Institute of Functional Materials Chemistry SSI "Institute for Single Crystals" of NAS of Ukraine,

Kharkiv, Ukraine

3,3'-BISINDOLYLMETHANE DERIVATIVES AS ANTIBIOTIC RESISTANCE DISRUPTORS

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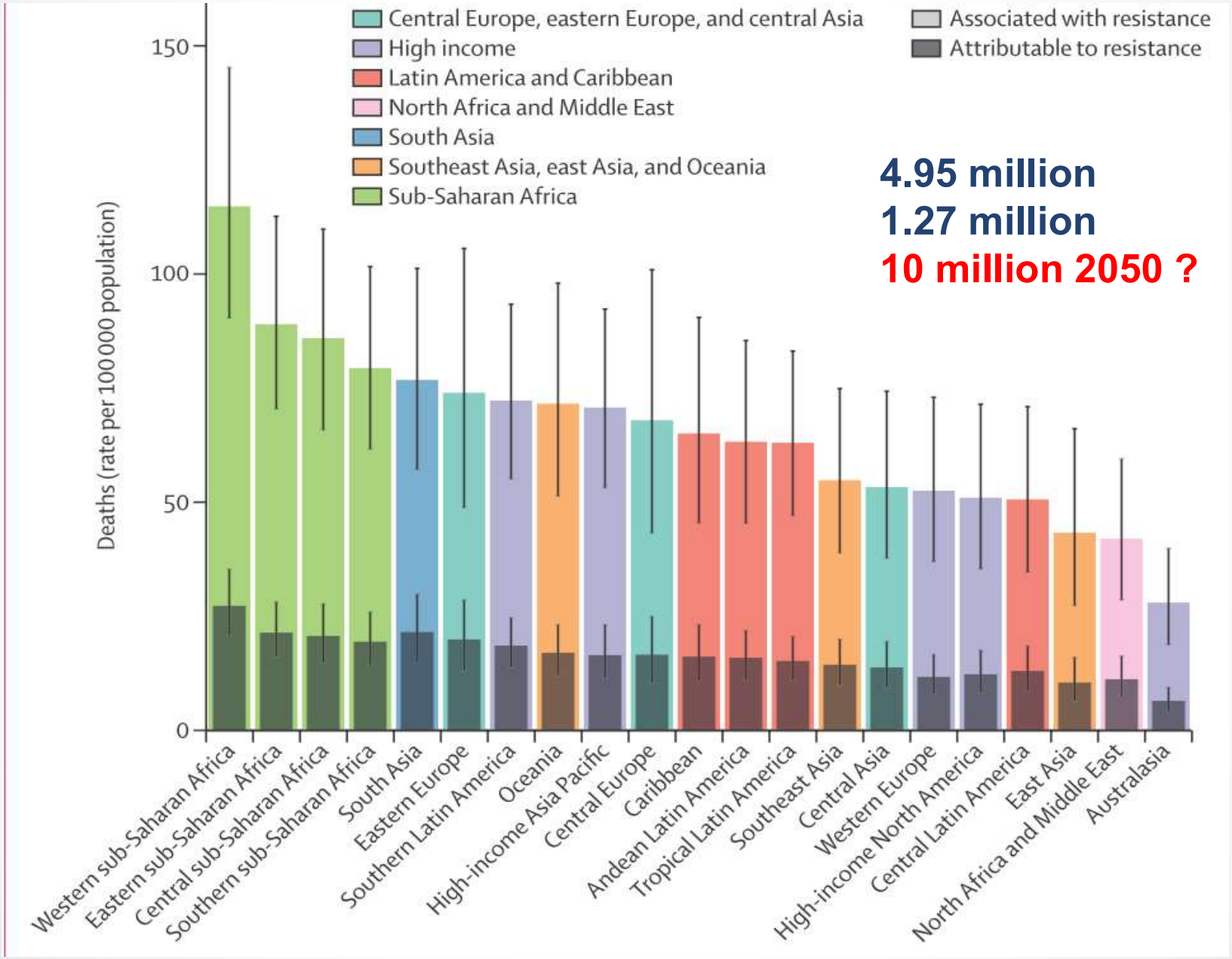
STATE OF THE PROBLEM

- Resistance of microorganisms to antibacterial agents in clinical practice is a global problem of modern medicine.
- Why has this problem become particularly acute now?
- Approaches to solve the problem of multidrug resistance
- Use of "auxiliary" agents to enhance the activity of existing drugs
- 3,3’-BIM and its derivatives as means of increasing the effectiveness of antibacterial drugs

Rate of deaths attributable to and associated with bacterial antimicrobial resistance, 2019



4.95 million
1.27 million
10 million 2050 ?

Lancet 2022; 399: 629–55

BACTERIAL RESISTANCE IS A GLOBAL PROBLEM OF MODERN MEDICINE

2017 WHO PRIORITIZATION OF PATOGENES:

CRITICAL PRIORITY:

E	*Enterococcus faecium*
S	*Staphylococcus aureus*
K	*Klebsiella pneumoniae*
A	*Acinetobacter baumanii*
P	*Pseudomonas aeruginosa*
E	*Enterobacter spp.*

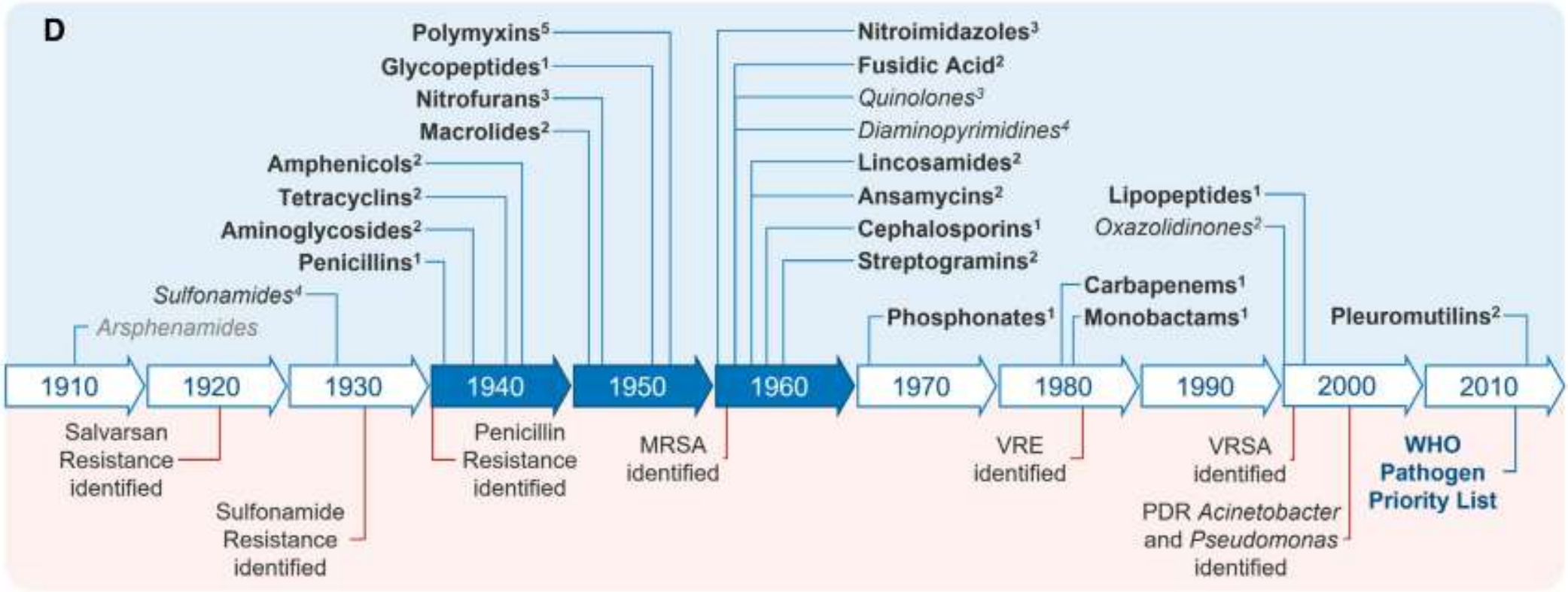
HIGH PRIORITY:

Helicobacter pylori,
Camphylobacter species,
Salmonella species,
Neisseria gonorrhoeae,
Escherichia coli (NDM-1)

MEDIUM PRIORITY:

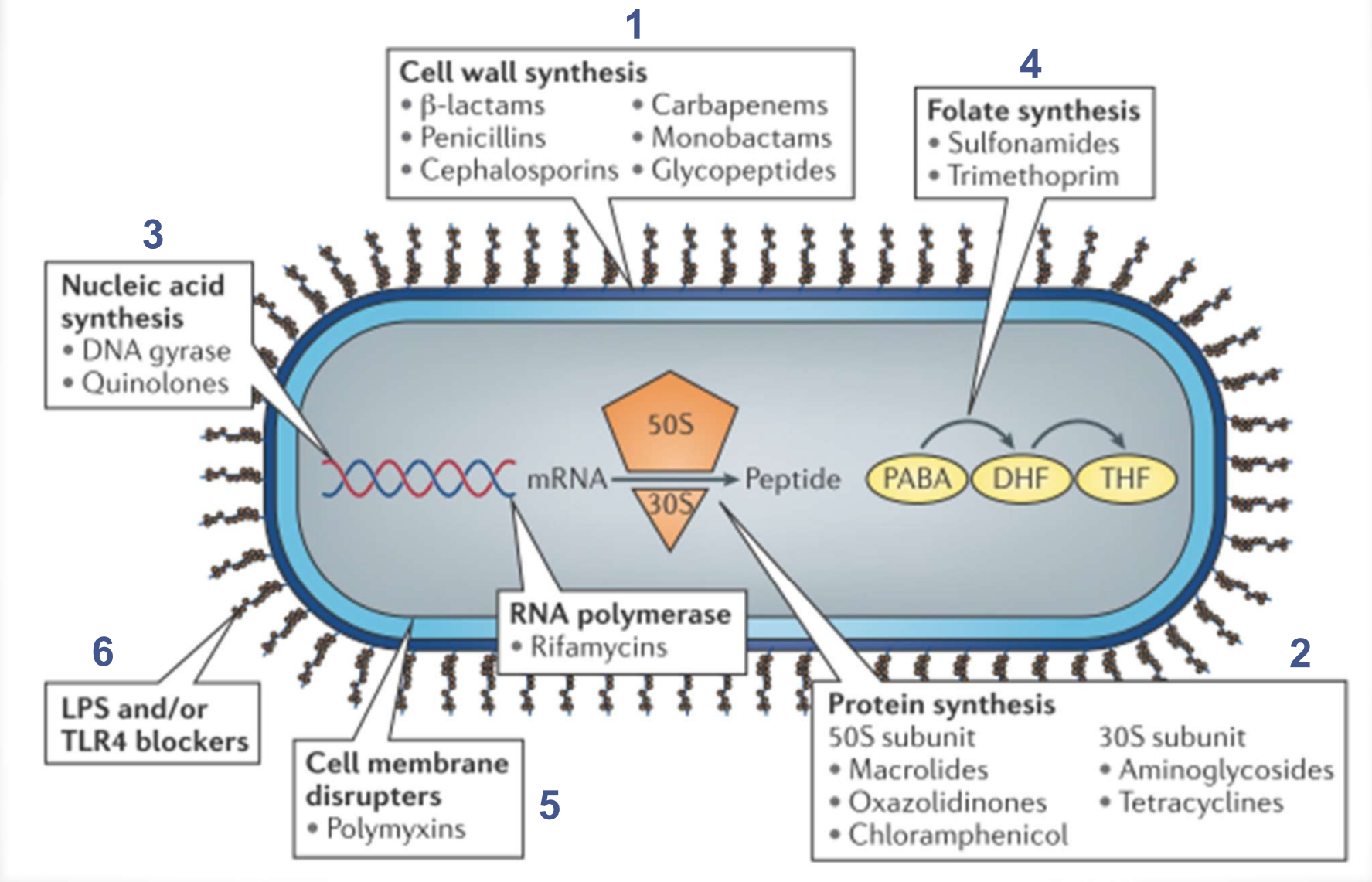
Haemophilus influenzae,
Shigella species,
Streptococcus pneumoniae

AVAILABLE ARSENAL OF ANTIBACTERIAL AGENTS



Walesch S. et al. EMBO reports, 2022, 24:56033

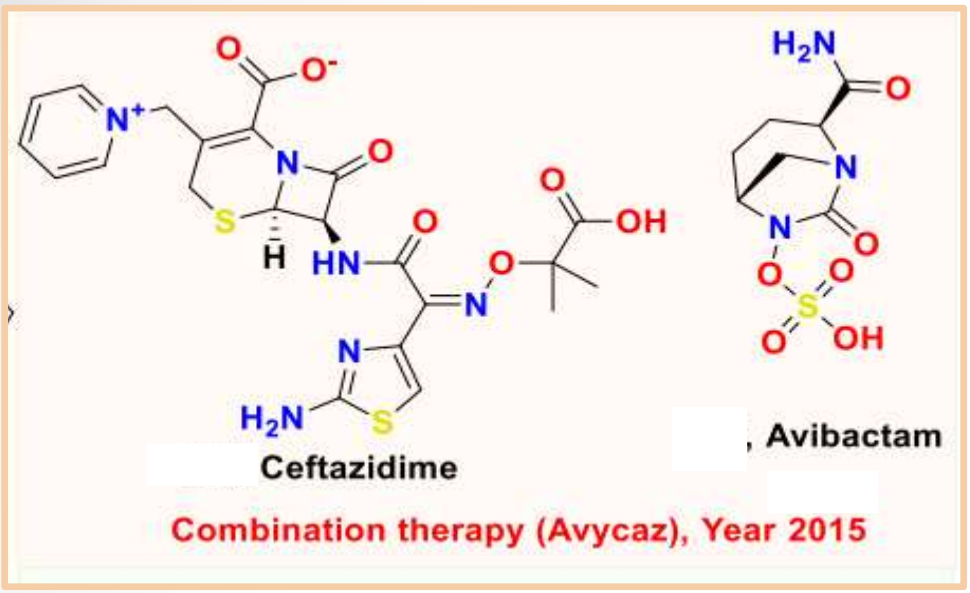
CELLULAR TARGETS OF ANTIBACTERIALS

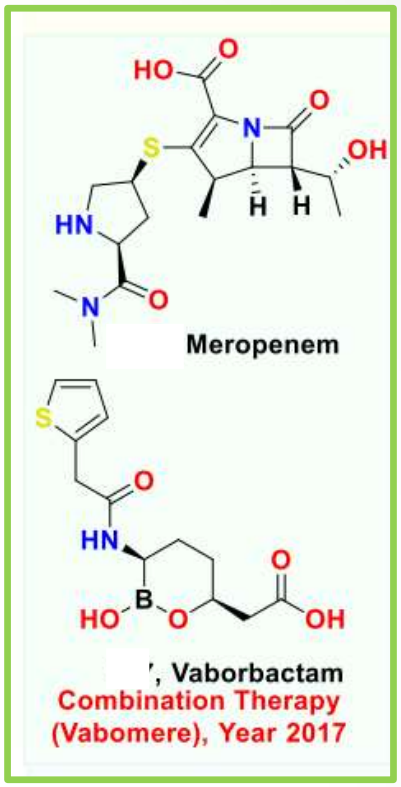


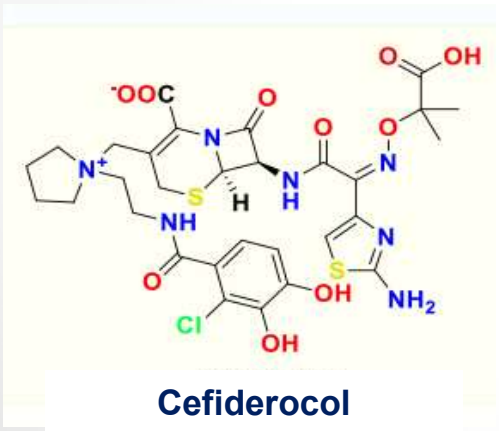
NEW TARGETS: **enoyl-acyl carrier protein reductase (afabicin); Toll-like receptor 4.**

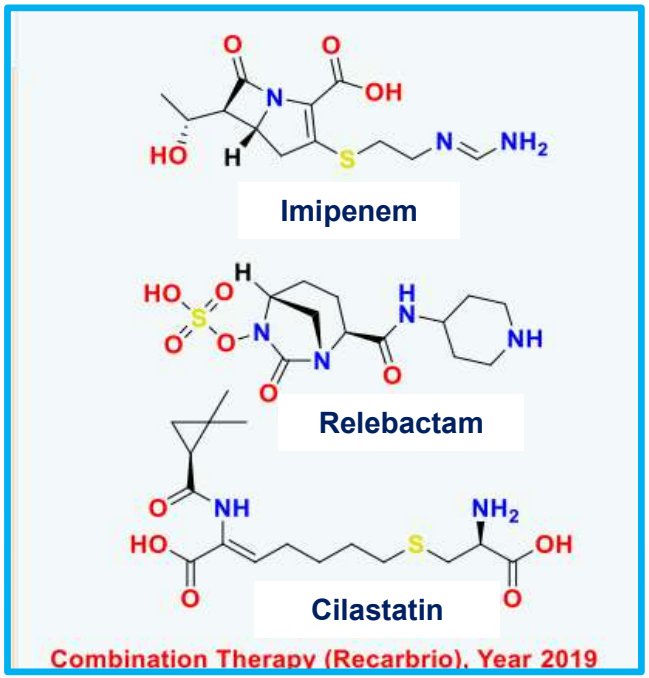
Brown D. Nat Rev, 14, 2015

FDA-APPROVED ANTIBACTERIAL DRUGS (2014-2020)

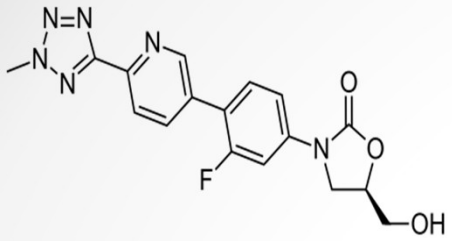




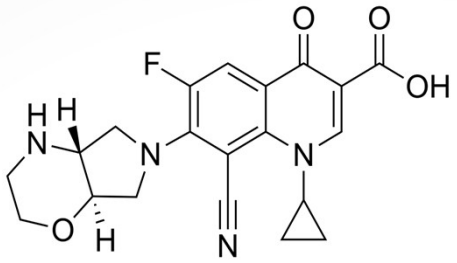




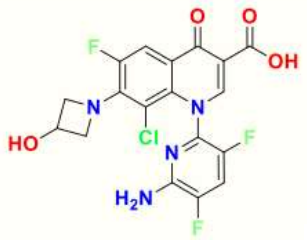
FDA-APPROVED ANTIBACTERIAL DRUGS (2014-2020)



Tedizolid (2014)

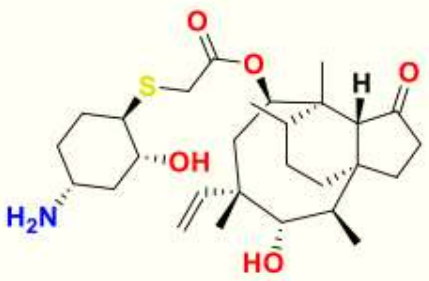


Finafloxacin (2014)



Delafloxacin (2015)

Ozenoxacin (2015)



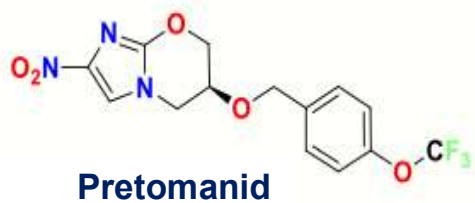
Lefamulin

Plazomicin

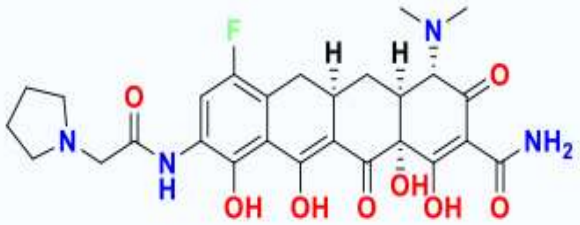
Benznidazole

Secnidazole

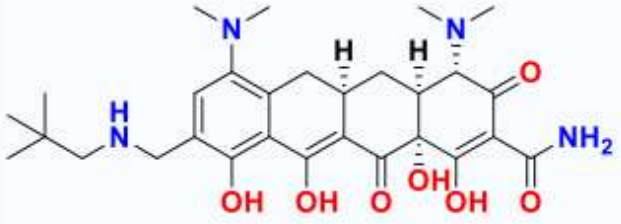
2017



Pretomanid (2019)



Eravacycline



Omadacycline

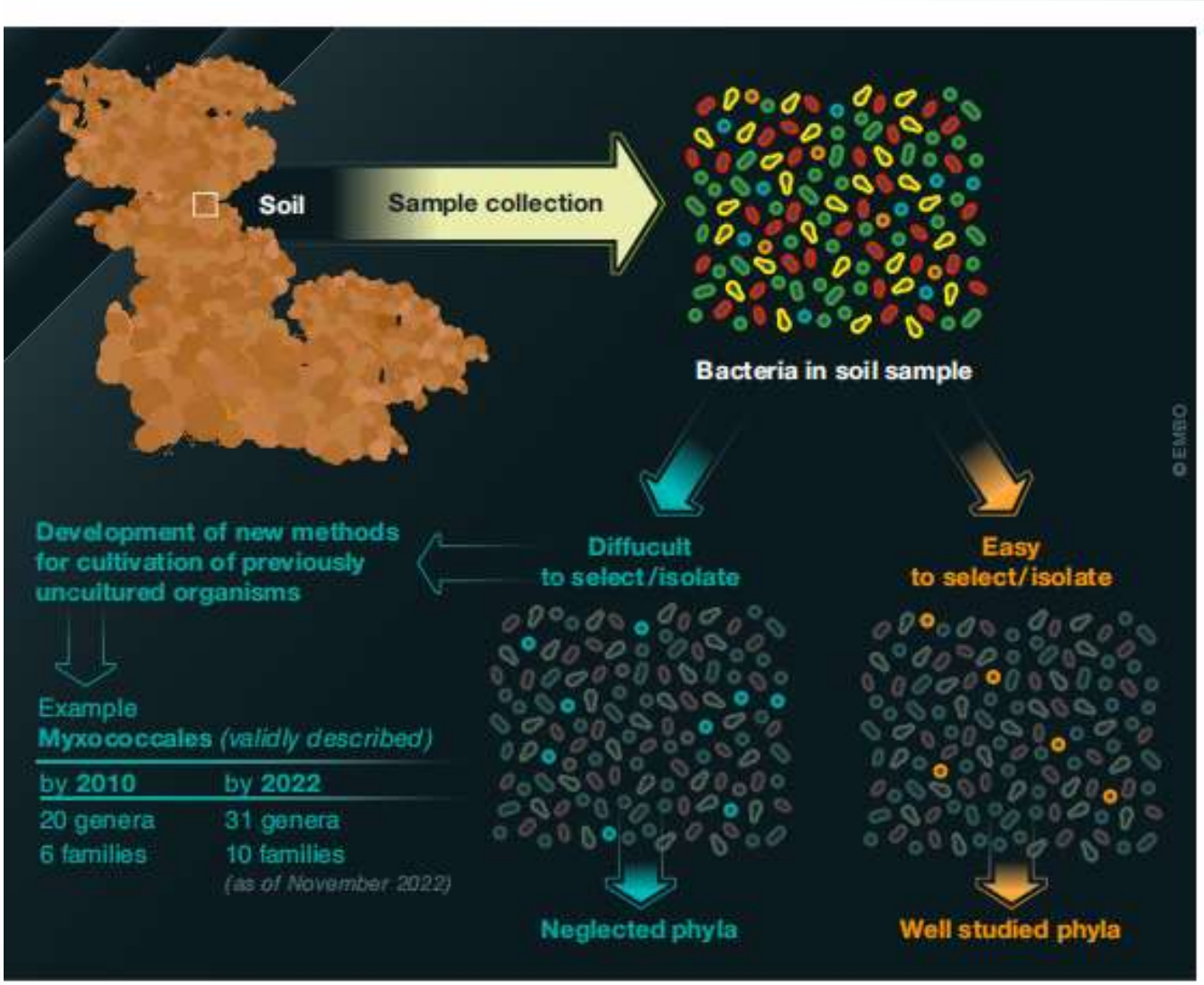
BASIC MECHANISMS OF BACTERIAL RESISTANCE

- The inability of the drug to diffuse through the extracellular matrix (changes in the structure of the cell wall and/or membrane).
- Enhanced gene transfer, which provides resistance to antibacterial agents (changes in the structure of targets, increased expression of enzymes capable of inactivating or modifying the molecular structure of a certain API, etc.).
- Expression of molecular pumps that carry out drug efflux outside the cell.
- Inactivation of the drug due to changes in the microenvironment.
- The presence of persistent bacteria.
- **Biofilm formation.**

POSSIBLE WAYS TO OVERCOME BACTERIAL RESISTANCE

- **Search for new producers of active metabolites with antibacterial activity among soil, marine microorganisms and other natural sources**

Soil samples can be a rich source of microbial diversity

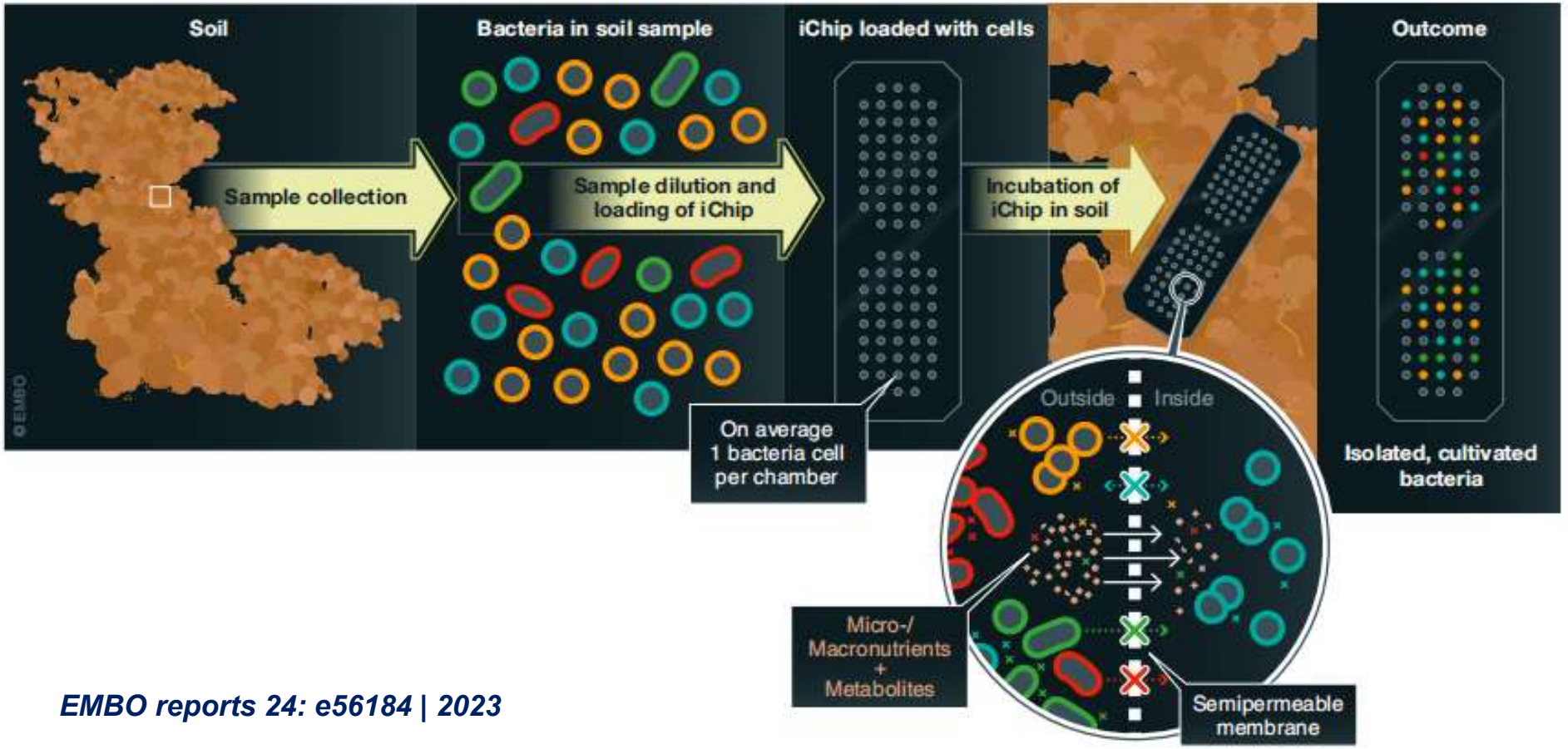


EMBO reports 24: e56184 | 2023

POSSIBLE WAYS TO OVERCOME BACTERIAL RESISTANCE

- **Development of innovative technologies for the cultivation and fermentation of microorganisms in order to identify previously unknown antibiotics**

iChip technology can be utilized to cultivate bacteria from the soil that could not be isolated under standard laboratory conditions before

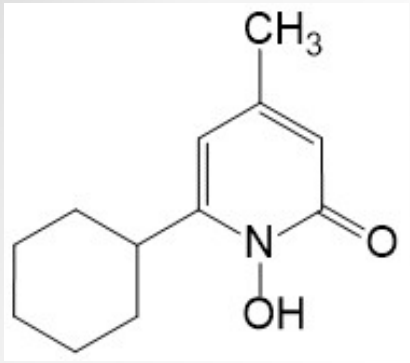


EMBO reports 24: e56184 | 2023

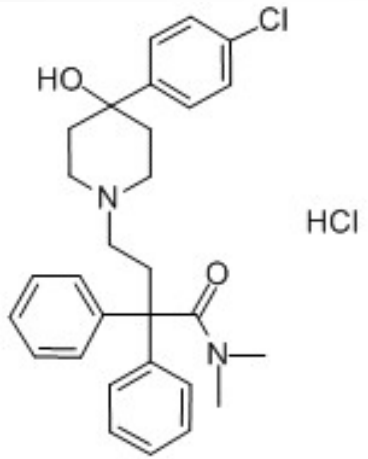
POSSIBLE WAYS TO OVERCOME BACTERIAL RESISTANCE

- **Nowel combinations of existing classes of antibiotics or co-administration them with new beta-lactamase inhibitors**
- **Simultaneous use of another drug or food component (resistance disruptors) that will restore sufficient antibacterial activity of the ineffective antibiotic**
- **Organic synthesis and screening of new compounds with the aim of discovering substances with antibacterial properties**

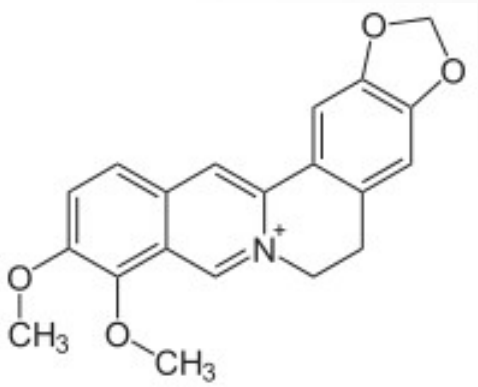
ANTIBIOTIC RESISTANCE DISRUPTORS



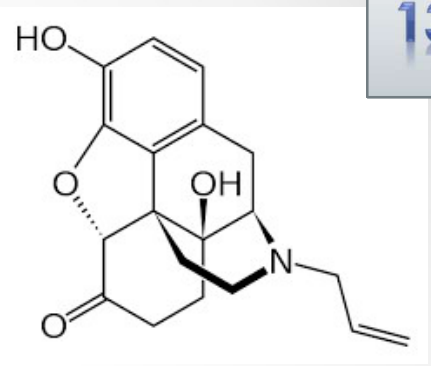
Ciclipirox
(LPS coat Gram (-) bacteria, (FTR1, FTR2 and FTH1))



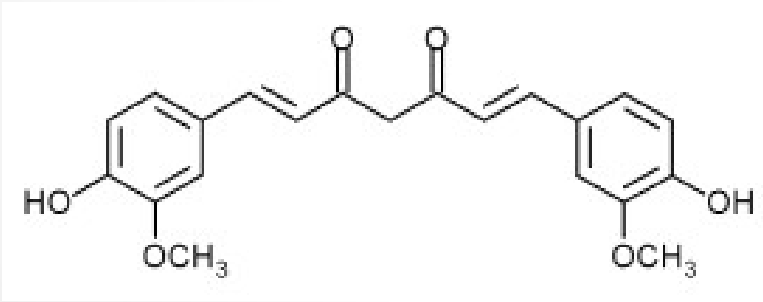
Loperamide
(inhibits H+ transport)



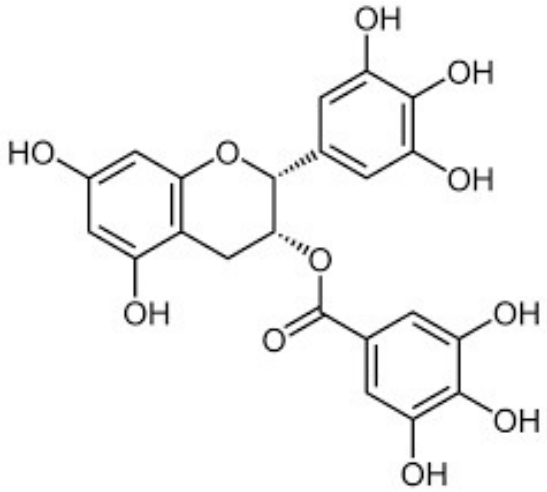
Berberine
(TNF, IL-1β, IL-6, MCP1, iNOS and COX2))



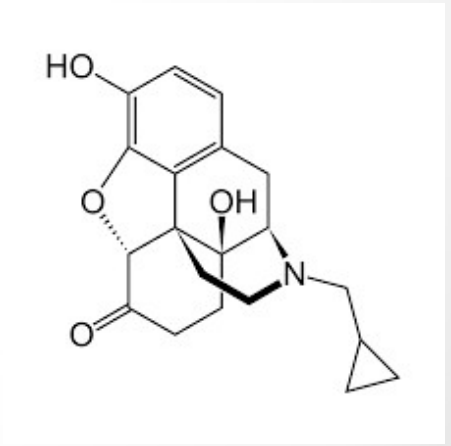
(+) Naloxone



Curcumin
(nhibits TLR2 and TLR4 signalling, induces autophagy by inhibiting the AKT–mTOR pathway)



Epigallocatechin-3-gallate (EGCG) (inhibition of DNA gyrase, blockade of TLR4 binding)



(+) Naltrexone
(both compounds block TLR4–MD2 signalling)

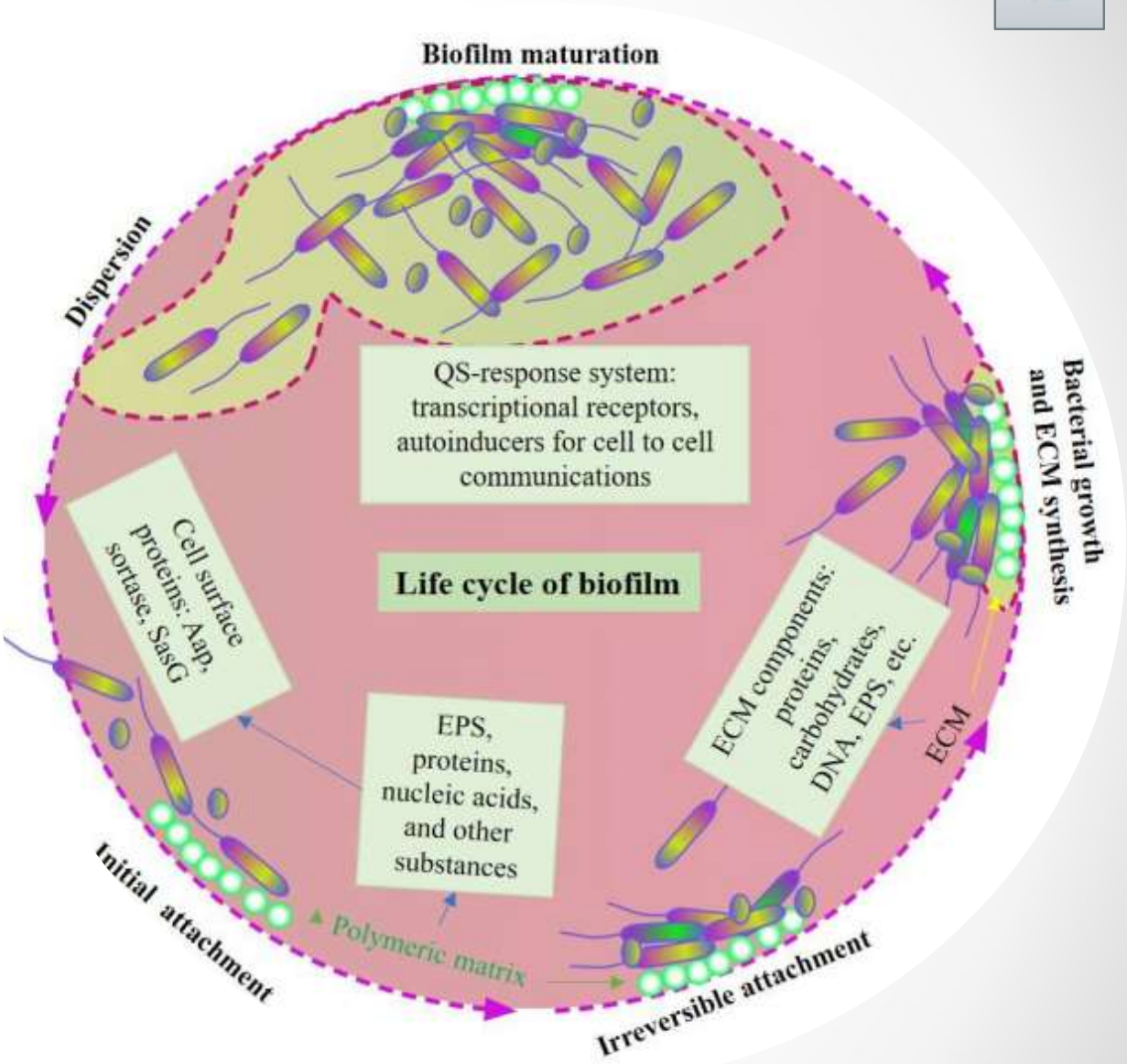
Brown D. Nat Rev, 14, 2015

SELECTIVE IMPACT OF RESISTANCE DISRUPTORS ON MAJOR CLASSES OF ANTIBIOTICS

DRUG	for Gram-negative bacteria	for Gram-positive bacteria
Carbapenems, cephalosporins and penicillins	Ciclopirox Loperamide (intravenous) Macrolides EGCG Naloxone, naltrexone, curcumin (for gut pathogens and LPS-driven endotoxic shock)	Curcumin EGCG Berberine
Polymyxins	Loperamide	-
Aminoglycosides	None identified	-
Fluoroquinolones	None identified	Curcumin
Tetracyclines	Loperamide	Curcumin
Glycopeptides	-	Naloxone, naltrexone, curcumin (with vancomycin or metronidazole for the treatment of *Clostridium difficile*-associated diarrhoea)
Macrolides	-	None identified

BIOFILM FORMATION, MATURATION AND DISPERSION

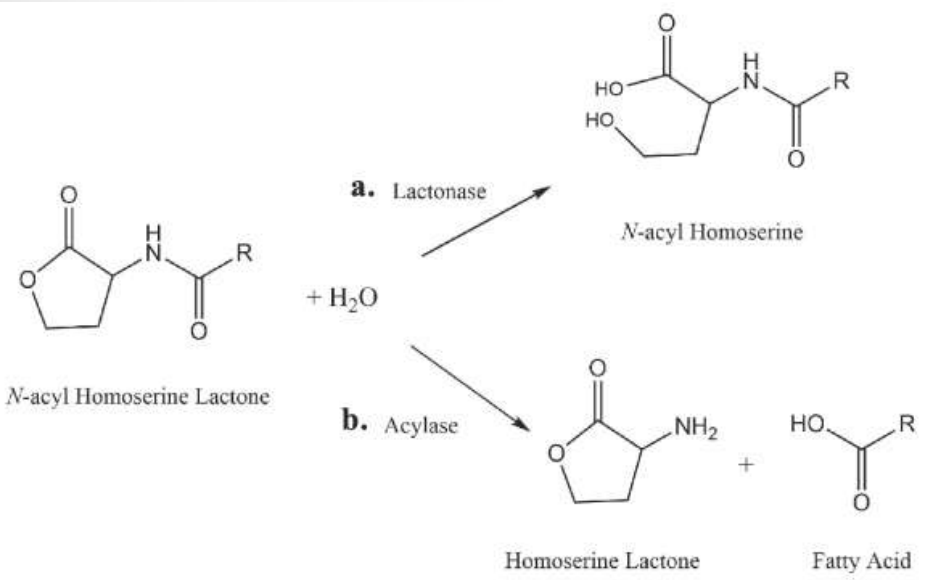
1. Reversible attachment of bacteria to an accessible surface
2. Irreversible fixation with the formation of the first layer of extracellular matrix (ECM)
3. The first stage of maturation (QS)
4. The second stage of maturation (build-up of ECM layers)
5. Biofilm dispersion (QQ)

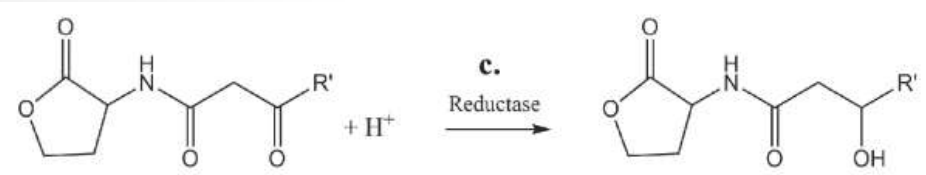


Mirghani R. et al. AIMS Microbiology, 2022, 8 (3): 239–277

WAYS OF INFLUENCE ON QS

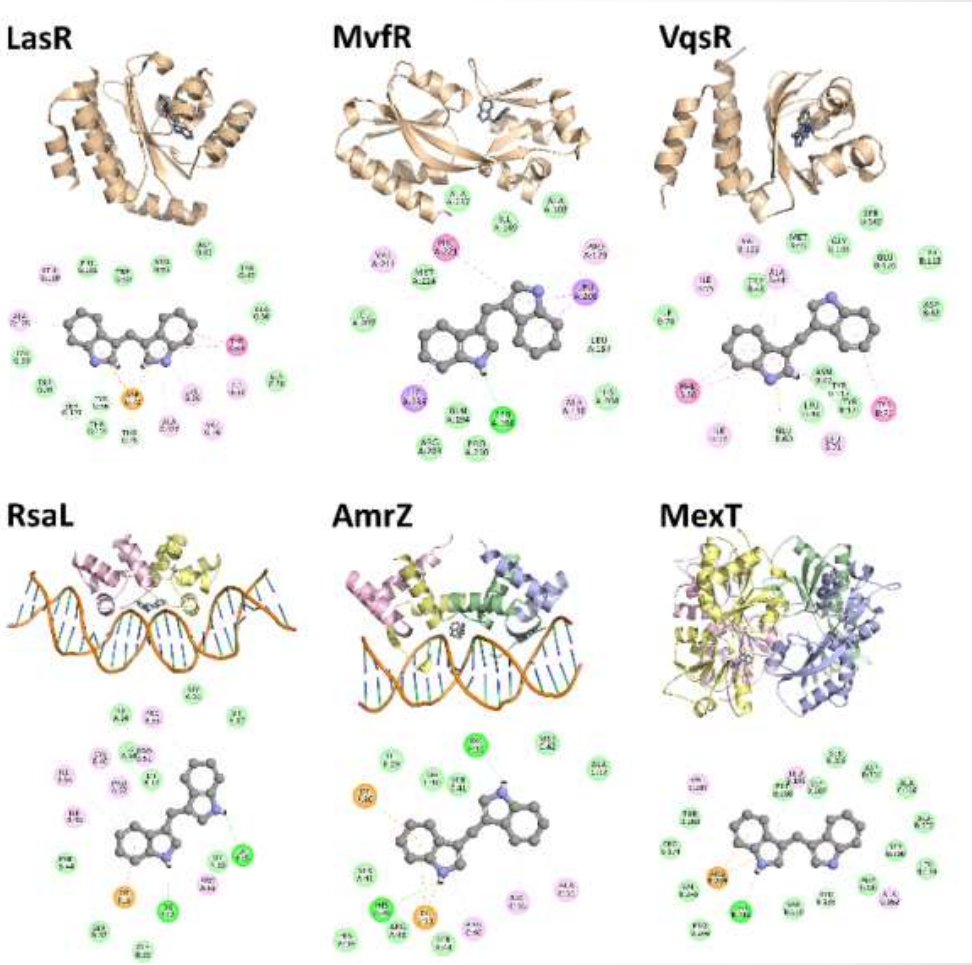
Enzyme destruction of signal molecules





EXPERT REVIEW OF ANTI-INFECTIVE THERAPY
https://doi.org/10.1080/14787210.2020.1794815

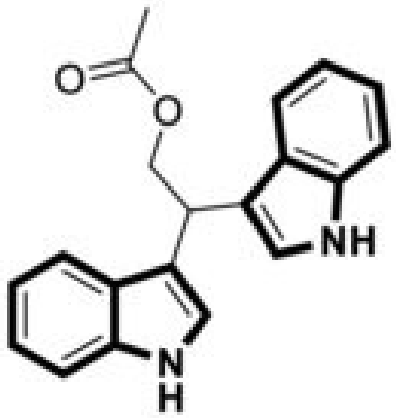
Effects on transcription factors (*P. aeruginosa* + *3,3'-BIM*)



Pharmaceutics **2022**, *14*, 967

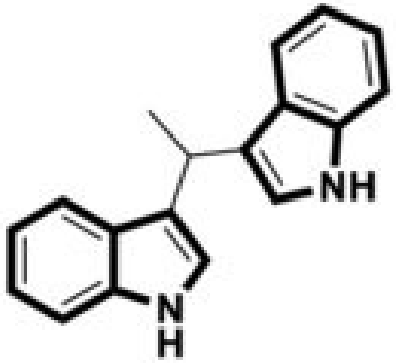
3,3'-BISINDOLILMETHANE ALKALOIDS

Streptococcus faecium IB37



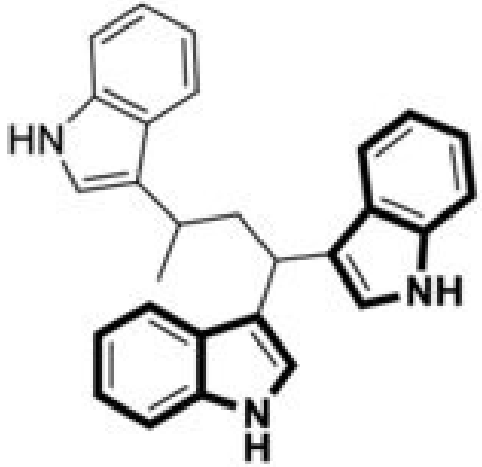
streptindole

Vibrio parahemolyticus

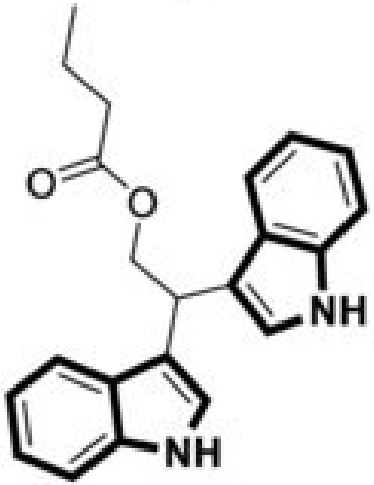


vibrindole A

Vibrio parahemolyticus bio249



1,1,3-tris(1-*H*-indol-3-yl)butane

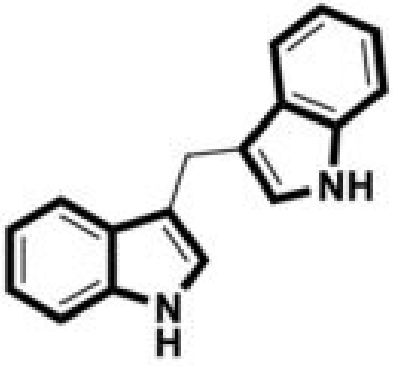


arsindole B

Xiamen sea bacterium strain CB101

tris(1*H*-indol-3-yl)methane

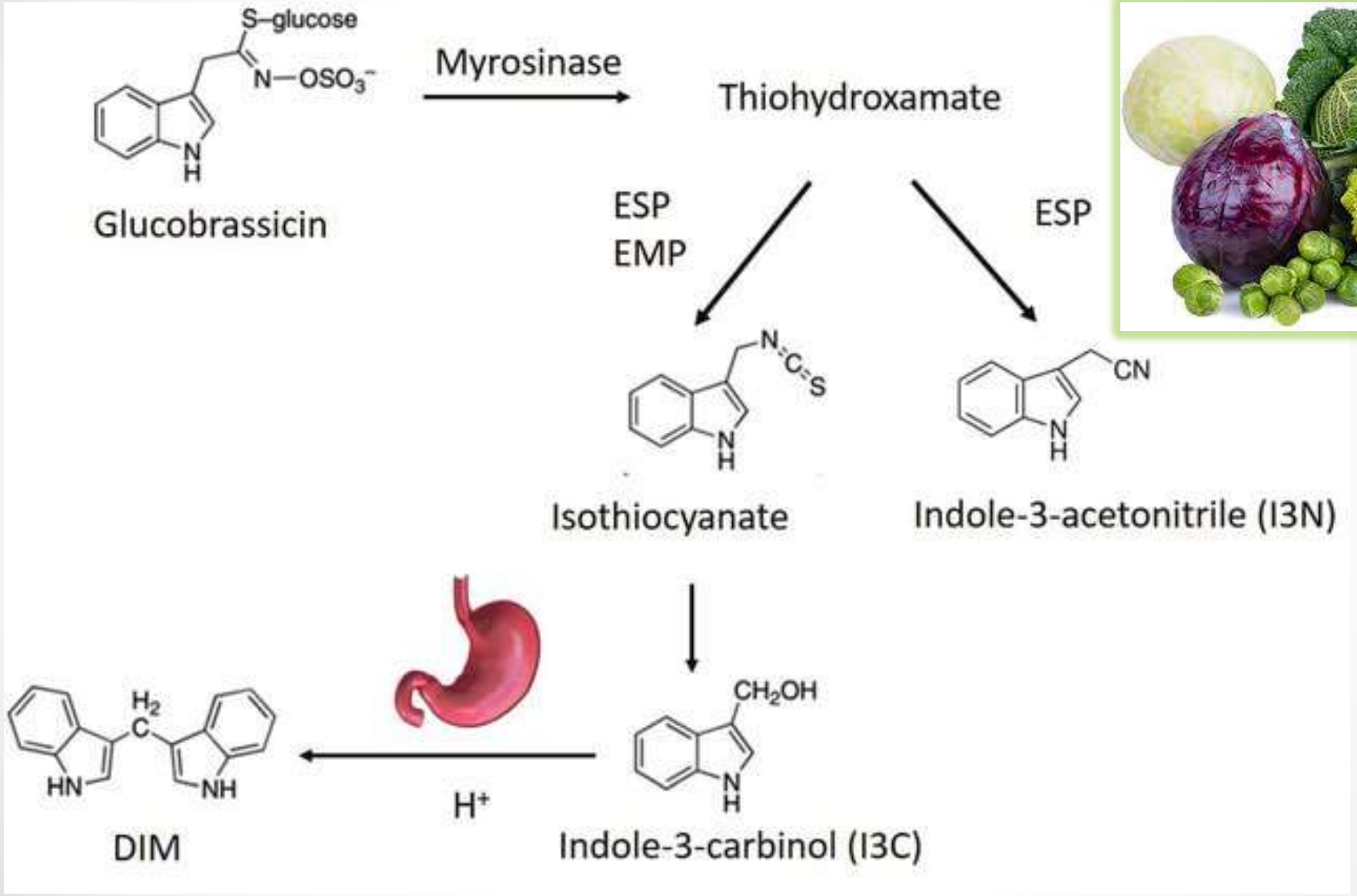
Vibrio sp.



arundine

Arundo donax

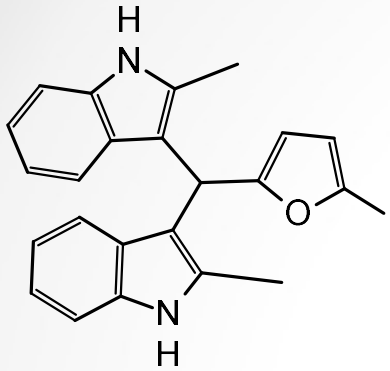
BIOSYNTHETIC PATHWAY TO FORM 3,3'-BIM FROM PLANT METABOLITES

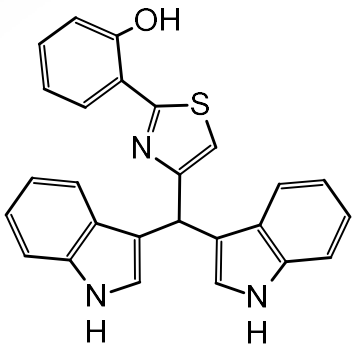


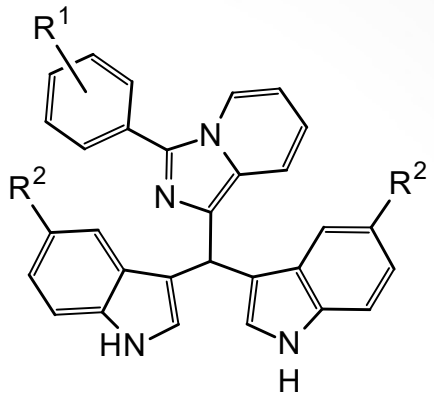
- *Koper JEB et al. Food & Function, 2020, 11(15)*

3,3'-BIMS OF SYNTHETIC ORIGIN AS BIOLOGICAL ACTIVE COMPOUNDS

ANTI-TUMOR ACTIVITY

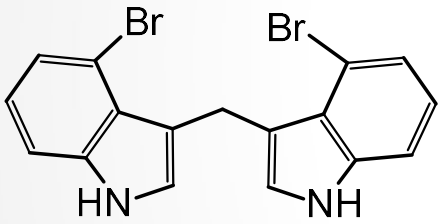


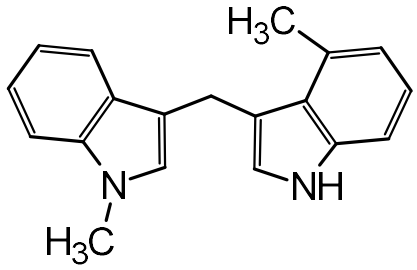




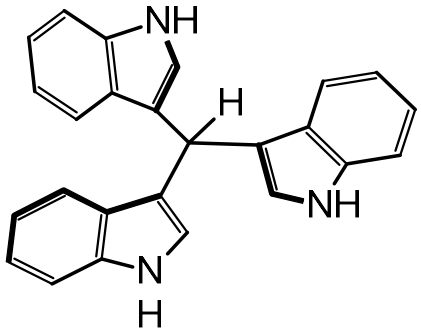
R^1 = 3,4-di-OMe, R^2= Br;
R^1 = 4-F, R^2= 5-OMe

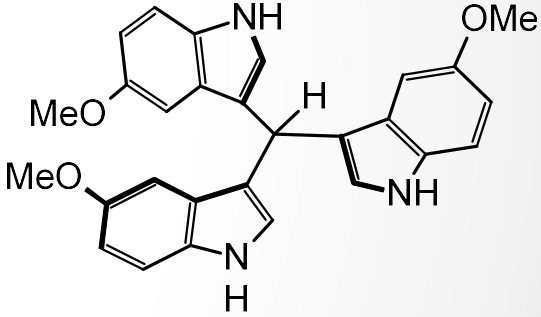
CANNABINOID CB2 RECEPTOR AGONISTS



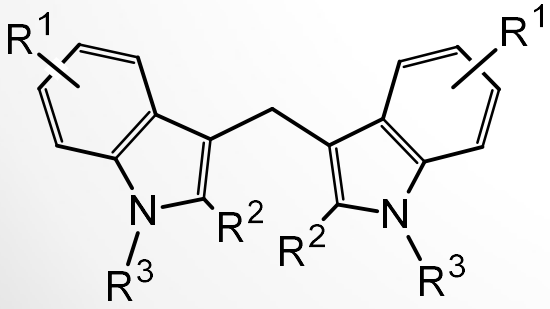


ANTI-INFLAMMATORY ACTIVITY



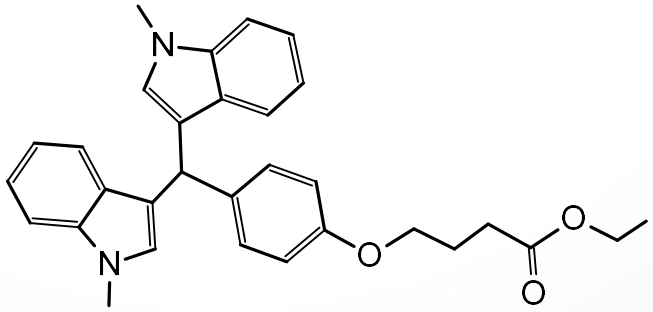


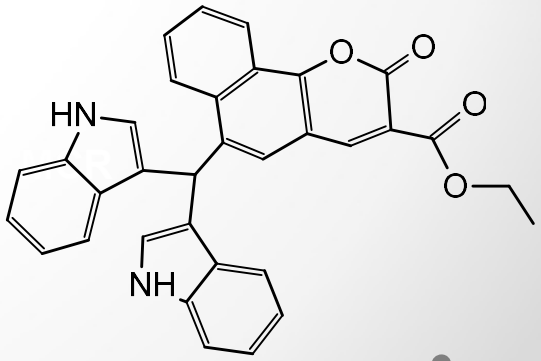
GPR84 RECEPTOR AGONISTS



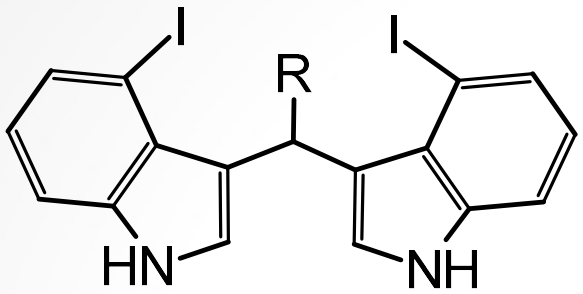
R^1 = 5-F, 7-F; R^2 =R^3 =H

ANTIHYPERLIPIDEMIC ACTIVITY



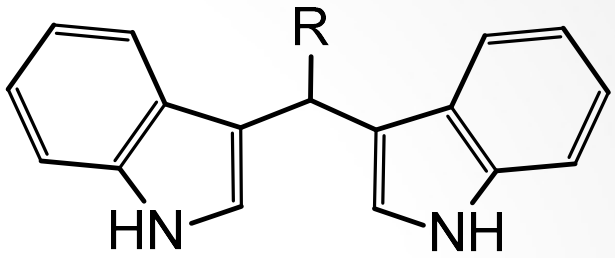


3,3'-BIMS WITH ANTIMICROBIAL, FUNGICIDAL AND ANTI-PARASITIC ACTIVITY



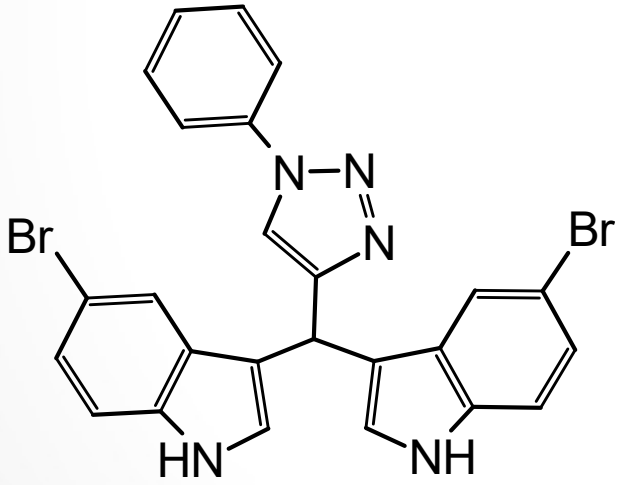
R: C_6H_4-4-OH; C_6H_3-3-OMe,-4-OH; C_6H_4-4-NO_2

IC_{50} 8.37 µM *L. donovani*



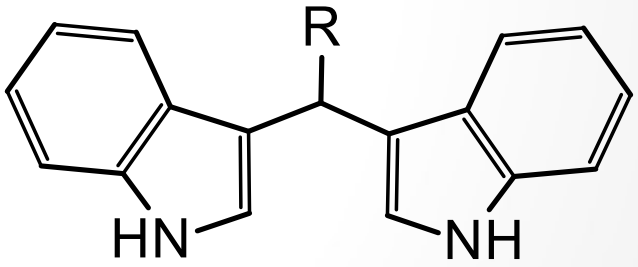
R: C_6H_3-3-Br,-4-F; C_6H_3-3,4-F_2

MIC 2.99-3.49 µM *C. neoformans*



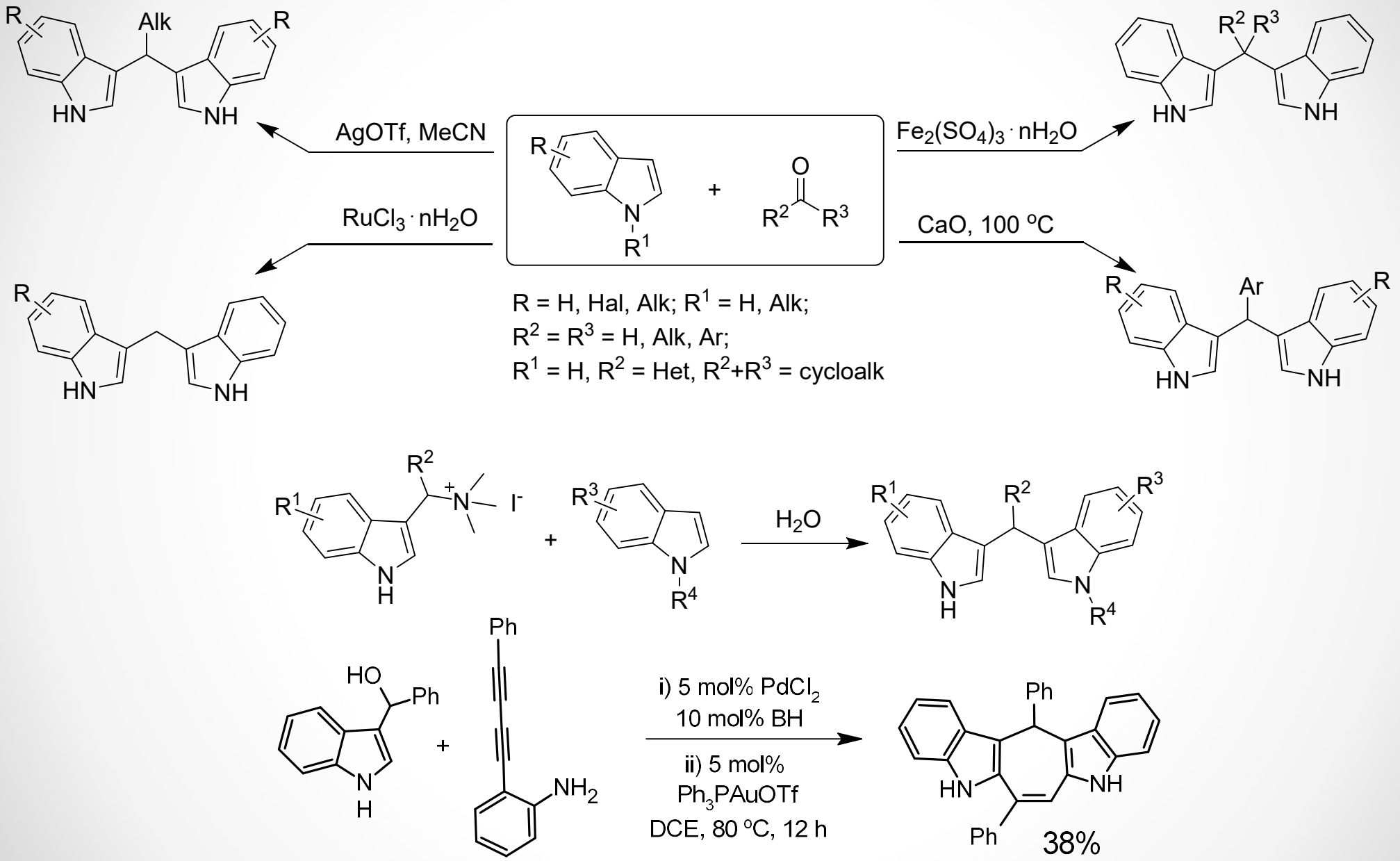
R: 4-NO_2; 4-NH-COMe; 2-NO_2; 3-Cl; 3-NO_2

(IC_{50} 0.22-14.81 µM/mL) *Mtb* H37Ra strain



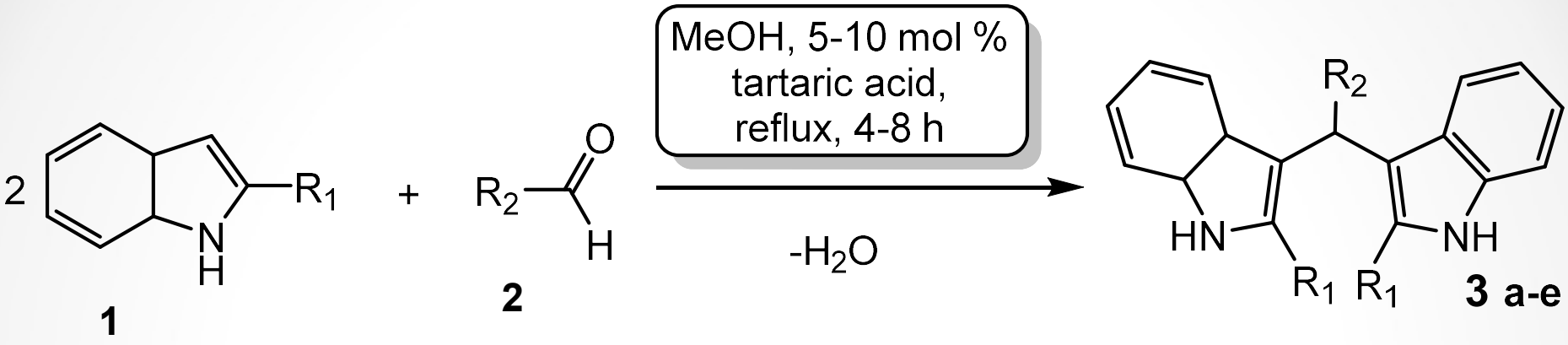
R: 5-NO_2-furyl; 5-NO_2-thienyl; Me; Et

METHODS FOR SYNTHESIS OF SUBSTITUTED BIMs



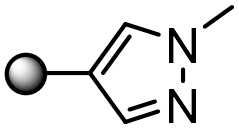
Shiri et al. 2010 *Chem. Rev.;* Deb et al. 2017 *Org. Biomol. Chem.;* Pillaiyar et al. 2018 *Org. Chem.* Noland et al. 2017 *Tetrahedron;* Liu et al. 2019 *Org. Lett.;* Lipson V., Vakula V., Shirobokova M. 2024 *ChemSelect*

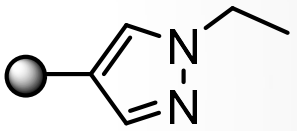
СИНТЕЗ ПОХІДНИХ 3,3’-БІМ

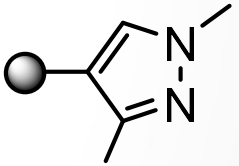


a (78%): R_1 = H; R_2 = 4-$MeOC_6H_4$

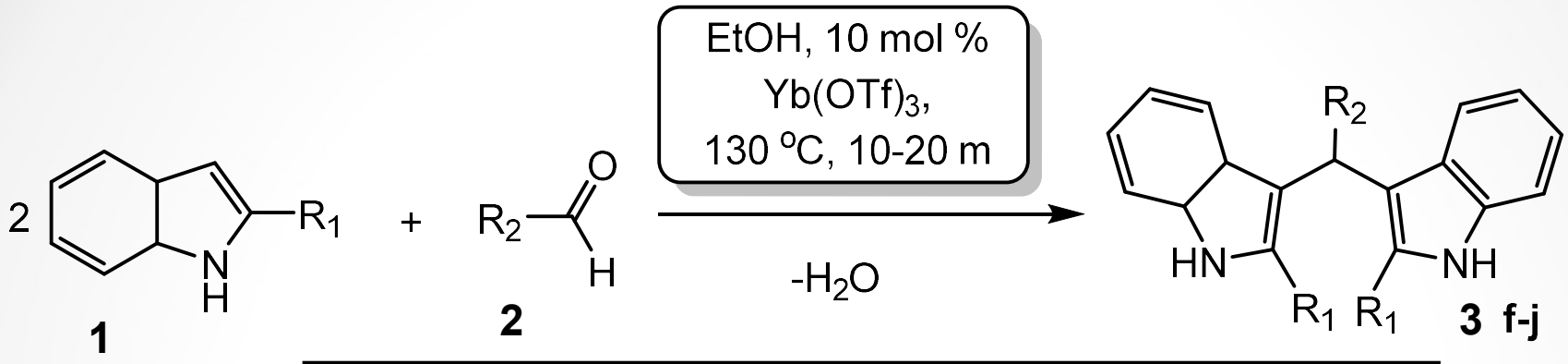
b (44%): R_1 = H; R_2 = 3,5-$F_2C_6H_4$

c (92%): R_1 = H; R_2 = 

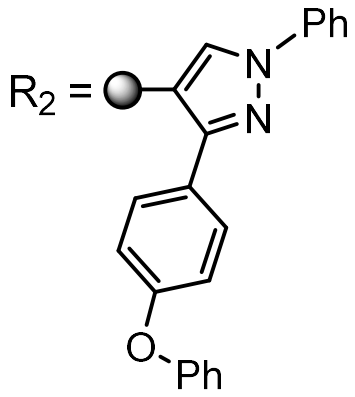
d (95%): R_1 = H; R_2 = 

e (86%): R_1 = H; R_2 = 

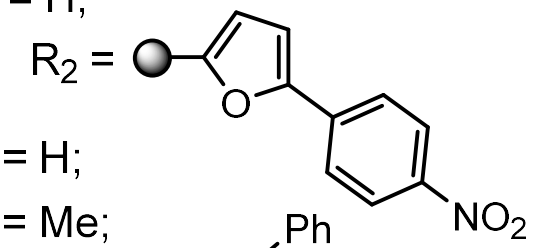
SYNTHESIS OF 3,3'-BIM DERIVATIVES UNDER MW ACTIVATION CONDITIONS



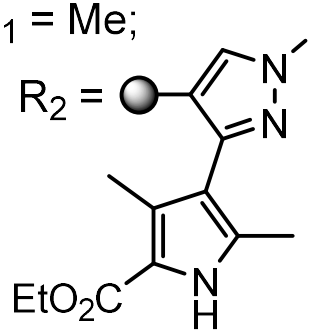
f (82%): R_1 = H;
g (78%): R_1 = Me;

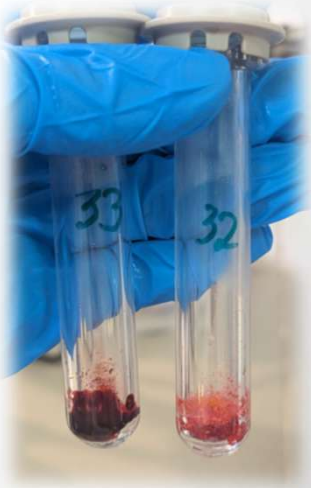


h (92%): R_1 = H;



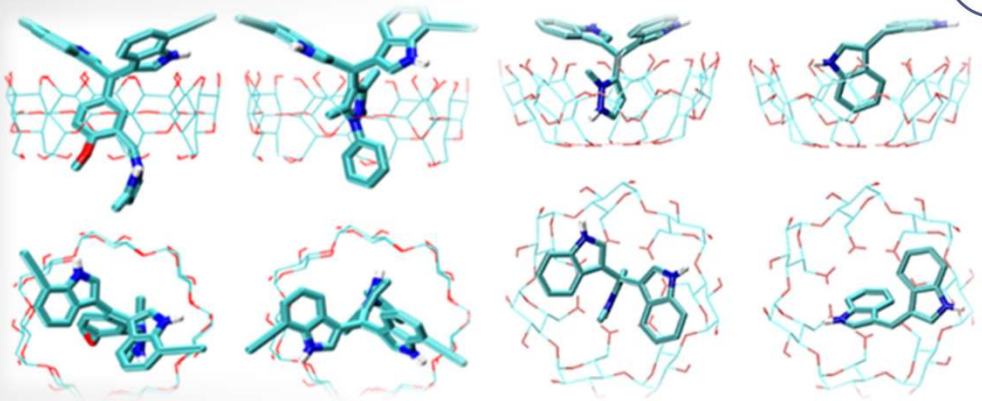
i (75%): R_1 = H;
j (84%): R_1 = Me;



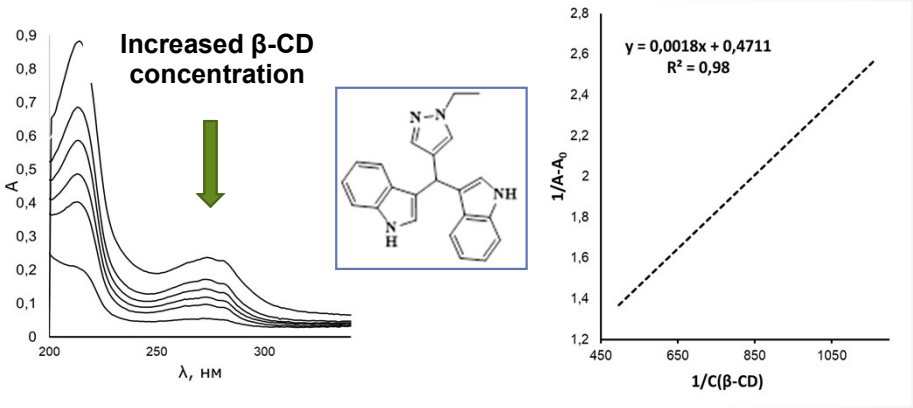


SUPRAMOLECULAR COMPLEXES BASED ON 3,3'-BIM DERIVATIVES WITH CYCLODEXTRINS

Molecular docking
showed the possibility of binding 3,3'-BIM to β-CD in its open (left) and closed (right) conformations

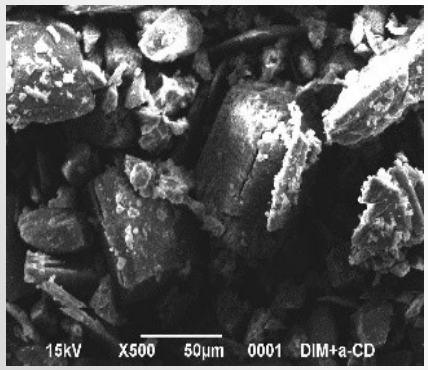


UV titration
was used to determine the binding constants of 3,3'-BIM with cyclodextrins using the **Beneschi-Hildebrand** model:
1/(A-Ao) = 1/{K(Amax–Ao)[CDn]} + 1/[Amax-Ao]

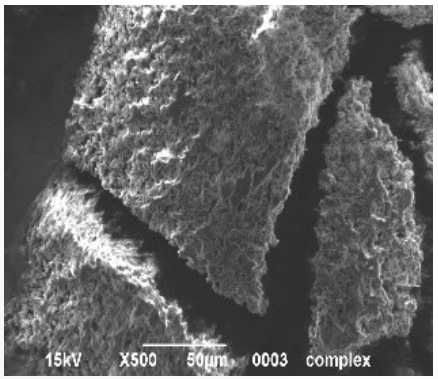


SEM analysis
of surface morphology revealed a significant difference between the mechanical mixture and the 3,3'-BIM complex with α-CD

Mechanical mixture

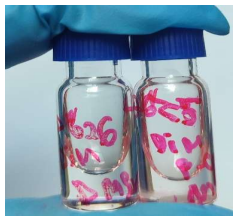


Complex



Storage stability:
Solutions of 3,3'-BIM in DMF with and without β-CD

On the day of preparation



In 2 weeks

In a month

Main results:

- The use of a combination of methods allowed us to establish the fact of complex formation of 3,3'-BIM with α-CD and with β-CD.
- The values of the binding constants of the complexes (≤103) indicate their moderate stability.
- Observation of the complexes in solutions during long-term storage showed a certain increase in the stability of 3,3'-BIM to oxidation.

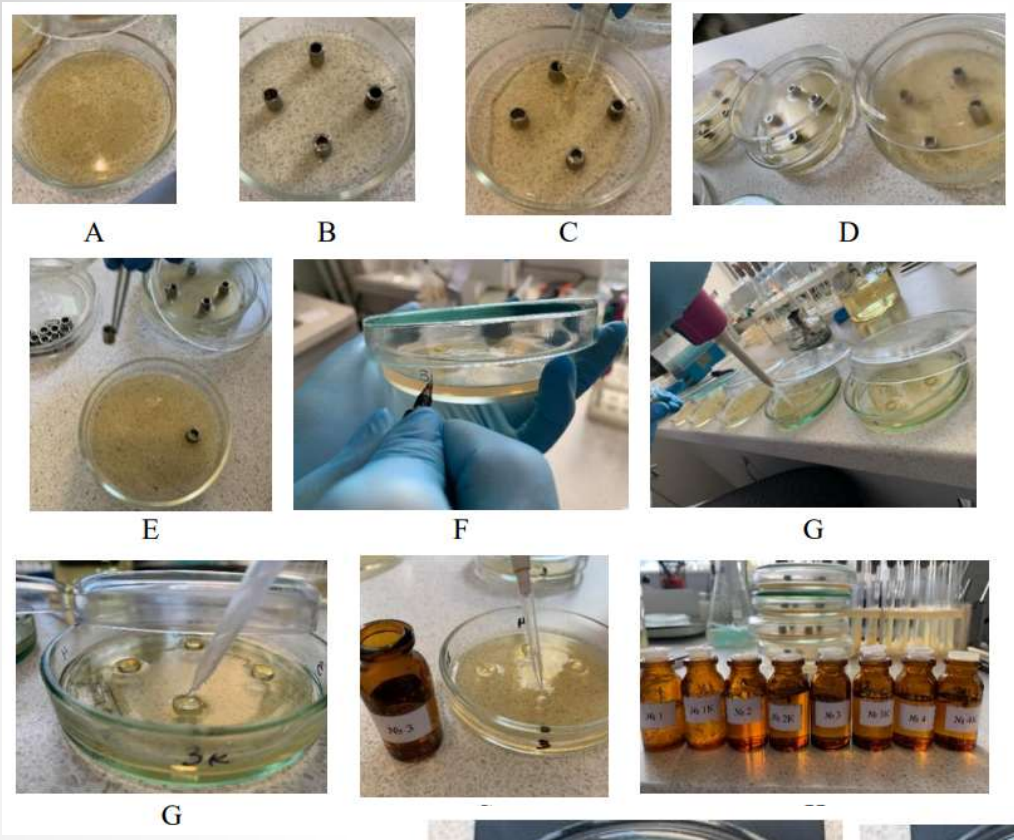
DETERMINATION OF 3,3’-BIM SOLUBILITY AND SELECTION OF THE OINTMENT BASE

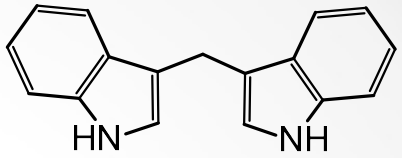
SOLVENT	SOLUBILITY OF 3,3’-BIM (µg in µL)	Qualitative description of the solubility of 3,3’-BIM
DMF	100 µg in 0,1 µL	very easily soluble
DMSO	100 µg in 1 µL	easily soluble
N-methylpyrrolidone	100 µg in 1 µL	easily soluble
ethyl acetate	100 µg in 1 µL	easily soluble
diethylene glycol monoethyl ether	100 µg in 1 µL	easily soluble
chloroform	100 µg in 10 µL	moderately soluble
ethanol (96 %)	10 µg in 10 µL	slightly soluble
hexane	–	practically insoluble
water	–	practically insoluble

1% SOLUTION OF 3,3'-BIM IN A MIXTURE OF N-METHYLPYRROLIDONE – PROPYLENE GLYCOL – MACROGOL (400) – H_2O

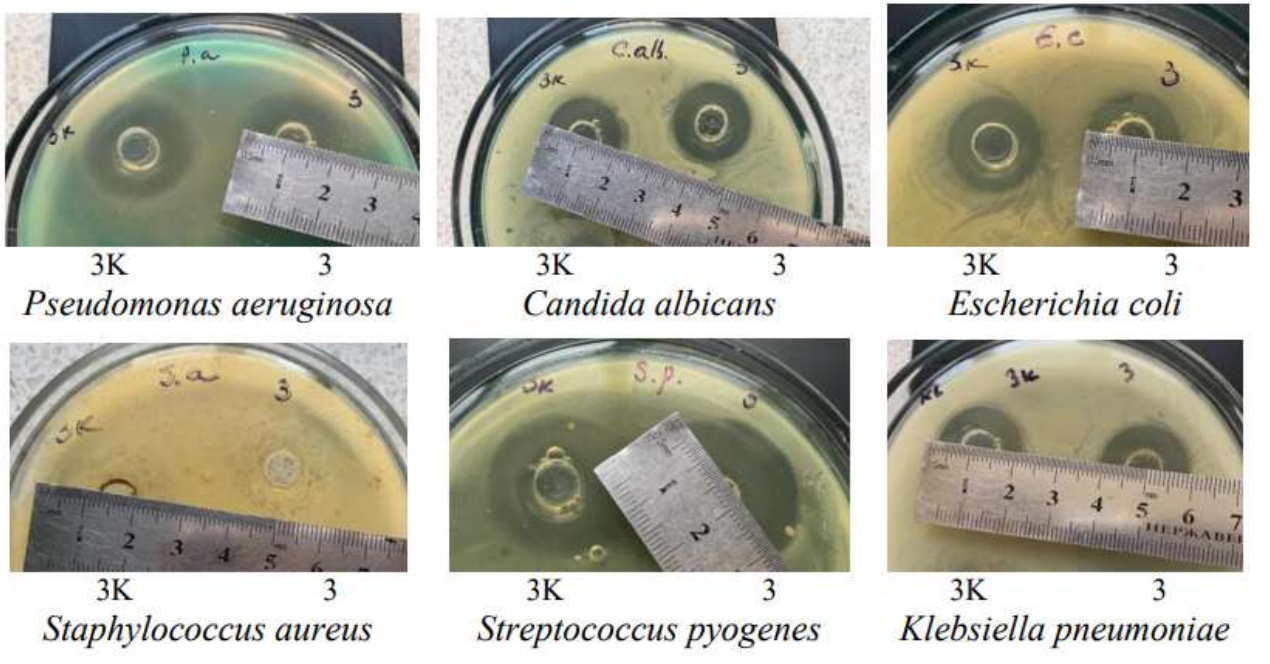
1% SOLUTION OF 3,3'-BIM IN A MIXTURE OF N-METHYLPYRROLIDONE – PROPYLENE GLYCOL – MACROGOL (400) – BUTYLHYDROXYANISOLE 0.05%, BUTYLHYDROXYTOLUENE 0.05%

RESEARCH ON THE ANTIBACTERIAL ACTIVITY OF 3,3’-BIM

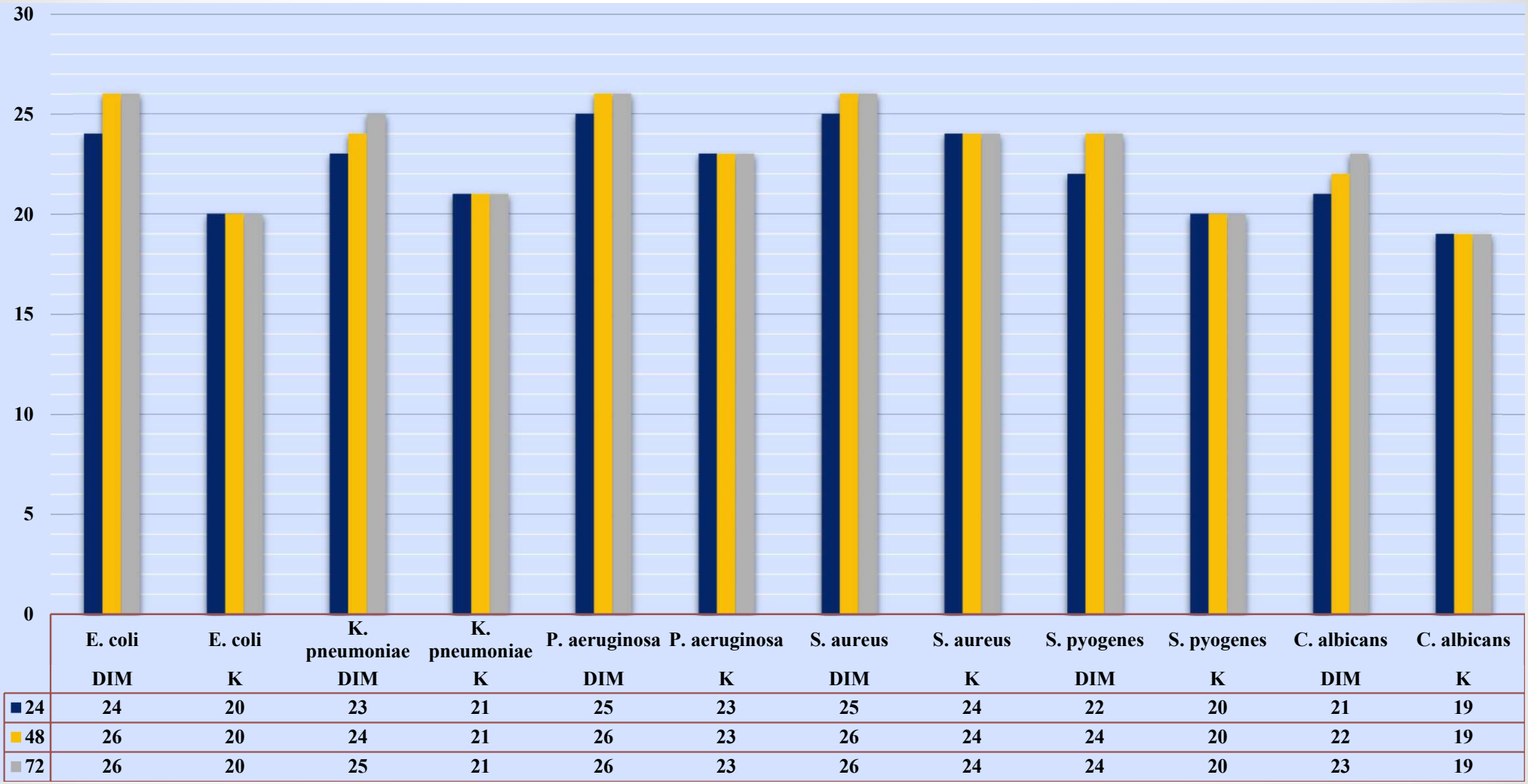




№1	0.5% solution of 3,3'-BIM in DMSO
№2	0.5% solution of 3,3'-BIM in diethylene glycol monoethyl ether
№3	0.5% solution of 3,3'-BIM in N-methylpyrrolidone
№4	0.5% solution of 3,3'-BIM in propylene glycol

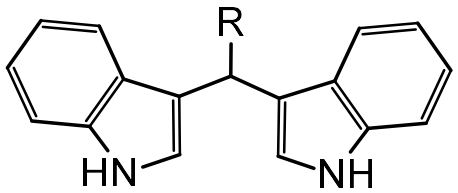


ANTIBACTERIAL ACTIVITY OF 0.5% SOLUTION OF 3,3'-BIM IN N-METHYLPYRROLIDONE



	E. coli DIM	E. coli K	K. pneumoniae DIM	K. pneumoniae K	P. aeruginosa DIM	P. aeruginosa K	S. aureus DIM	S. aureus K	S. pyogenes DIM	S. pyogenes K	C. albicans DIM	C. albicans K
24	24	20	23	21	25	23	25	24	22	20	21	19
48	26	20	24	21	26	23	26	24	24	20	22	19
72	26	20	25	21	26	23	26	24	24	20	23	19

ACTIVITY OF 0.5% SOLUTION OF COMPOUNDS 3a, 3b AND 3d COMPARED TO N-METHYLPYRROLIDONE (K)

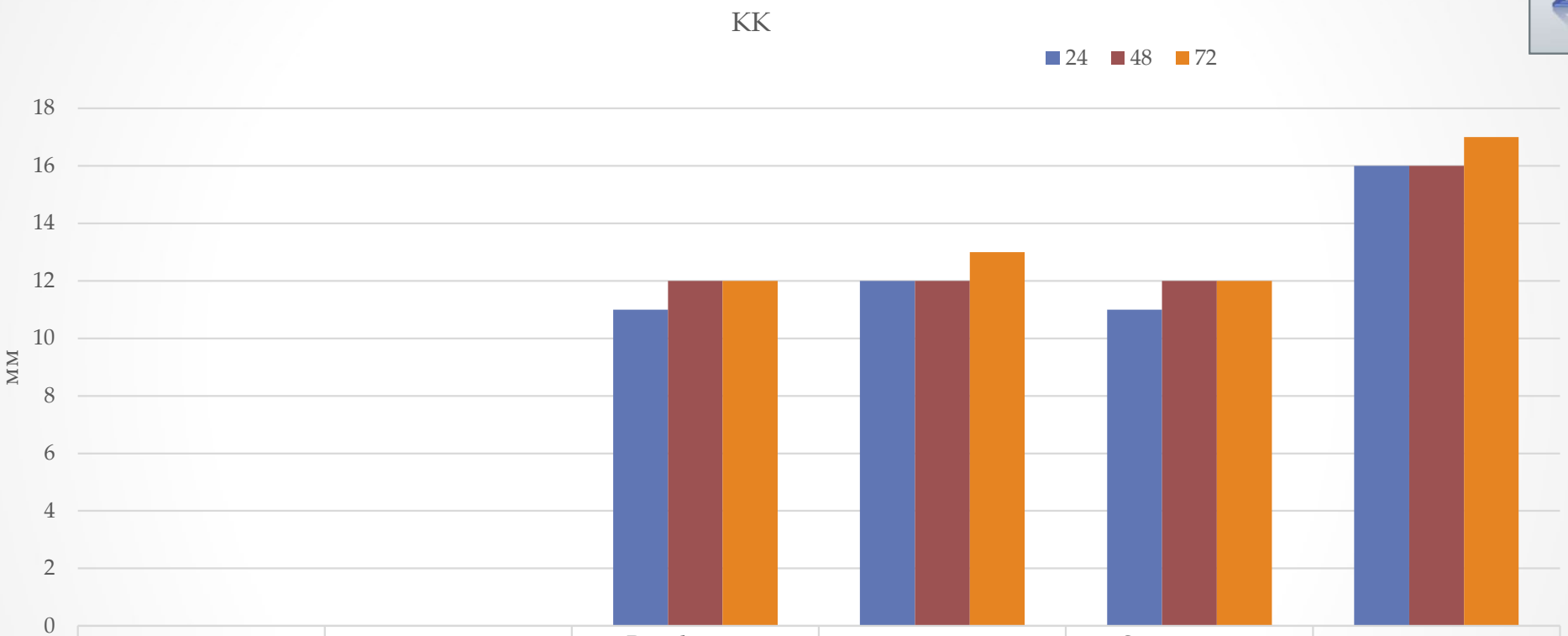


MM

	E. coli				K. pneumoniae				P. aeruginosa				S. aureus				S. pyogenes				C. albicans			
	b	d	a	K	b	d	a	K	b	d	a	K	b	d	a	K	b	d	a	K	b	d	a	K
24	27	26	25	18	28	26	27	20	30	29	29	20	28	26	27	17	28	25	27	16	27	25	26	19
48	28	27	27	18	30	29	28	20	31	30	30	20	30	27	29	17	29	27	29	16	29	27	28	19
72	30	28	27	18	32	31	29	20	32	31	31	20	32	29	31	17	31	29	31	16	31	30	30	19

3a R = 4-$MeOC_6H_4$ **3b** R = 3,5-$F_2C_6H_4$ **3d** R = 1-Et-pyrasol-4-yl

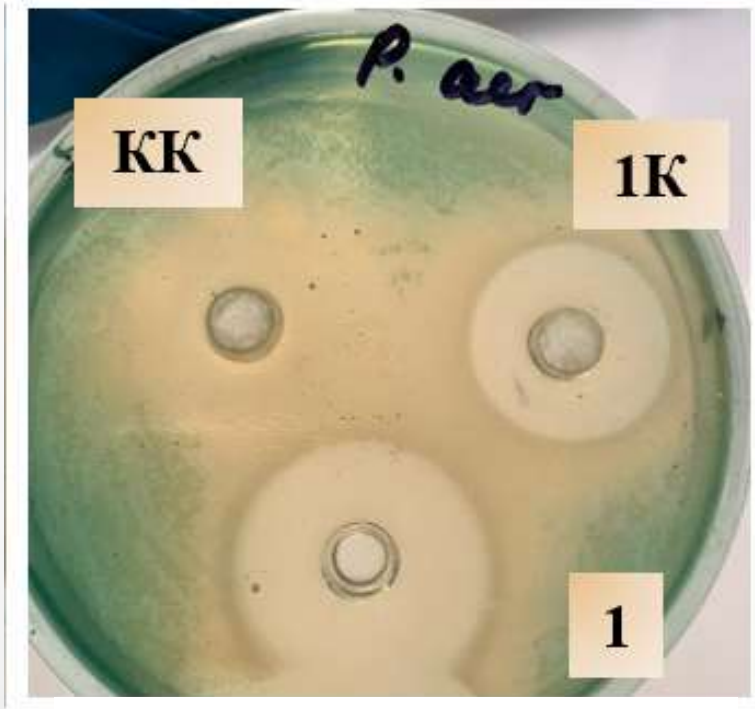
ANTIBACTERIAL ACTIVITY OF 1% SOLUTION OF 3,3'-BIM IN THE MIXTURE OF N-METHYLPYRROLIDONE – PROPYLENE GLYCOL – MACROGOL (400) – H_2O



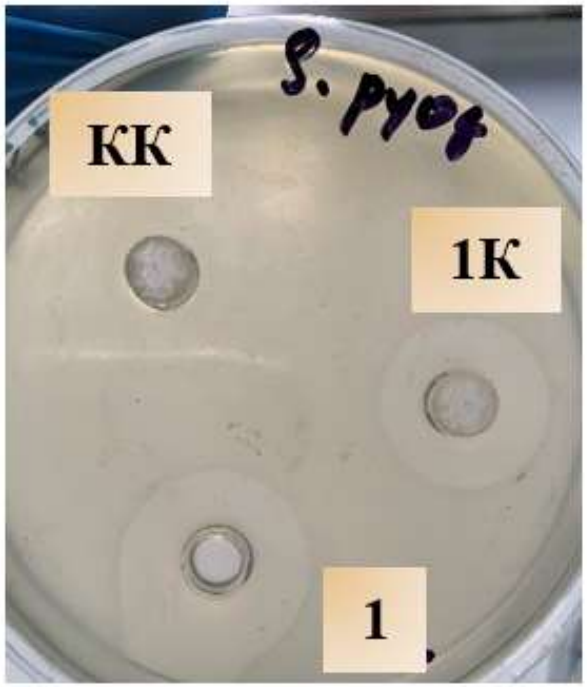
мікроорганізми	Escherichia coli	Klebsiella pneumoniae	Pseudomonas aeruginosa	Staphylococcus aureus	Streptococcus pyogenes	Candida albicans
24	0	0	11	12	11	16
48	0	0	12	12	12	16
72	0	0	12	13	12	17

SENSITIVITY OF POLYRESISTANT CLINICAL STRAINS TO SOLUTIONS OF 1% 3,3'-BIM IN THE MIXTURE OF N-METHYLPYRROLIDONE – PROPYLENE GLYCOL – MACROGOL (400) – H_2O + ANTIBACTERIAL DRUG

№ 1 + 0,1 % gentamicin sulfate solution
№ 2 + 1,0 % ceftriaxone solution
№ 3 + 1,0 % dioxidine solution
№ 4 + 0,1 % ofloxacin solution
№ 5 + 0,75 % chloramphenicol solution



Pseudomonas aeruginosa

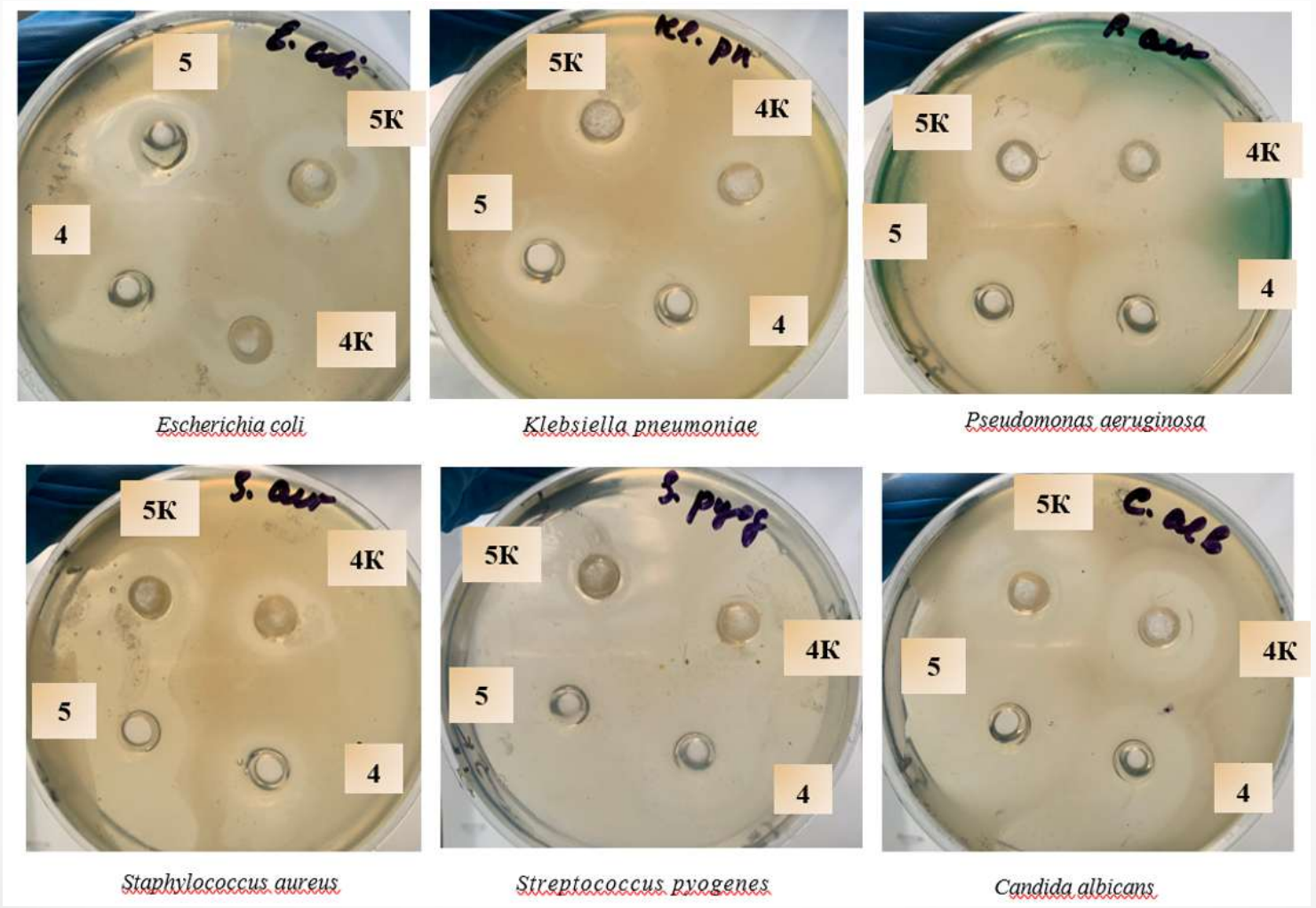


Streptococcus pyogenes

SENSITIVITY OF POLYRESISTANT CLINICAL STRAINS TO SOLUTIONS OF 1% 3,3'-BIM IN THE MIXTURE OF N-METHYLPYRROLIDONE – PROPYLENE GLYCOL – MACROGOL (400) – H_2O + ANTIBACTERIAL DRUG

№ 4 + 0,1 % ofloxacin solution

№ 5 + 0,75 % chloramphenicol solution



Escherichia coli | *Klebsiella pneumoniae* | *Pseudomonas aeruginosa*

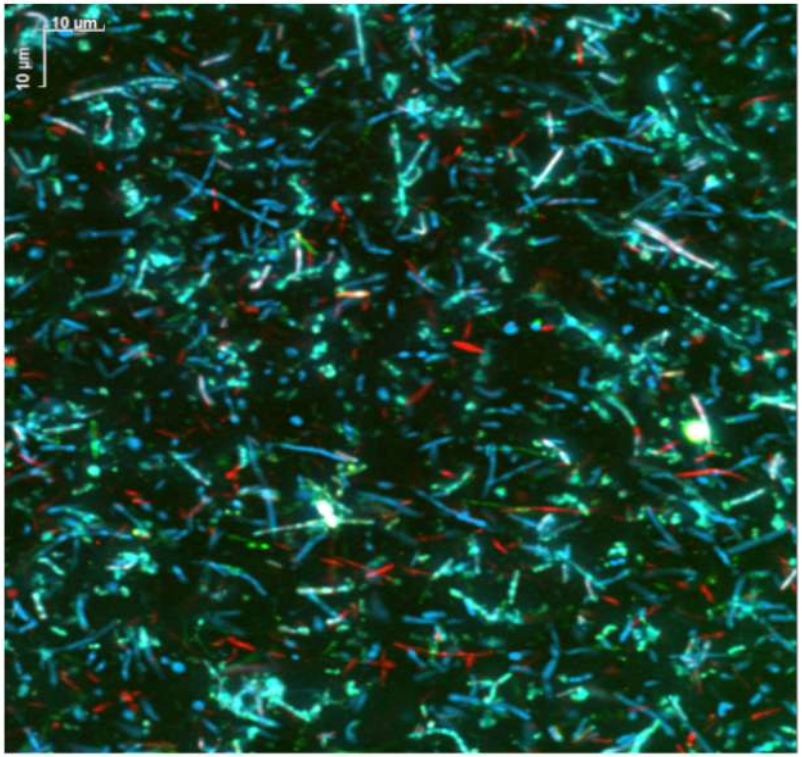
Staphylococcus aureus | *Streptococcus pyogenes* | *Candida albicans*

DETERMINATION OF THE EFFECT OF MIXTURES OF 3,3'-BIM WITH ANTIMICROBIAL APIs ON THE FORMATION OF BACTERIAL BIOFILMS

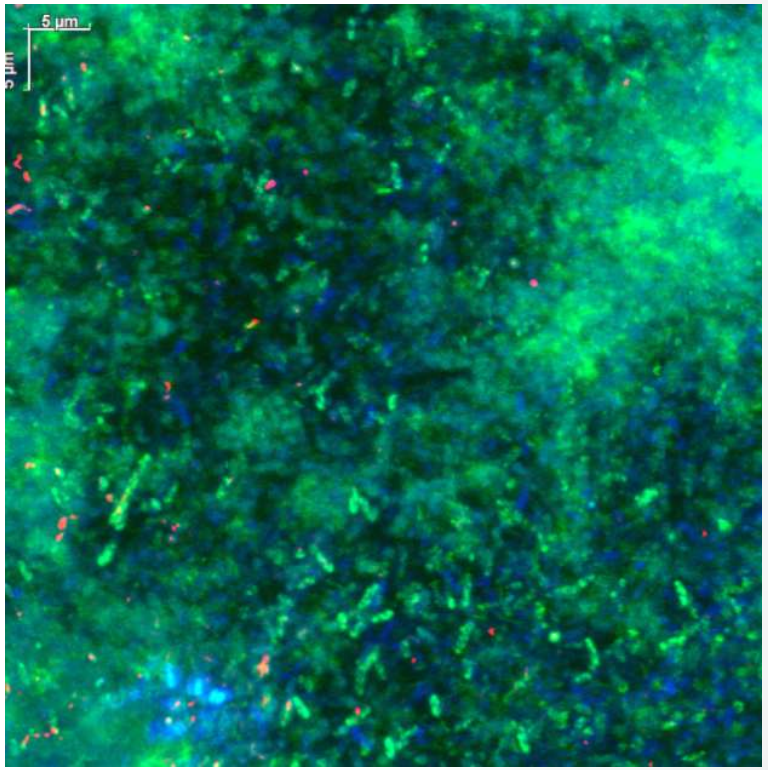
Bacteria strain	Decrease in optical density of daily biofilms (times) compared to optical density of control biofilms			
	Solution ofloxacin 0.1% + BIM 1%	Solution ofloxacin 0.1%	Dioxidine solution 1.0% + BIM 1%	Dioxidine solution 1.0 %
Klebsiella pneumoniae NCTC 5055 = SS B 5055	19.9	6.5	9.5	5.3
Klebsiella pneumoniae 1745	11.5	5.8	18.2	6.5
Pseudomonas aeruginosa 27853 = NCDCF-51 (7419)	16.3	6.4	9.8	6.0
Pseudomonas aeruginosa 10	5.9	4.1	4.3	2.3
Pseudomonas aeruginosa 3	8.2	4.7	8.4	4.7
Escherichia coli 25922 (F50) = NCDC F 50	10.1	6.0	11.8	5.0
Escherichia coli S:CL10TZR	8.1	4.7	12.2	4.7
Staphylococcus aureus 25923 = NCDC 25923 = F-49	9.3	6.1	7.3	5.0
Staphylococcus aureus 551	8.2	5.1	9.7	5.6

THE DAILY *Pseudomonas aeruginosa 10* BIOFILM AFTER ADDITION OF 0.1% OFLOXACIN SOLUTION + 1% 3,3'-BIM (A) AND WITHOUT 3,3'-BIM (B)

(за результатами лазерної скануючої конфокальної мікроскопії)



(A)



(B)

Formulations of ointments on water-soluble base for microbiological study using agar diffusion test

Components	Content (%)					
	Ointment base	Base with 1% DIM	Sample №1	Sample №2	Sample №3	Sample №4
Fluoroquinolone*	-	-	0.10	0.10	0.10	0.10
3,3'-BIM	-	1.00	-	0.10	0.50	1.00
Butylhydroxyanisole	-	0.02	-	0.02	0.02	0.02
Butylhydroxytoluene	-	0.10	-	0.10	0.10	0.10
N-methylpyrrolidone	6.82	6.82	6.82	6.82	6.82	6.82
Poloxamer 338	3.70	3.70	3.70	3.70	3.70	3.70
Macrogol 1450	20.00	20.00	20.00	20.00	20.00	20.00
Macrogol 6000	5.00	5.00	5.00	5.00	5.00	5.00
Macrogol 400	22.60	22.16	2.54	22.47	22.31	22.11
Propylene glycol	41.88	41.20	41.84	41.69	41.45	41.15

Note: * Ofloxacin, or levofloxacin, or moxifloxacin

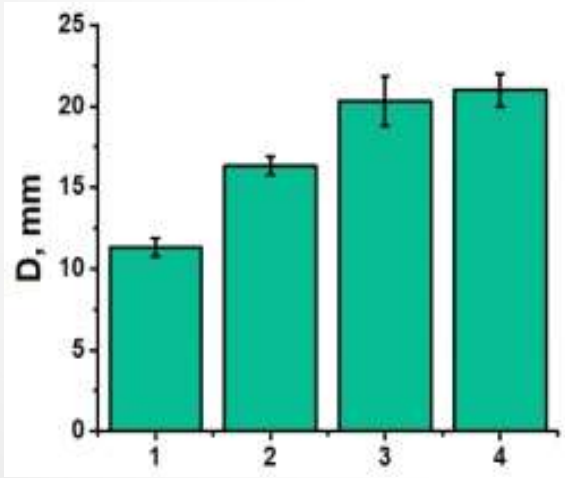
ZONES OF GROWTH INHIBITION FOR *C. amalonaticus 1574* WITH EXPOSURE TO 0.1% MOXIFLOXACIN OINTMENT: (*a*) OINTMENT WITHOUT DIM; (*b*) OINTMENT CONTAINING 1.0% 3,3'-BIM

(a)

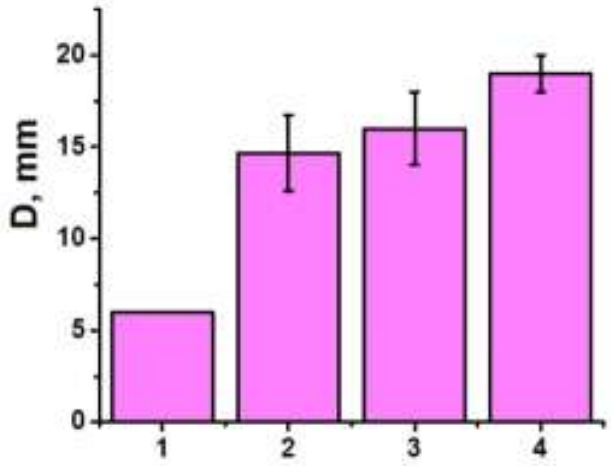
(b)

Note: Wells without zones of growth inhibition were filled with ointment base

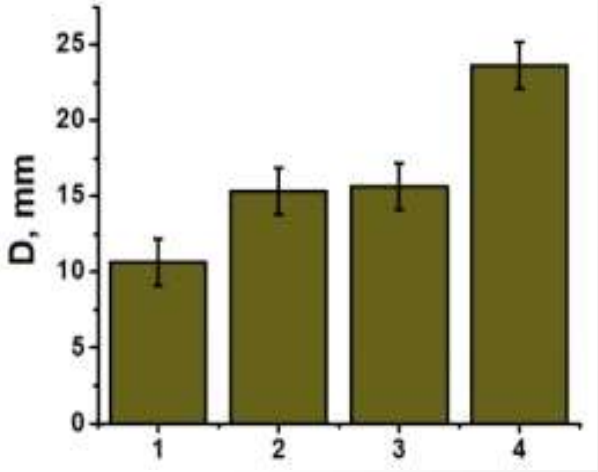
DIAMETERS (D) OF GROWTH INHIBITION ZONES OF *P. aeruginosa 4791* (a), *K. pneumoniae 2820* (b) AND *C. amalonaticus 1574* (c) WITH EXPOSURE TO 0.1% OFLOXACIN OINTMENT WITHOUT 3,3'-BIM (1) AND WITH 3,3'-BIM AT CONCENTRATIONS: 0.1% (2), 0.5% (3), 1.0% (4)



(a)



(b)



(c)

CONCLUSIONS

- 43 new 3,3'-BIM derivatives were synthesized
- The antibacterial activity of 16 synthesized derivatives was studied on standard strains of *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pyogenes, Candida albicans.*
- 3,5-$F_2C_6H_4$-3,3'-BIM showed high antimicrobial activity against *Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pyogenes, Candida albicans;* 1-Et-pyrazol-4-yl-3,3'-BIM was active against *Klebsiella pneumoniae.*
- It has been shown that colloidal nonionic surfactant solution containing 0.1% ofloxacin and 1.0% 3,3'-BIM effectively prevented biofilm formation of both clinical strains and a standard strain of *P. aeruginosa.*
- Our study highlights the potential of the combined use of fluoroquinolones and 3,3'-BIM in water-soluble ointments for the local treatment of purulent wounds infected with resistant bacterial strains in the inflammatory phase

THANK YOU FOR YOUR ATTENTION