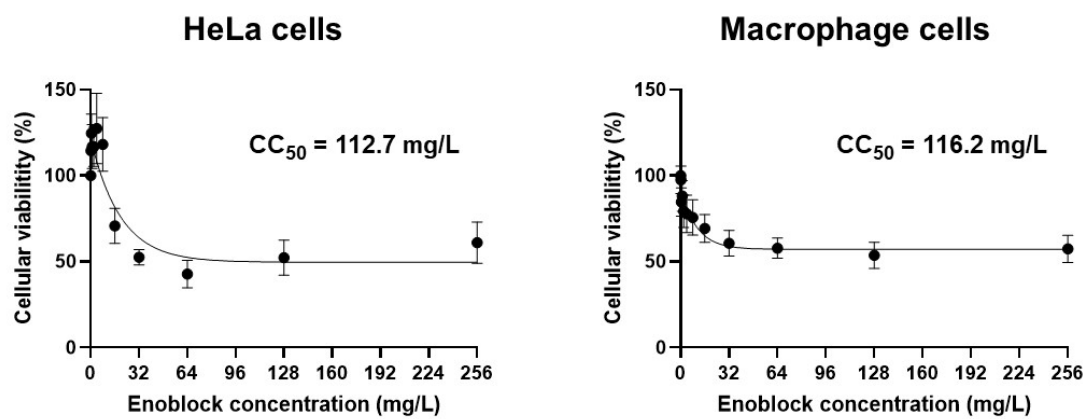


Appendix for “ENOblock synergizes with colistin to treat *Acinetobacter baumannii* infections”

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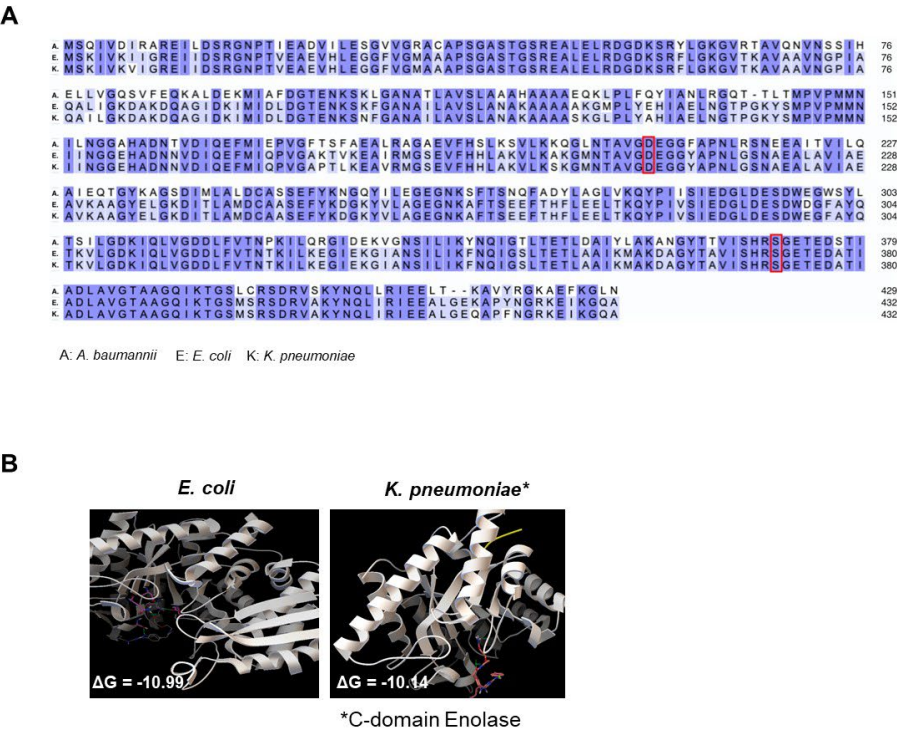
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Appendix Figure S1



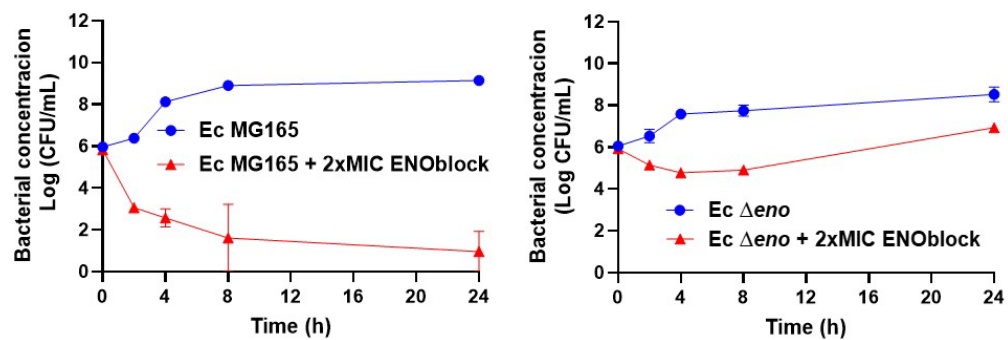
Appendix Figure S1. Cellular viability of epithelial and macrophage cells in presence of different concentration of ENOblock.

Appendix Figure S2



Appendix Figure S2. ENOblock acts on *E. coli* and *K. pneumoniae* through the inhibition of enolase. (A) The enolase sequences of *A. baumannii*, *E. coli* and *K. pneumoniae*. (B) Structural models generated by docking of ENOblock into *E. coli* and *K. pneumoniae* enolase. ENOblock is displayed as sticks. ΔG: Glide score.

Appendix Figure S3



Appendix Figure S3. ENOblock acts on *E. coli* through the inhibition of enolase. Time-kill curves of *E. coli* *Ec* MG165⁺ and *Ec* Δeno strains in the presence of 2xMIC ENOblock for 24 hours.

Appendix Figure S4

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Homo sapiens msiekiwareildsrngnptvevdlytakglfraavpsgastgiyealelrdgdkqrylgkgv lkavdhinstiapa 76
A. baumannii msiekiwareildsrngnptvevdlytakglfraavpsgastgiyealelrdgdkqrylgkgv lkavdhinstiapa 76
G. mellonella mpiksikarqifdsrgnptvevdlytelglfraavpsgastgvhealelrdnknqyhgkgv ltaikninei iapa 76

Homo sapiens lissglsvveqekldnlmleldgtenkskfganaillgvs lavckagaaere lplyrhiaqlagnsdli lvpvafnv 152
A. baumannii lissglsvveqekldnlmleldgtenkskfganaillgvs lavckagaaere lplyrhiaqlagnsdli lvpvafnv 152
G. mellonella l lkqnidvtqqneidqfmisldgtenkskl ganaillgvs lavakagaaaskgvplykhla dls gnnn i v l p v p a f n v 152

Homo sapiens ing-shagnklamqefmilpvgaesfrdamrlgaevyhtlkgvikdkkygkdatnvvgdeggfapnilensealelvk 227
A. baumannii ing-shagnklamqefmilpvgaesfrdamrlgaevyhtlkgvikdkkygkdatnvvgdeggfapnilensealelvk 227
G. mellonella inggshagnklamqefmilptgatsfseamrmgsevyhylkkii kekf gldstavgdeggfapnilnnkdal fliq 228

Homo sapiens eaidkagytekivigmdvaasefyrdgkydlldfkspt-dpsryitgdqlgalyqdfvrdypvvsiedpfdqddwaa 302
A. baumannii eaidkagytekivigmdvaasefyrdgkydlldfkspt-dpsryitgdqlgalyqdfvrdypvvsiedpfdqddwaa 302
G. mellonella daiqqagytgkieigmdvaasefykngtydlldfknpsnpadylpsdkladlylefikefplvsiedpfdqddwaa 304

Homo sapiens wskftanvgiqivgddltvtnpkrieraveekacnc lllkvnqigs vtea iqacklaqengwgvmvshrsgetedt 378
A. baumannii wskftanvgiqivgddltvtnpkrieraveekacnc lllkvnqigs vtea iqacklaqengwgvmvshrsgetedt 378
G. mellonella wsgltsrtpiqivgddltvtnpkriatavekkacnc lllkvnqigs vtesiqahllakknwggtmvshrsgetedt 380

Homo sapiens fiadlvvg lctgqiktgapcrserlakynqlmr ieeelgd earfagh nfrnpsv l 433
A. baumannii fiadlvvg lctgqiktgapcrserlakynqlmr ieeelgd earfagh nfrnpsv l 433
G. mellonella fiadlvvg l stgqiktgapcrserlakynqilr ieeelgaaakyagknfr rpv - - 433

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Appendix Figure S4. The enolase sequences of *Homo sapiens*, *Acinetobacter baumannii* and *Galleria mellonella*.

Appendix Table S1

Appendix Table S1. Plasmids and primers used in this study.

Plasmids, and primers	Relevant features and use	References
pGEM-T	Suicide plasmid for <i>A. baumannii</i> . Amp ^R	Promega (Spain)
enolase intUp	5-TGACGGCGACAAATCTCGTT-3	Present study
enolase intLw	5-GCGCAAAACCCACCTTCATCA-3	Present study
enolase extUp	5-CTTGAGCCATTCTTGGCACA-3	Present study
enolase extLw	5-TTAAAAACCCGGCGCTTAGG-3	Present study
M13F	5-GTAAACGACGGCCAGT-3	Smani et al. 2013
M13R	5-CAGGAAACAGCTATGAC-3	Smani et al. 2013

Appendix Table S2

Appendix Table S2. EIIP and AQVN parameters to analyze the electronic properties of drugs tested for synergy against *A. baumannii* Ab ATCC 17978 strain

Molecule	AQVN	EIIP
ENOblock	2.628	0.078
Colistin	2.592	0.084
Imipenem	2.973	0.034
Ceftazidime	3.288	0.127
Tigecycline	2.815	0.025

AQVN: and average quasi-valence number, EIIP: Electron-ion interaction potential.

Appendix Table S3

Appendix Table S3. Antibacterial activity of ENOblock in colistin-resistant *E. coli* strains.

Strain#	Colistin resistance mechanism	Colistin MIC (mg/L)	ENOblock MIC (mg/L)
CRA5	-	8	16
CRA7	-	8	16
CRA8	MCR1	8	16
CRA17	-	8	32
CRA20	MCR1	8	32
CRA32	-	8	8
CRA57	-	4	16
R2	MCR1	8	32
R5	MCR1	4	32
R6	MCR1	8	16
R34	MCR1.5	8	64
R37	MCR3.2	8	32
MCR1+	MCR1	4	64

Appendix Table S4

Appendix Table S4. Antibacterial activity of ENOblock in carbapenem-resistant *K. pneumoniae* strains.

Strain#	Meropenem resistance mechanism	Meropenem MIC (mg/L)	ENOblock MIC (mg/L)
Kp6	KPC-3	2	32
Kp7	KPC-2	-	16
Kp8	KPC-2	-	64
Kp9	KPC-41	-	64
Kp10	KPC-2	16	32
Kp11	KPC-2	4	16
Kp12	KPC-2	16	64
Kp13	KPC-3	>16	64
Kp14	KPC-2	4	64
Kp15	KPC-11	4	64
Kp16	KPC-3	>16	64
Kp17	KPC-3	16	16
Kp18	KPC-2	4	32
Kp19	KPC-3	4	64

Appendix Table S5

Appendix Table S5. ISM frequencies for ENOblock intermolecular interactions and corresponding signal – to – noise (S/N) ratios.

Interaction	F	S/N
Enolase - ENOblock	0.271	12.44
	0.435	3.40
Fibronectin - enolase - ENOblock	0.271	51.498
	0.435	11.96
Fibrinogen - enolase - ENOblock	0.271	18.161
	0.435	8.78