.6 (64)**	**>688.4 (>256)**	1.2 (32)	**1.2 (16.5)**	**41.9 (15.6)**
P. aeruginosa 259*,##	**9.3 (128)**	**>688.4 (>256)**	9.6 (256)	**9.1 (124.4)**	**35.4 (131.6)**
S. maltophilia 2 COI	**2.3 (32)**	**>688.4 (>256)**	1.2 (32)	**1.2 (16.5)**	**41.9 (15.6)**
S. maltophilia 280 COI	**4.6 (64)**	**>688.4 (>256)**	1.2 (32)	**1.2 (16.5)**	**41.9 (15.6)**
S. maltophilia 384COR	**2.3 (32)**	**>688.4 (>256)**	1.2 (32)	**1.2 (16.5)**	**41.9 (15.6)**
S. maltophilia 390COR	2.3 (32)	**>688.4 (>256)**	2.4 (64)	2.3 (32.9)	**83.6 (31.1)**

[1] average molecular mass (Mn); [2] MW; [3] refers to CB1H-P7 NPs; # denotes KPCs-producing isolates; § denotes New Delhi metallo carbapanemases (NDMs) producing isolate; *P. aeruginosa, S. maltophilia* and *A. baumannii* were all MDR bacteria; 1V, 5V, 6V, 7G = MDR strains isolated from patients with cystic fibrosis; CR = MDR (*P. aeruginosa*) or KPCs-producing (*K. pneumoniae*) strains resistant also to colistin; PY pyomelanin producers; * resistant to the combination avibactam-ceftazidime; ## resistant to carbapenems; COR co-trimoxazole resistant; COI co-trimoxazole intermediate

Time-Killing Curves and Bactericidal Effects on Representative MDR Isolates of Gram-positive and Gram-negative Species



Cytotoxicity of P7, CB1H and CB1H-P7 NPs on HaCaT Human Keratinocytes Cells



The statistical significance of differences between experimental and control groups was determined via a two-way analysis of variance (ANOVA) with the Bonferroni correction. Asterisks indicate the following p-value ranges: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

LD_{50} and Sis of P7, CB1H and CB1H-P7 NPs

Sample	Equations	R^2	LD_{50} (μM)	SI	
				Gram-positive	Gram-negative
P7	y = -25.7640x + 103.4	0.8972	2.1	0.5-1.8	0.1-1.8
CB1H	y = -0.6059x + 84.712	0.8648	57.3	≤0.4.	≤0.2.
CB1H-P7 NPs	y = -31.0430x + 94.949	0.9842	1.5	0.3-2.4	0.2-1.3

Conclusions

- CB1H-P7 NPs previously prepared were evaluated *in vitro* for assessing their antibacterial effects, for investigating their cytotoxicITY profile, and for determining their selectivity indices (SIs).
- CB1H-P7 NPs were essayed on 36 MDR clinical isolates, including different genera of Gram-positive and Gram-negative species obtaining excellent results. The antibacterial potency ranged from being considerable (MIC = 4.8 μM) to very potent (MICs = 0.6-2.4 μM) against 34 out of 36 isolates tested.
- CB1H-P7 NPs displayed very low MICs against colistin-resistant isolates of *P. aeruginosa* (1.2 μM) and *K. pneumoniae* (2.4 μM), currently untreatable by the presently available antibiotics.
- CB1H-P7 NPs were effective against an isolate of *E. coli* producing NDM β-lactamases, which are capable to hydrolyse all β-lactam antibiotics except for aztreonam and against which no approved inhibitor works.

Conclusions

- Time kill experiments carried out on different MDR strains of *P. aeruginosa*, *S. aureus*, *E. coli*, and *K. pneuomniae*, evidenced that after 24 h of exposure, CB1H-P7 NPs were capable of killing all bacteria tested.
- Dose-dependent cytotoxicity studies performed for 24 hours on human keratinocyte cells (HaCaT) and the determination of the relative SIs of CB1H-P7 NPs, showed that, by complexing CB1H with P7, not only the antibacterial effects of both the ingredients (CB1H and P7) were improved, but also their SIs.
- CB1H-P7 NPs have SIs up to 3.3-fold higher than those of P7 and up to 6.5-fold higher than those of BC1H.

Future Perspectives

Although further investigations are needed to evaluate the actual clinical applicability of CB1H-P7 NPs developed here, the preliminary results obtained in this study suggest that they may be used in the future as powerful new agents capable of rapidly killing even the most alarming MDR bacteria and to cure difficult-to-treat bacterial infections.

Thank you!

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