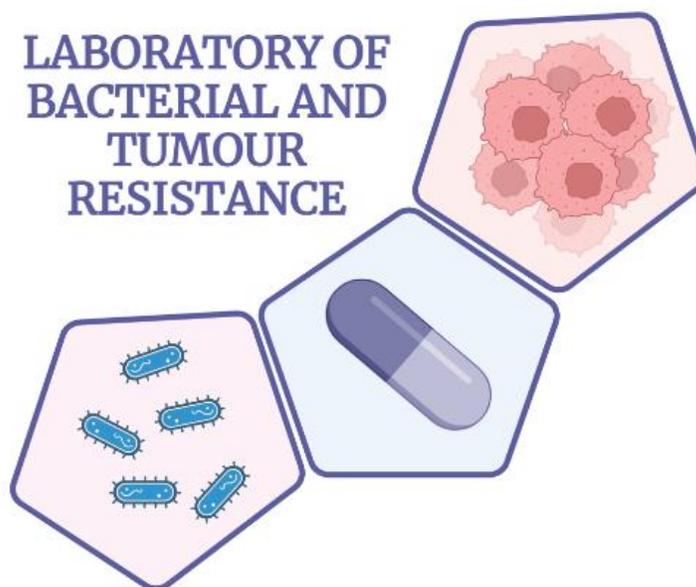




Steroidní hormony – nové terapeutické strategie při bakteriálních infekcích

Jitka Viktorová

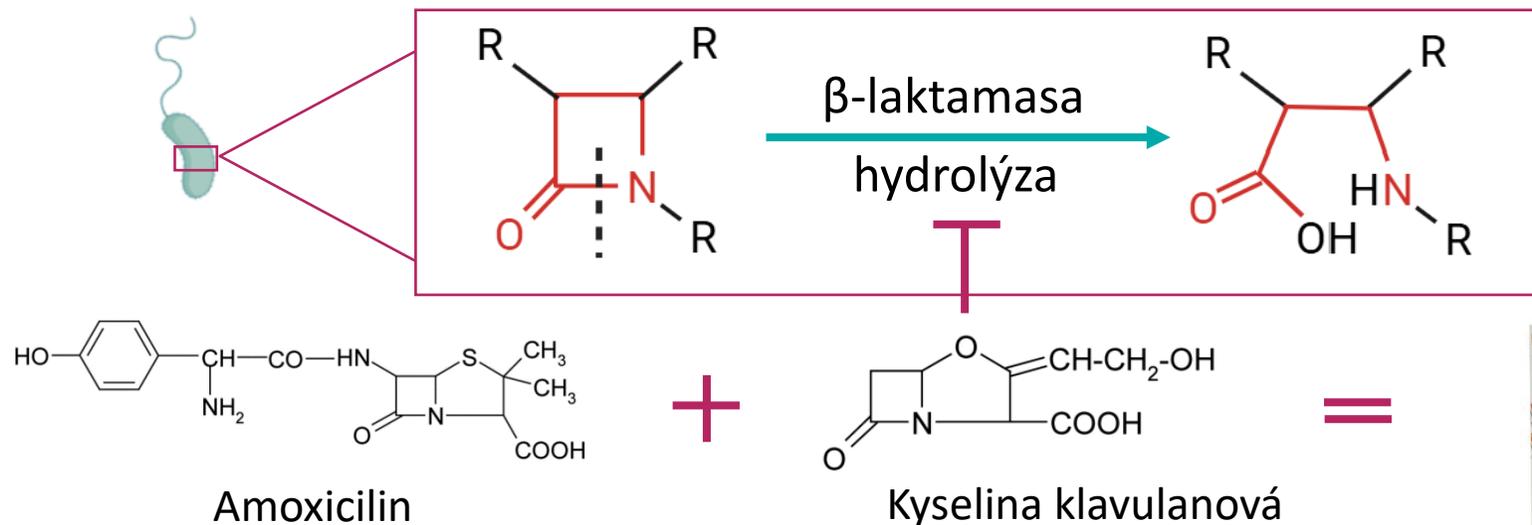


Adjuvantní terapie



β -laktamové antibiotikum

Nemá antimikrobiální aktivitu



V experimentálním vývoji

- Anhydrotetracyklinové deriváty (TetX, monooxygenasa)
- Osthol (mcr-1, fosfoethanolamin transferasa)
- Inhibitory aminoglykosid-modifikujících enzymů
- Nové inhibitory β -laktamas

V klinické praxi (EUCAST)

Piperacillin/Tazobactam, Ticarcillin/Clavulanic acid, Ceftazidime/Avibactam, Ceftolozane/Tazobactam, Imipenem/Relebactam, Meropenem/Vaborbactam
....

Hypotéza



Steroidní hormony a neurosteroidy mohou být potenciálními inhibitory bakteriálních efluxních pump a zvyšovat účinnost antibiotik

Na základě důkazů:

- **Nadprodukce efluxních pump** – základní mechanismus rezistence
- U lidí jsou endogenní steroidy **substráty efluxních pump**, které vykazují značnou **homologii s bakteriálními efluxními pumpami**.
- Nové přístupy v personalizované medicíně založené na pohlaví a hormonálním stavu.

Regulation of ABC transporters by sex steroids may explain differences in drug resistance between sexes

Rafael Mineiro¹, Cecília Santos¹, Isabel Gonçalves¹, Manuel Lemos¹, José Eduardo B Cavaco¹, Telma Quintela^{2,3}

Mammalian Steroid Hormones Are Substrates for the Major RND- and MFS-Type Tripartite Multidrug Efflux Pumps of *Escherichia coli*

Authors: Christopher A. Elkins ✉, Lisa B. Mullis | AUTHORS INFO & AFFILIATIONS

The effects of hormones on sex differences in infection: from genes to behavior

S.L. Klein 👤 ✉

Role of Sex Steroid Hormones in Bacterial-Host Interactions

Elizabeth García-Gómez, Bertha González-Pedrajo, Ignacio Camacho-Arroyo ✉

Sexual dimorphism in bacterial infections

Edgar Ricardo Vázquez-Martínez¹, Elizabeth García-Gómez², Ignacio Camacho-Arroyo¹ and Bertha González-Pedrajo^{3*}

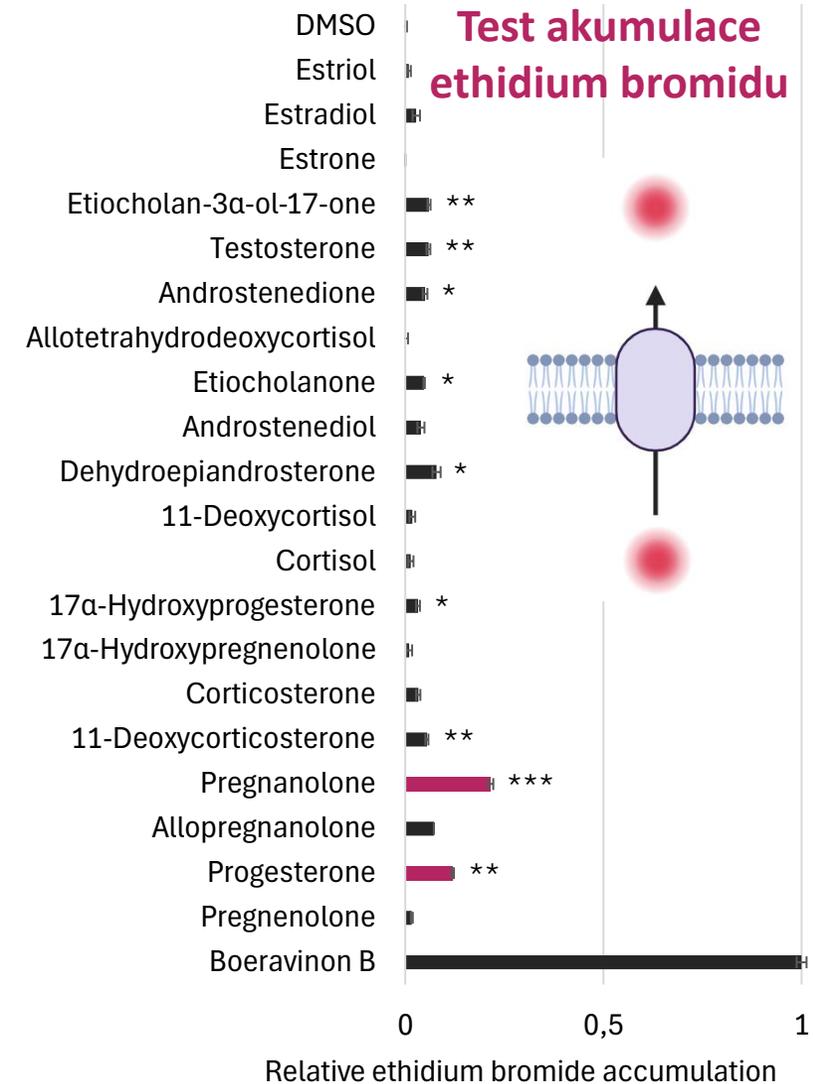
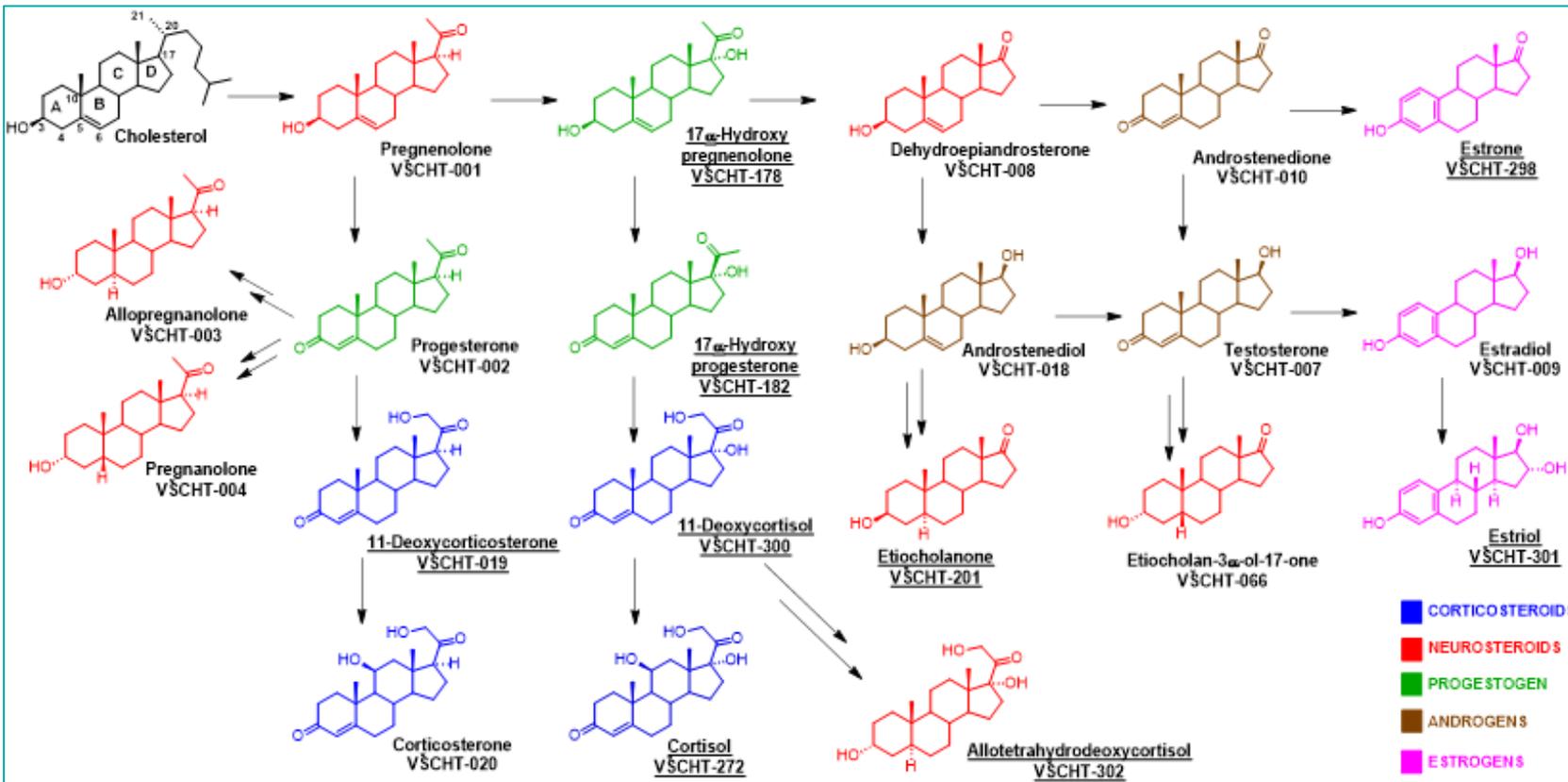
Effect of Androgens and Glucocorticoids on Microbial Growth and Antimicrobial Susceptibility

Balbina J. Plotkin,¹ Robert J. Roose,² Quenby Erikson,¹ Susan M. Viselli²

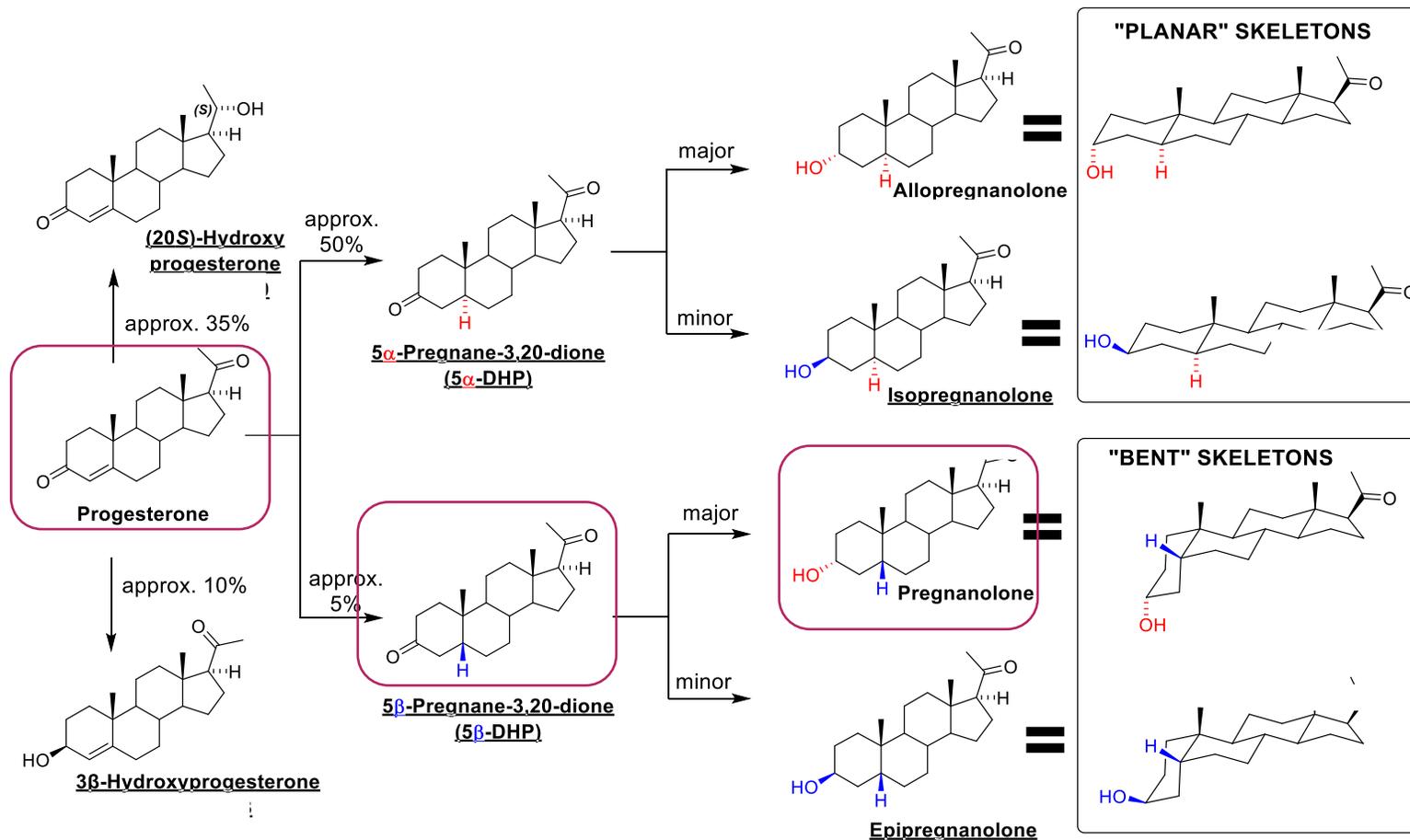
Mohou endogenní steroidy ovlivnit efluxní systém?



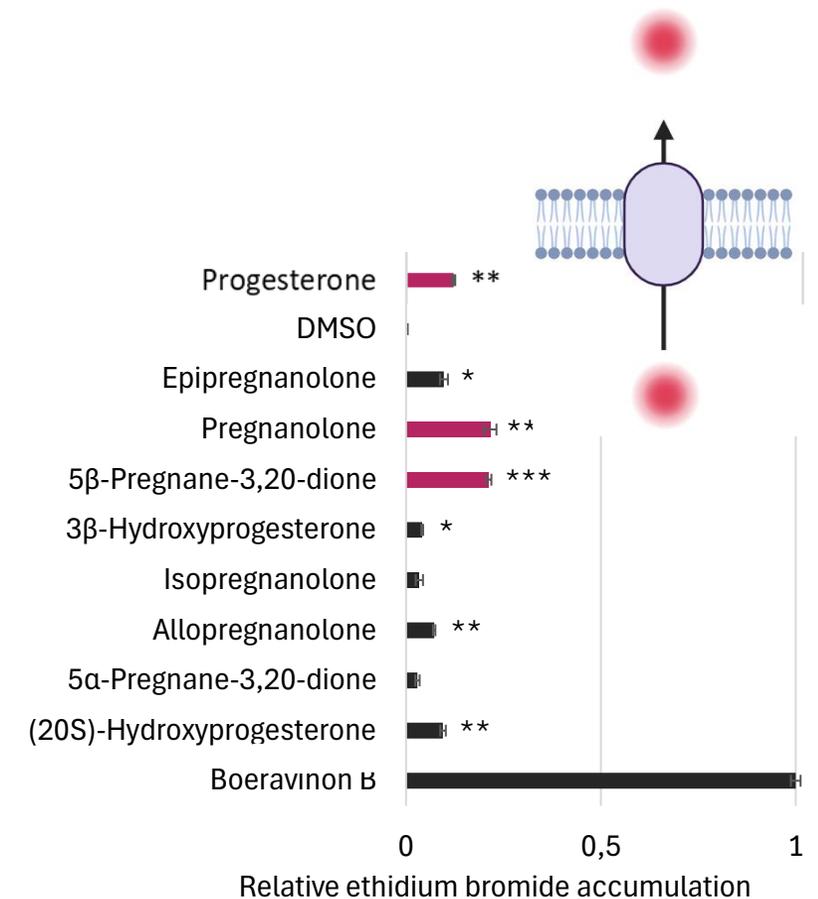
Methicilin-rezistentní *Staphylococcus aureus* kmen SA4



Mohou endogenní steroidy ovlivnit efluxní systém?



Test akumulace ethidium bromidu



Senzitizace MRSA k antibiotikům

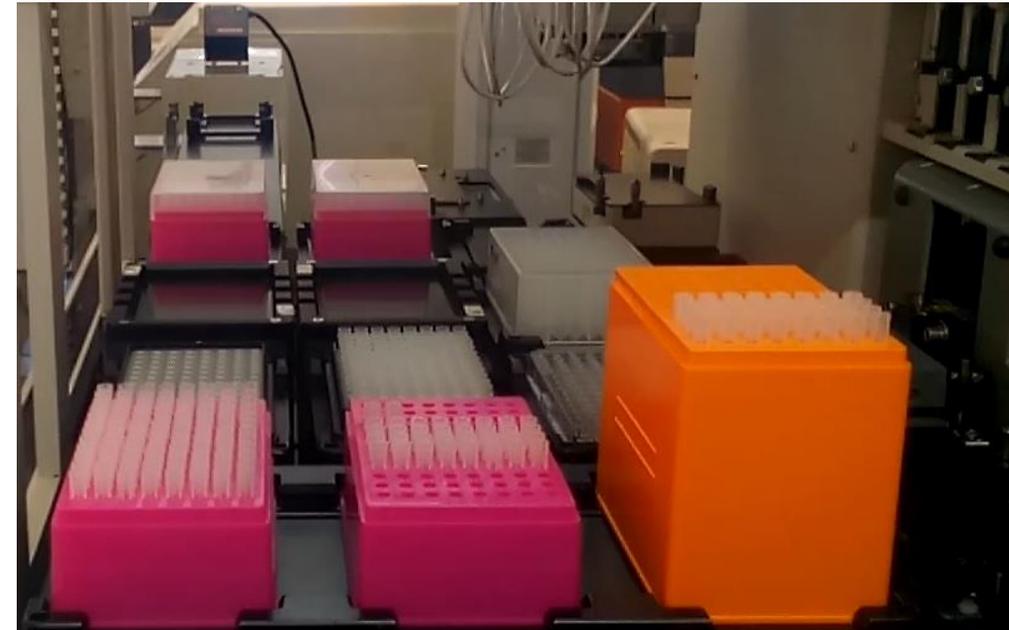


Estriol
Estradiol
Estrone
Etiocholan-3 α -ol-17-one
Testosterone
Androstenedione
Allotetrahydrodeoxycortisol
Etiocholanone
Androstenediol
Dehydroepiandrosterone
11-Deoxycortisol
Cortisol
17 α -Hydroxyprogesterone
17 α -Hydroxypregnenolone
Corticosterone
11-Deoxycorticosterone
➔ Pregnanolone
➔ Allopregnanolone
➔ Progesterone
Pregnenolone

Methicilin-rezistentní *Staphylococcus aureus* kmen SA4

Ciprofloxacin
Tetracycline
Chloramphenicol
Erythromycin
Gentamicin
Oxacillin

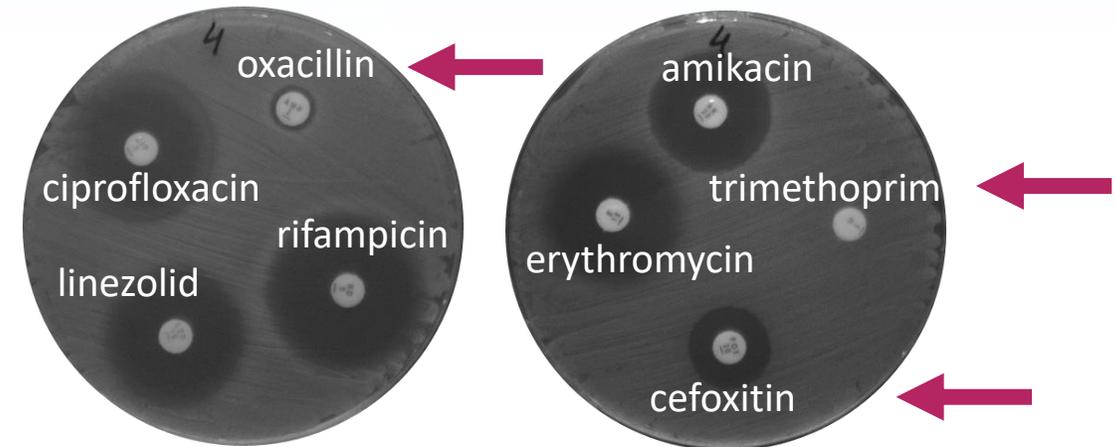
➔
**Ciprofloxacin
Erythromycin**



Celogenomové sekvenování (WGS)

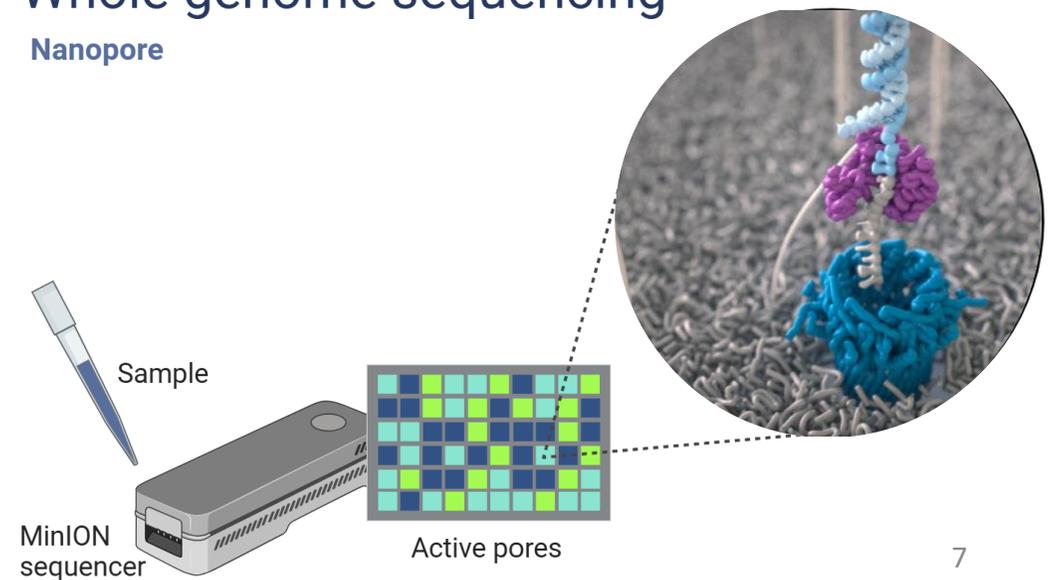


bla _I _of_Z	penicillinase repressor BlaI
bla _{R1}	β-lactam sensor/signal transducer BlaR1
bla _Z	penicillin-hydrolyzing class A β-lactamase BlaZ
mecA	β-lactam-resistant peptidoglycan transpeptidase (PBP2a)
dfrC	trimethoprim-resistant dihydrofolate reductase DfrC
dfrS1	trimethoprim-resistant dihydrofolate reductase DfrS1
LmrS	major facilitator superfamily (MFS) transporter
tet(38)	major facilitator superfamily (MFS) transporter
norA	major facilitator superfamily (MFS) transporter
arlR	major facilitator superfamily (MFS) transporter
arlS	major facilitator superfamily (MFS) transporter
kdpD	kdpDE
mepA	multidrug and toxic compound extrusion (MATE) transporter
mepR	multidrug and toxic compound extrusion (MATE) transporter
mgrA	ATP-binding cassette (ABC) transporter
norC	major facilitator superfamily (MFS) transporter
sdrM	major facilitator superfamily (MFS) transporter
sepA	small multidrug resistance (SMR) transporter



Whole genome sequencing

Nanopore

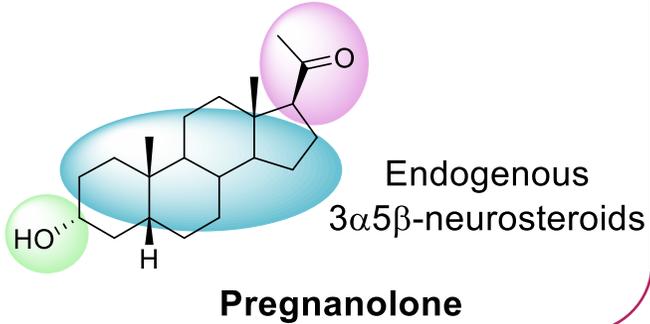


SAR studie



C-17 substituent (D-ring)

C-3 substituent (A-ring)



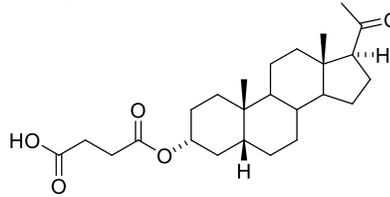
Compounds with substitution at C-3 (A-ring)

Negatively charged

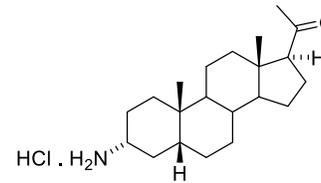
Positively charged

Zwitterionic

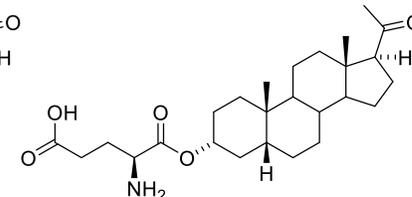
Uncharged



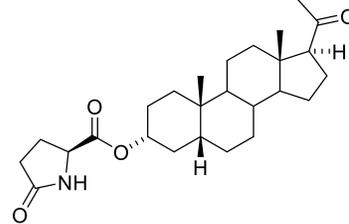
1



2

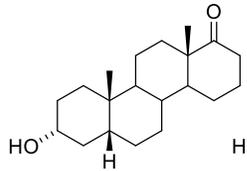


3

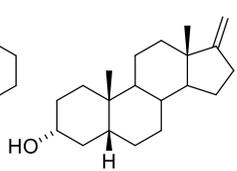


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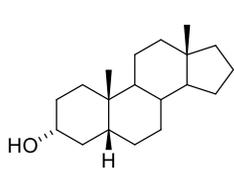
Compounds with substitution at C-17 (D-ring)



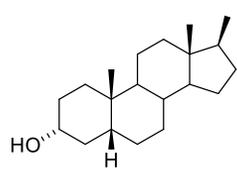
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6

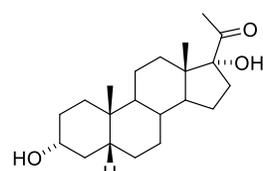


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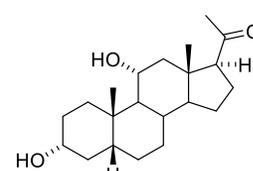


8

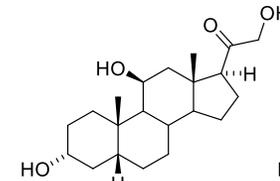
Endogenous $3\alpha 5\beta$ -neurosteroids



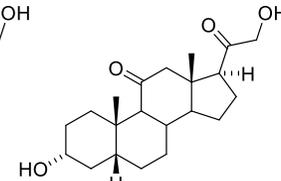
9



10



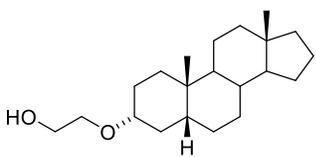
11



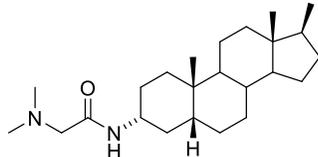
12

Compounds with substitution at C-3 (A-ring) and C-17 (D-ring)

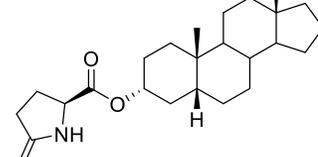
Compounds with substitution at C-3 (A-ring)



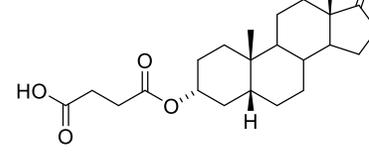
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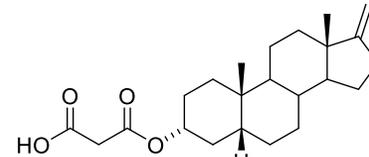
14



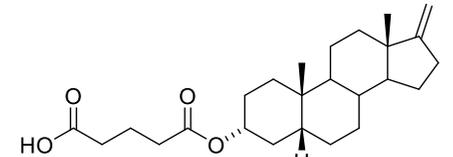
15



16



17



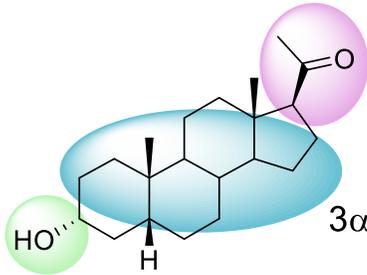
18

SAR studie



C-17 substituent (D-ring)

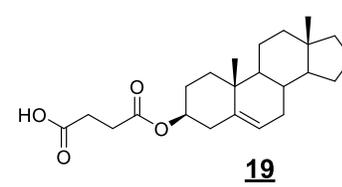
C-3 substituent (A-ring)



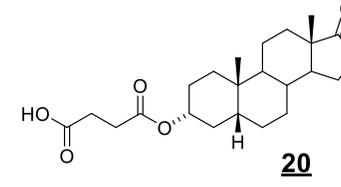
Endogenous
3 α 5 β -neurosteroids

Pregnanolone

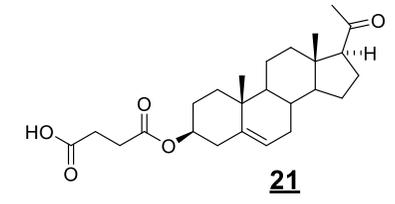
Compounds with substitution at C-17 (D-ring)



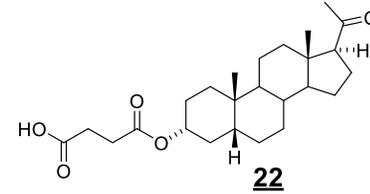
19



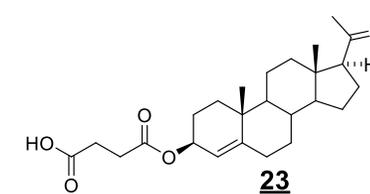
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21

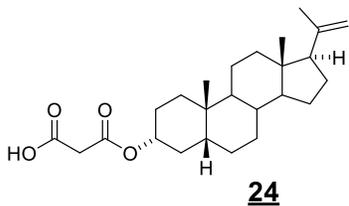


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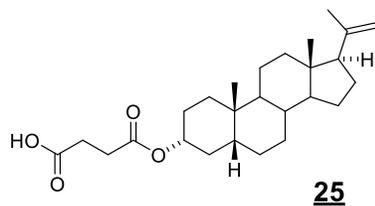


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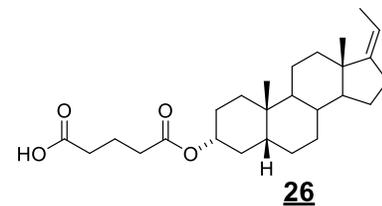
Compounds with substitution at C-3 (A-ring) and C-17 (D-ring)



24

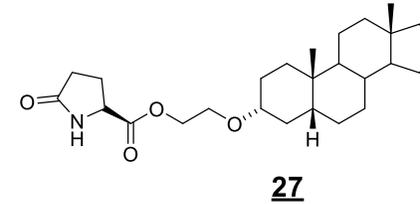


25

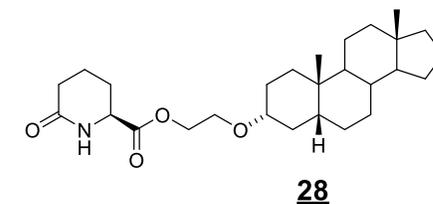


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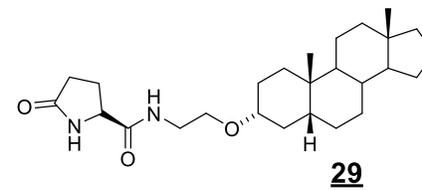
Compounds with substitution at C-3 (A-ring)



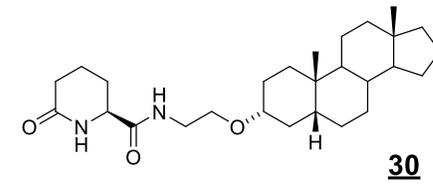
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28

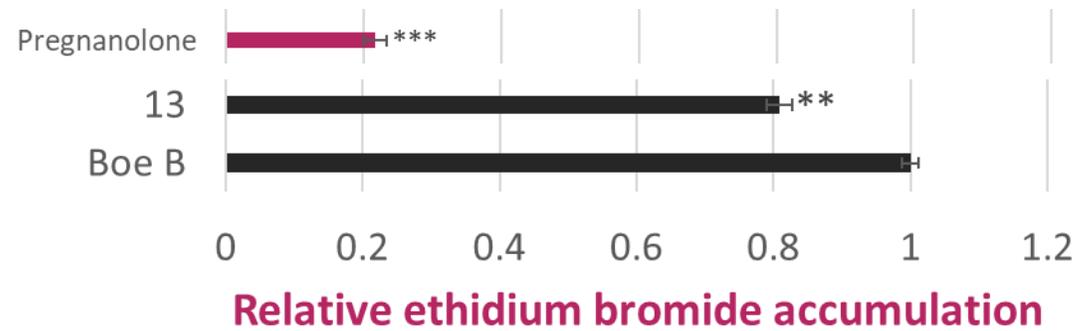
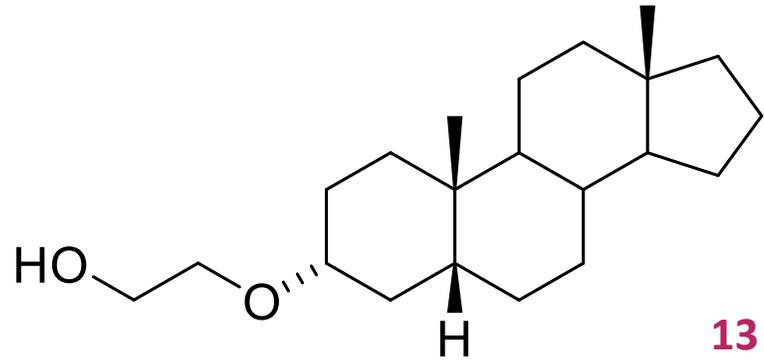


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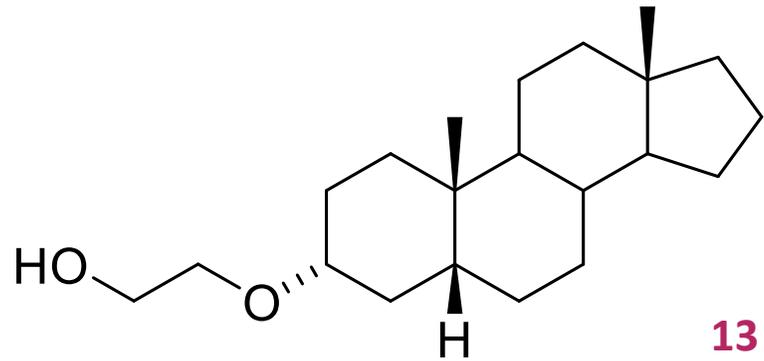


30

Lead compound



Lead compound

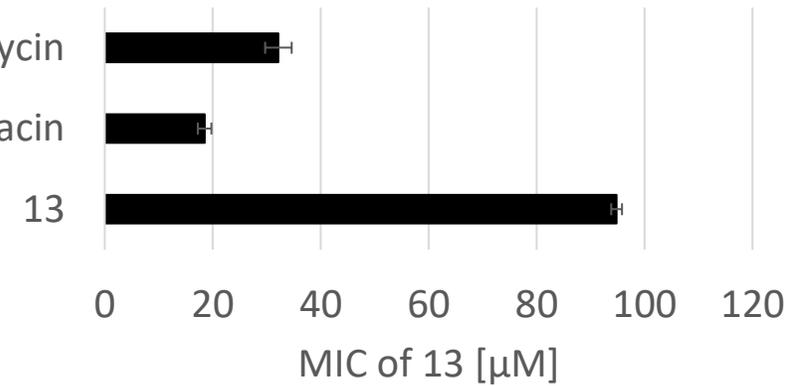


Zvýšená citlivost k antibiotikům u MRSA kmen SA4

13 + ½ MIC of erythromycin

13 + ½ MIC of ciprofloxacin

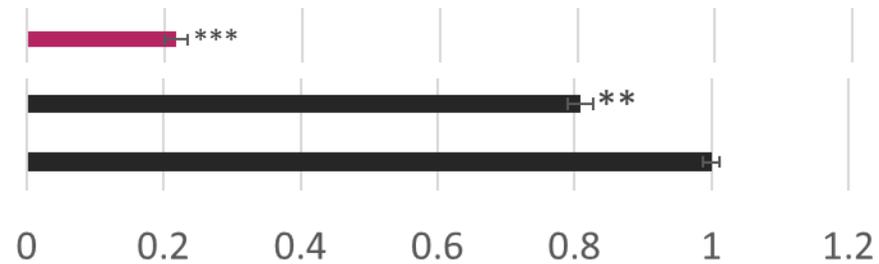
13



Pregnanolone

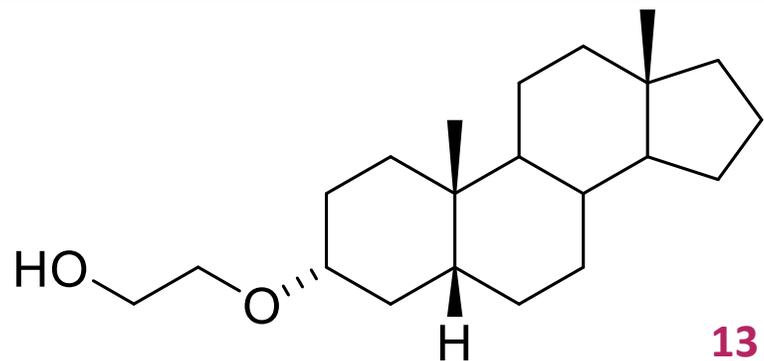
13

Boe B

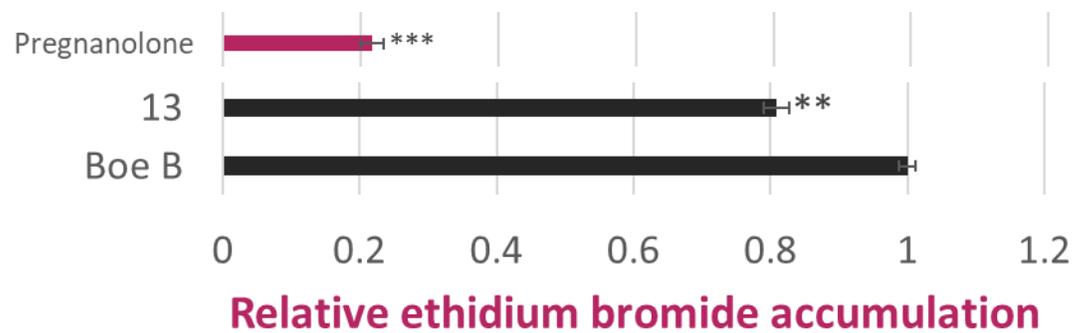
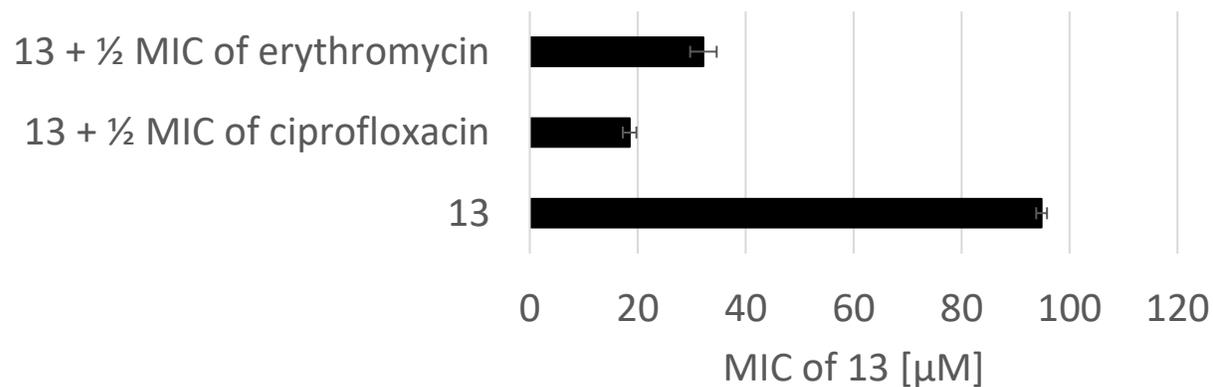


Relative ethidium bromide accumulation

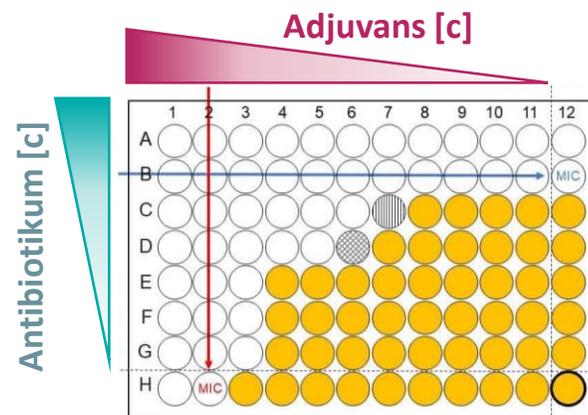
Lead compound



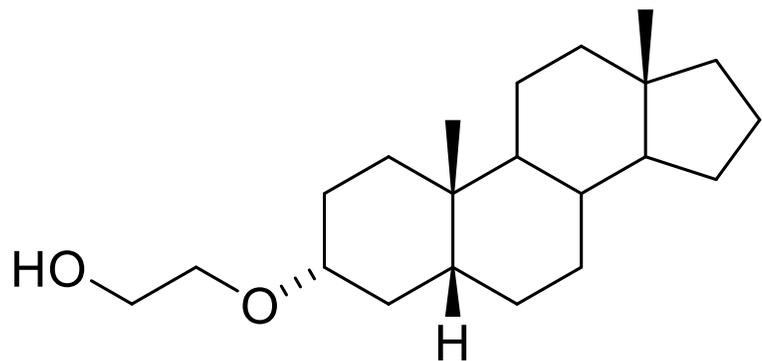
Zvýšená citlivost k antibiotikům u MRSA kmen SA4



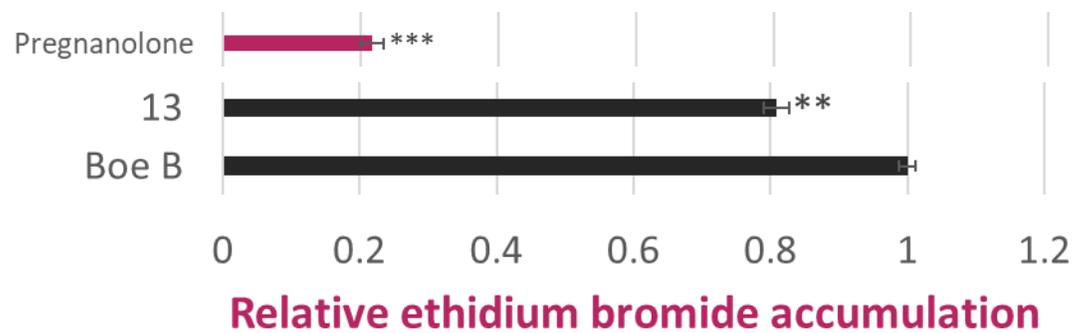
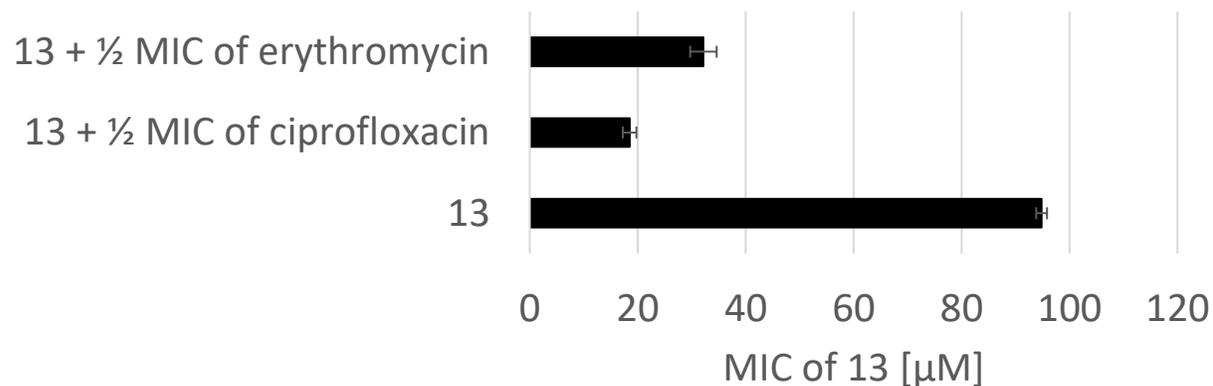
Šachovnicový test



Lead compound



Zvýšená citlivost k antibiotikům u MRSA kmen SA4



Šachovnicový test



$$\text{FICI} = \text{FIC}_A + \text{FIC}_B$$

$$\text{FIC}_A = \text{MIC [A+B]} / \text{MIC [A]}$$

$$\text{FIC}_B = \text{MIC [A+B]} / \text{MIC [B]}$$

MRSA kmen	cip	ery
SA4	0.75	0.83
M53	0.83	0.92

➔ **Aditivní účinek**

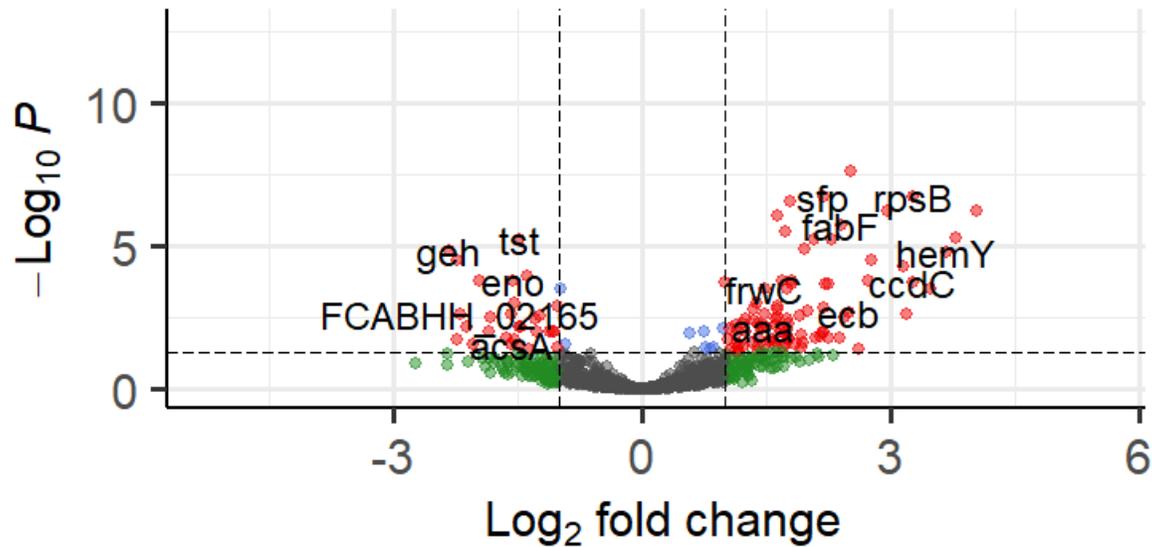
Transkriptomová analýza - erythromycin



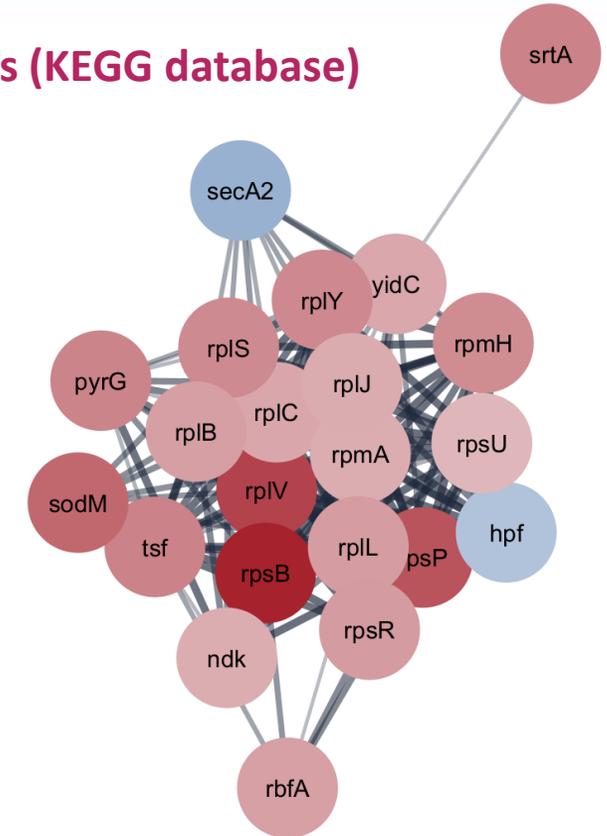
U lidí se steroidní hormony vážou na intracelulární receptory, které regulují genovou expresi.

Volcano plot

● NS ● Log_2 FC ● p-value ● p-value and log_2 FC



Function units (KEGG database)

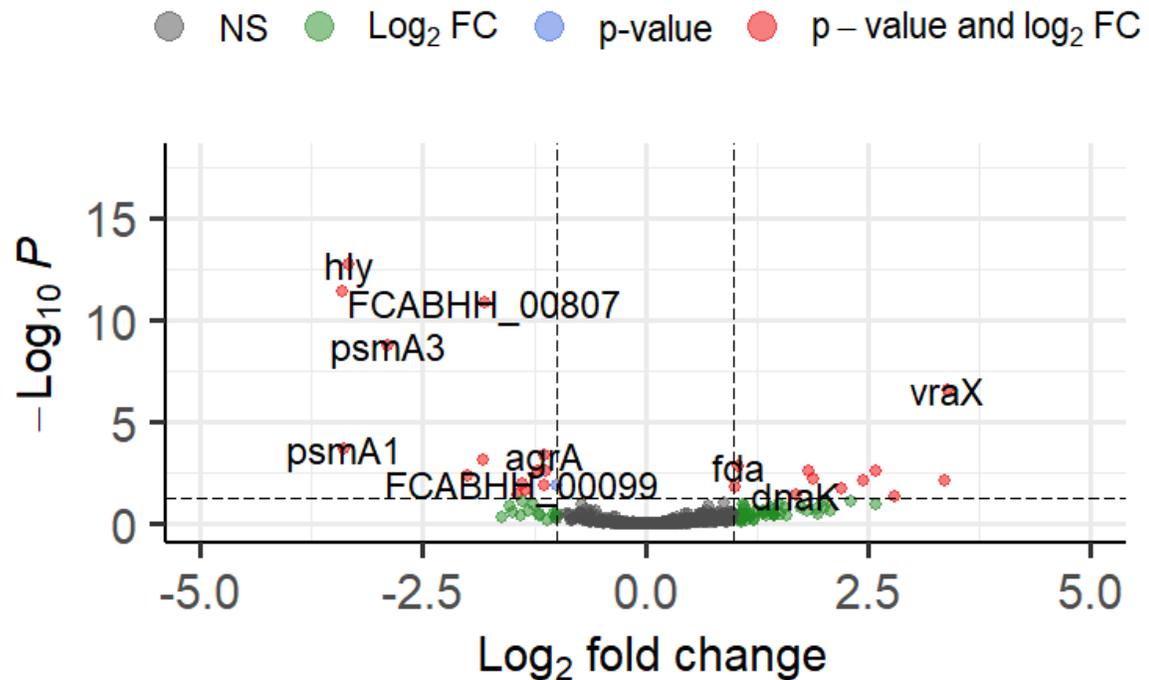


➔ Bakterie se chrání před erythromycinem zvýšenou expresí ribozomálních proteinů.

Transkriptomová analýza – sloučenina 13



Volcano plot



Function units (KEGG database)

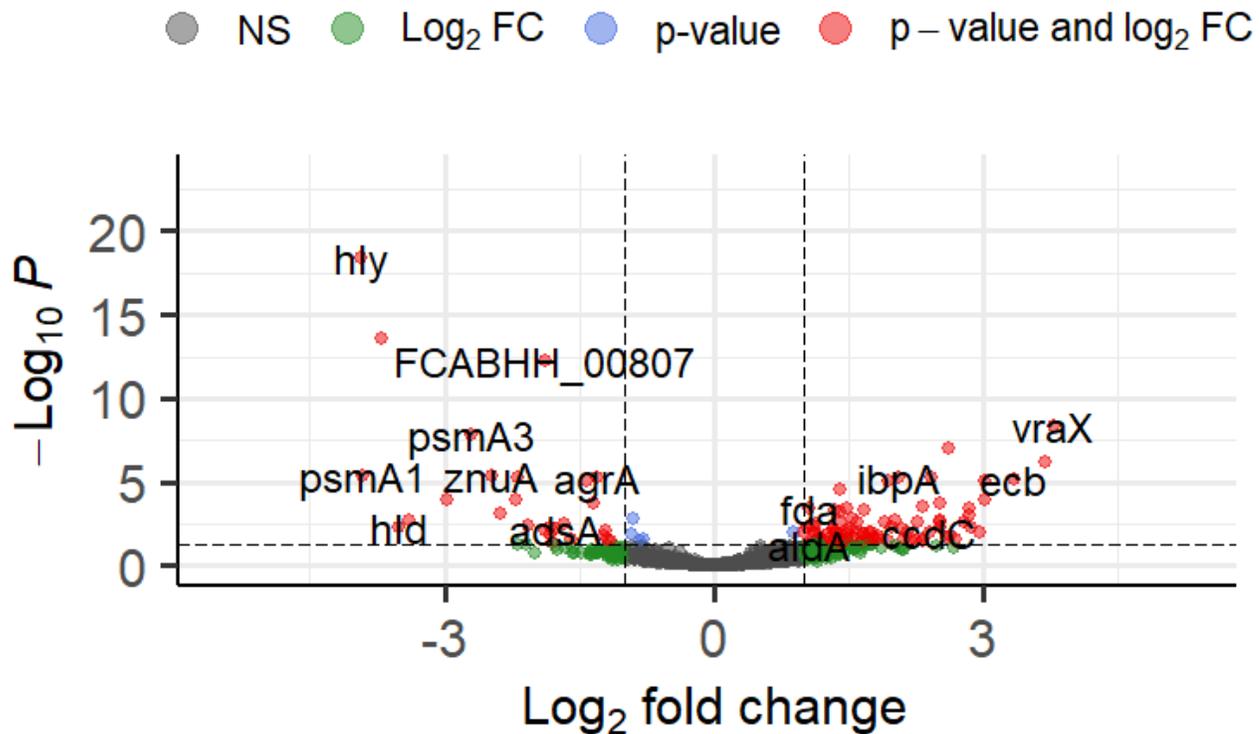
Gene	Log ₂ FC	Function
<i>psmA2</i>	-3,4	Neutrophils lysis – peptide 11 ×
<i>psmA1</i>	-3,4	Neutrophils lysis – peptide
<i>hly</i>	-3,3	Alpha-hemolysin
<i>psmA3</i>	-2,9	Neutrophils lysis – peptide
<i>agrC</i>	-2,0	Synthesis of autoinducing protein (quorum sensing) 4 ×

➔ **Sloučenina 13 snižuje bakteriální virulenci a quorum sensing**

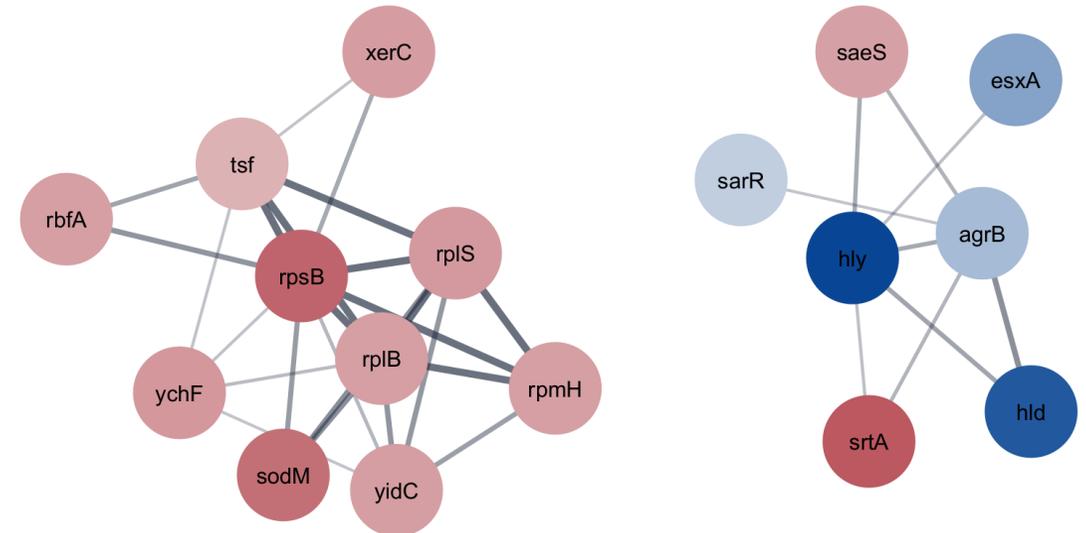
Transkriptomová analýza – kombinace



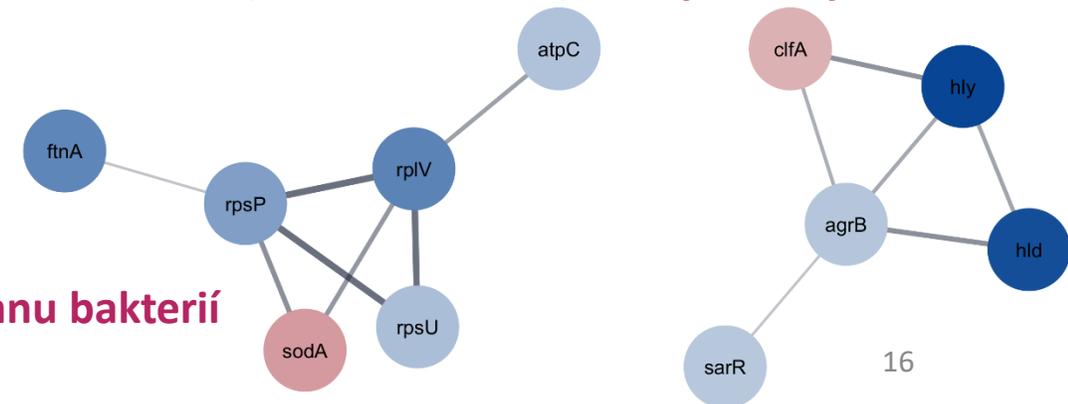
Volcano plot



Function units (KEGG database) vs non-treated



Function units (KEGG database) vs erythromycin-treated



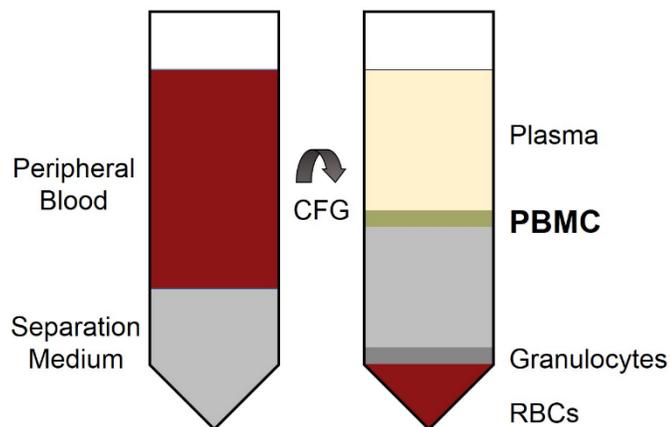
➔ Kombinace erythromycinu a sloučeniny 13 snižuje jak ochranu bakterií proti erythromycinu, tak i bakteriální virulenci.

Toxicita



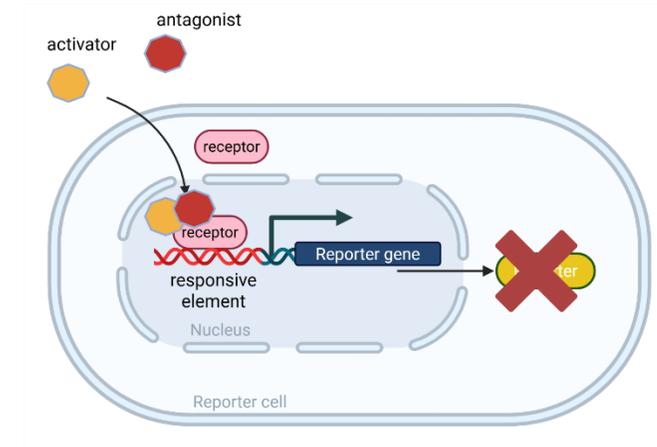
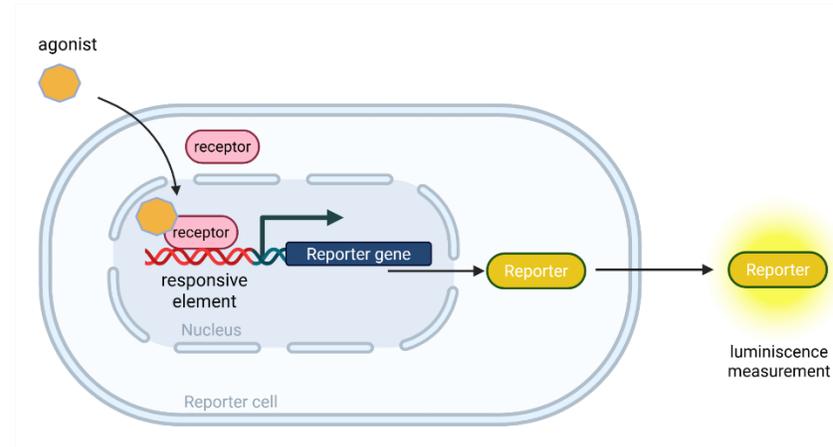
Cytotoxicita

- Mononukleární buňky periferní krve (**PBMC**)
- Monocyty, lymfocyty, NK buňky
- Separace gradientovou metodou



Endokrinní disrupce

- Interference s hormonálním systémem
- Buněčný reportérový test *in vitro* – produkce luciferasy
- **hER α** (buněčná linie HeLa9903)
- **hAR, hGR** (buněčná linie MDA-kB2)
- **hER β , hPR** (Eurofins)



➔ Žádná cytotoxicita až do 100 μM

➔ Při koncentraci 10 μM nevykazuje agonistickou ani antagonistickou aktivitu



Steroidní hormony a neurosteroidy jsou inhibitory efluxních pump a zvyšují účinnost antibiotik

- Steroidy zapojené do syntézy **pregnanolonu** z prekursoru **progesteronu** inhibují bakteriální efluxní systém, pravděpodobně LmrS.
- Mnohem účinnější deriváty lze připravit substitucí na C3.
- Lead compound:
 - inhibuje efluxní systém
 - zvyšuje citlivost kmenů MRSA na ciprofloxacin a erythromycin
 - snižuje virulenci bakterií a quorum sensing
 - snižuje bakteriální ochranu proti antibiotikům
 - má nízkou cytotoxicitu a neovlivňuje endokrinní systém



Poděkování



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