

2-AMINO-4,6,7,8-TETRAHYDROTHIOPYRANO[3,2-b]PYRAN-3-CARBONITRILE 5,5-DIOXIDE VP-4535 AS AN ANTIMICROBIAL AGENT SELECTIVE TOWARD METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

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The antibacterial activity of 2-amino-4,6,7,8-tetrahydrothiopyrano[3,2-b]pyran-3-carbonitrile 5,5-dioxide toward five key ESKAPE pathogenic bacteria, methicillin-resistant *Staphylococcus aureus* (ATCC 43300), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 700603), *Acinetobacter baumannii* (ATCC 19606), and *Pseudomonas aeruginosa* (ATCC 27853) was evaluated. The antifungal activity was studied towards pathogenic fungal strains *Candida albicans* (ATCC 90028) and *Cryptococcus neoformans* var. *Grubii* (ATCC 208821). Compound VP-4535 bearing 5-methylindolin-2-one motif possessed the highest antibacterial activity and excellent selectivity toward methicillin-resistant *Staphylococcus aureus* but was inactive against non-resistant *Staphylococcus aureus* strain. The compound in therapeutic concentration was safe to human red blood cells, human lymphocytes, HaCaT, Balb/c 3T3 and HEK-293 cells.

Key words: cyclic sulfones, thiopyrano[3,2-b]pyranes, antimicrobial, antibacterial, ESKAPE pathogenic bacteria, cytotoxicity.

Cyclic sulfoxides and sulfones are important pharmacophores with a wide range of pharmacological activities owing to a range of mechanisms of action and are widely used in drug design [1]. The cyclic sulfone moiety is frequently utilized in medicinal chemistry to optimize the physicochemical properties of lead compounds. Their ability to act as a conformational constraint, H-acceptor and electron-withdrawing functionality has been used to improve affinity and potency by optimizing the interactions with the target proteins. The polarity associated with both sulfones and sulfoxides helps to lower the overall lipophilicity which in many cases translates into improvements in ADME and pharmaceutical properties. In addition, the sulfone moiety can reduce the basicity of cyclic amines and ameliorate liabilities such as human Ether-a-go-go-Related-Gene (hERG) and phospholipidosis, resulting in improved toxicity profiles [1].

There are many examples of commercial drugs and clinical compounds containing six-membered thiopyrane ring. Depending on the substitution pattern of the core thiopyrane ring, this class of compounds has demonstrated a diverse range of biological activities ranging from anti-inflammatory and antiviral to ATP-sensitive potassium channel (KATP) openers [2-7]. Many compounds are now under active investigation as selective inhibitors of different enzymes and kinases, and also agonists/antagonists of receptors [8-14]. Antiglaucoma agent Dorzolamide [15], diuretic Metikran [16] and anti-herpesvirus agent Amenamevir (ASP-2151) [17, 18] even became marketed drugs (Fig. 1).

Recently, we turned our attention to β -keto-sulfones since they have been established as versatile reagents useful for the preparation of a multitude of sulfur-containing compounds both synthetic and biological importance [19-22]. In this context, cyclic

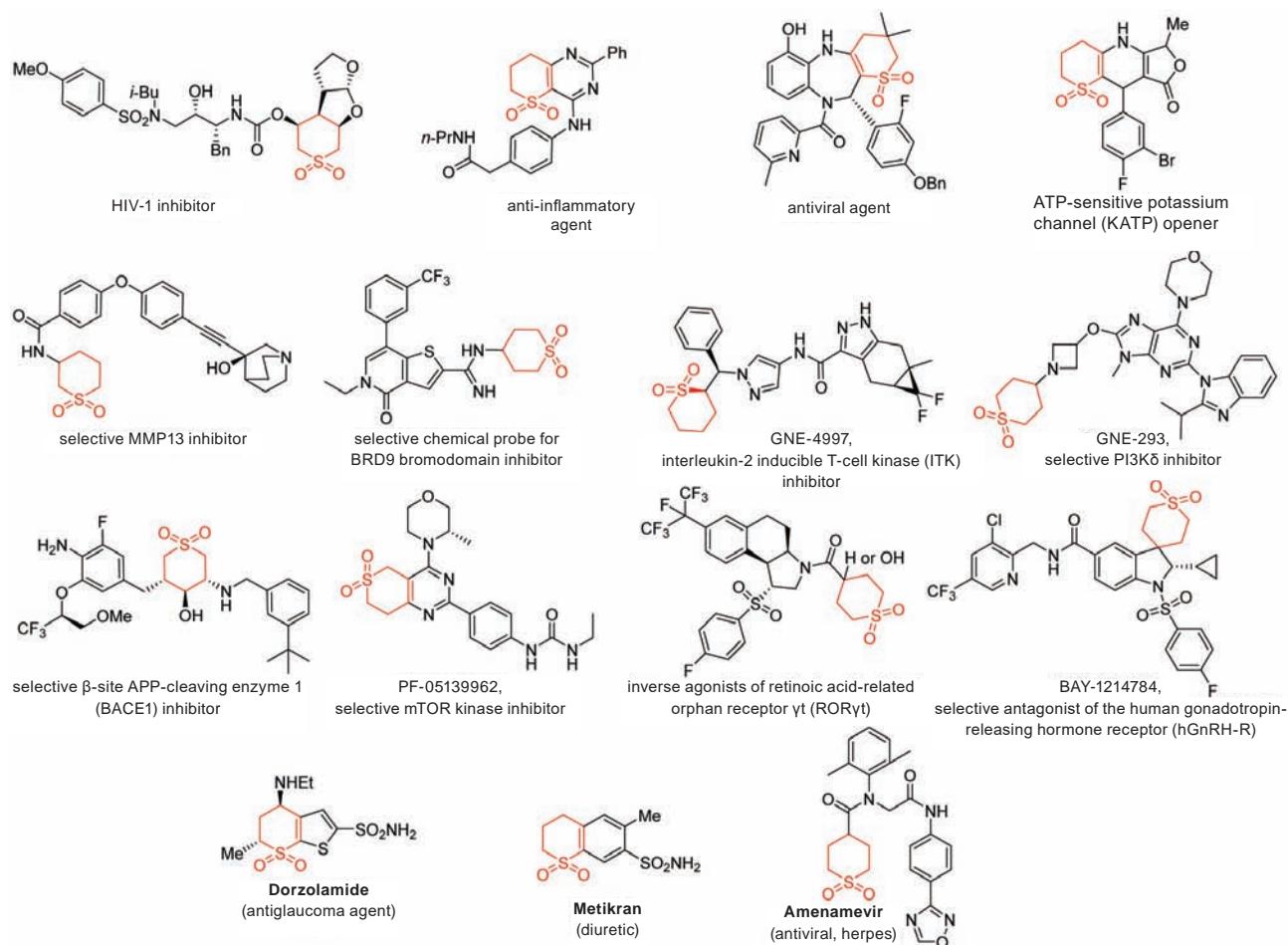


Fig. 1. Selected examples of bioactive molecules with six-membered cyclic sulfone motif (showed in red)

β -ketosulfones are among the most promising reagents and particularly useful due to their availability and possible application in the synthesis of a diverse range of polycyclic sulfones [21]. Our interest in dihydro-2*H*-thiopyran-3(4*H*)-one-1,1-dioxide **1** has arisen due to its high reactivity in multicomponent reactions (MCR) and wide applicability in the synthesis of various sulfur and nitrogen-containing heterocycles [23]. In this work in the continuation of our studies on the synthesis of biologically active heterocycles [24–27], we decided to use building block **1** in the synthesis of a small set of biologically relevant sulfones and test them for antimicrobial activity.

Materials and Methods

Compound preparation. Initially, the tests were carried out at a single compound concentration of 32 $\mu\text{g/ml}$ in duplicate, to identify any active compound. Furthermore, a hit confirmation of the active compounds by a dose–response test, using

eight concentrations at 1:2 dilution, in duplicate, to determine the MIC against bacteria and yeasts, CC_{50} (concentration at 50% cytotoxicity) against mammalian cells, and HC_{10} (concentration at which 10% hemolysis is induced) against human red blood cells was performed. All substances were dissolved in DMSO to form a stock concentration of 10 mg/ml. Aliquots were diluted in water and 5 μl were dispensed into empty 384-well plates in duplicate for each strain and cell-assayed. As soon as cells were added to the plates, this gave a final compound concentration of 32 $\mu\text{g/ml}$, or in case of a serial dilution assay compound concentrations from 32 to 0.25 $\mu\text{g/ml}$, in both cases with a maximum DMSO concentration of 0.3%.

Primary antimicrobial assays via CO-ADD [28]. The compounds have been investigated for activity towards one Gram-positive bacteria (*S. aureus* ATCC 43300 MRSA), four Gram-negative bacteria (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC 700603, *A. baumannii* ATCC

19606), and two yeasts (*C. albicans* ATCC 90028 and *C. neoformans* H99 ATCC 208821), and this research was performed by the Community for Open Antimicrobial Drug Discovery (CO-ADD).

All bacteria were overnight cultured in cation-adjusted Q14 Mueller–Hinton broth (CAMHB) at 37°C. The resultant mid-log phase cultures were added to each well of the compound containing plates (384-well nonbinding surface plates-Corning 3640), giving a cell density of 5×10^5 CFU/ml (colony-forming units/ml). All plates were covered and incubated at 37°C for 18 h without shaking. Inhibition of bacterial growth was determined measuring absorbance at 600 nm. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate as references. Growth inhibition of *C. albicans* was determined measuring absorbance at 530 nm, while the growth inhibition of *C. neoformans* was determined measuring the difference in absorbance between 600 and 570 nm, after the addition of resazurin (0.001% final concentration) and incubation at 35°C for additional 2 h. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate as references. Percentage growth inhibition of an individual sample is calculated based on Negative controls (media only) and Positive Controls (bacterial/fungal media without inhibitors). Negative inhibition values indicate that the growth rate (or OD_{600}) is higher compared to the negative control (bacteria/fungi only, set to 0% inhibition). The growth rates for all bacteria and fungi have a variation of $\pm 10\%$, which is within the reported normal distribution of bacterial/fungal growth. Any significant variation (or outliers/hits) is identified by the modified Z-Score, and actives are selected by a combination of inhibition value and Z-Score. Growth inhibition was evaluated as a percentage between untreated cells (positive growth control) and media only (negative growth control). Compounds with $\geq 80\%$ growth inhibition were selected as active compounds in the initial screening, and MIC was determined following EUCAST recommendations. Also, 80% growth inhibition was used as a threshold for full inhibition.

Antimicrobial methods. Antibacterial effect was determined using MTT test. Experiments were conducted at pH 7.2. Subsequent bacterial culture in logarithmic phase of growth in Sabouraud medium,

pH 7.2, was centrifuged 10 min at $500 \times g$, sediment of bacteria was washed with sterile saline and resuspended in small volume of sterile saline. A defined volume of this suspension was introduced into Sabouraud medium with pH 7.2 for achievement of OD 0.4-0.6 at 590 nm (optical path 1.0 cm). 100 μ l of each suspension were introduced into series of 1.5 ml Eppendorf tubes and thereafter inoculated with 10, 5 and 2 μ l of tested sample solution. Each point was repeated in triplicate. Tubes were incubated 4 h at 37°C. Thereafter 10 μ l of MTT solution (5 mg/ml) was introduced and incubation was continued for 1 h. Cells were harvested by centrifugation 5 min at 1500 g, supernatant was discarded, small sediment was suspended in 1 ml of DMSO. After the incubation for 1 h at 37°C the OD of liquid was measured at 580 nm using spectrophotometer ULAB 102 UV (Ukraine) [29].

Cytotoxicity assay toward HEK-293 cells. HEK-293 (human embryonic kidney) ATCC CRL-1573 cells were counted manually in a Neubauer hemocytometer and then plated in 384-well tissue culture-treated plates (Corning 3712) containing the compounds to give a density of 5,000 cells/well in a final volume of 50 μ l. Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum was used as growth media and the cells were incubated at 37 °C with the compounds for 20 h in 5% CO₂. Cytotoxicity (or cell viability) was determined by fluorescence, ex: 560/10 nm, em: 590/10 nm (F560/590), after the addition of 5 μ l of 25 μ g/ml resazurin (2.3 μ g/ml final concentration) and after incubation at 37°C for further 3 h in 5% CO₂. Tecan M1000 Pro monochromator plate reader was used for the fluorescence intensity measurement, using automatic gain calculation. The IC₅₀ was calculated by means of curve fitting the inhibition values versus log (concentration) using a sigmoidal dose–response function, with variable fitting values for bottom, top, and slope.

Hemolysis assay. Human whole blood was washed three times with three volumes of 0.9% NaCl and then resuspended in the same with a concentration of 0.5×10^8 cells/ml, as determined by manual cell count in a Neubauer hemocytometer with further addition of washed cells to the 384-well compound containing polystyrene plates (Corning 3657) for a final volume of 50 μ l. The plates were incubated for 1 h at 37°C after 10-min shaking on a plate shaker. The next step was centrifugation of plates at 1000 g for 10 min to pellet cells and debris; 25 μ l of

the supernatant was then transferred to a polystyrene 384-well assay plate (Corning 3680). Hemolysis was defined by the supernatant absorbance at 405 nm (OD_{405}) using a Tecan M1000 Pro monochromator plate reader. HC10 was established by curve fitting the inhibition values versus log (concentration) using a sigmoidal function with variable fitting values for top, bottom, and slope. The use of human blood (sourced from the Australian Red Cross Blood Service) for hemolysis trials was approved by the University of Queensland Institutional Human Research Ethics Committee (Approval Number: 2014000031).

Cells culture and cytotoxicity assay (cell proliferation (MTT) and Trypan Blue assays). Human keratinocytes of HaCaT line and murine fibroblasts of Balb/c 3T3 line were obtained from a Collection at the Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine (Kyiv, Ukraine). Cells were grown in the DMEM (Biowest, Nuaille, France) culture medium supplemented with 10% of fetal bovine serum (Biowest, Nuaille, France) at 37°C and 5% CO₂. *In vitro* evaluation of cytotoxic activity of the compound **VP-4535** and a reference drug doxorubicin was conducted using the MTT test [30]. Briefly, cells were seeded for 24 h in 96-well microtiter plates at a concentration of 5,000 cells/100 µl/well; after that, cells were incubated for 72 h with various additions of the synthesized compounds or DMSO (0.357; 3.57; 35.7; 357; 892.5 µg/ml), or Dox (0.58; 5.8 µg/ml). MTT, which is converted to dark blue, water-insoluble formazan by the mitochondrial dehydrogenases, was used to determine viable cells according to the Sigma-Aldrich protocol. Formazan was dissolved in DMSO, and the results of the reaction were determined by an Absorbance Reader BioTek ELx800 (BioTek Instruments, Inc., Winooski, VT, USA).

The study protocol with human lymphocytes isolated from healthy adult human peripheral blood was approved by Ethics Committee of the Institute of Cell Biology of National Academy of Sciences of Ukraine (protocol no 2 dated by January 27, 2019). Lymphocytes of human peripheral blood were isolated from blood consisting of anti-coagulant sodium heparin solution 10 U/ml (B.Braun Medical, S.A., Spain) from a healthy adult donor on density gradient of Histopaque 1077 (Merck, Germany) using a modified protocol [31]. The blood : Histopaque 1077 mixture (1:1) was centrifuged at 400×g at room temperature for 30 min. The cells were washed in the phosphate buffered saline (PBS). The residual erythrocytes were removed from the lym-

phocytes population by the hypotonic lysis. Lymphocytes were cultured in the RPMI-1640 (Biowest, Nuaille, France) medium supplemented with 20% fetal bovine serum (Biowest, France) at 95% air and 5% CO₂, and 37°C. The lymphocytes were activated using phytohemagglutinin-L (PHA-L, 1 µg/ml, Sigma-Aldrich, USA) mitogen and incubated for next 24 h before treatment with studied compounds.

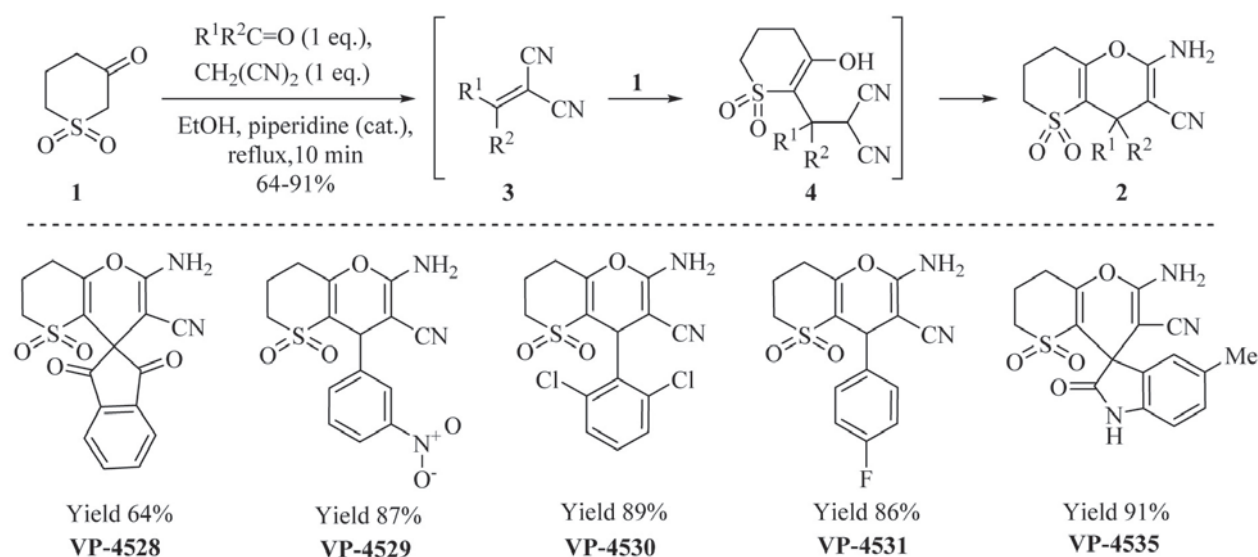
The evaluation of the anti-proliferative activity *in vitro* of the studied compound or DMSO (0.357; 3.57; 35.7; 357; 892.5 µg/ml), or Dox (0.58; 5.8 µg/mL) towards mitogen-activated lymphocytes (150,000 cells/100 µl) of human peripheral blood was conducted on 48 h using MTT assay (EZ4U, Biomedica, Vienna, Austria). The optical density was measured with the Absorbance Reader at 490 nm with 630 nm as a reference wavelength. The reduction of cells growth (in percentages, %) was calculated as ratio of absorbance in treated cells relative to absorbance in control cells. The anti-proliferation activity of the studied compounds was expressed as an IC₅₀ value (the concentration of sample that reduces the 50% of cells growth).

Statistical analysis. Z-Score analysis is done to investigate outliers or hits among the samples. The Z-Score is calculated based on the sample population using a modified Z-Score method which accounts for possible skewed sample population. The modified method uses median and median average deviation (MAD) instead of average and Standard deviation (SD), and a scaling factor [32]: $M(i) = 0.6745 \cdot (x(i) - \text{median}(x)) / \text{MAD}$. All screening is performed as two replicas ($n = 2$), with both replicas on different assay plates, but from single plating and performed in a single screening experiment (microbial incubation). Two values are used as quality controls for individual plates: $Z\text{-Factor} = 1 - [3 \cdot (\text{SD}(\text{Negative controls}) + \text{SD}(\text{Positive controls})) / (\text{average}(\text{Positive controls}) - \text{average}(\text{Negative controls}))]$.

Cytotoxicity data are presented as the mean ± SD. Results were analysed and illustrated with GraphPad Prism (version 6; GraphPad Software, San Diego, CA, USA). Statistical analyses were performed using two-way ANOVA with Dunnett's multiple comparisons test (cells growth inhibition). A $P < 0.05$ was considered as statistically significant.

Results and Discussion

Chemistry. We have recently found that compound **1** easily reacts with aromatic aldehydes and



Scheme. Synthetic route to the target compounds **VP-4528**, **VP-4529**, **VP-4530**, **VP-4531**, **VP-4535**

malononitrile in a 1:1:1 molar ratio in EtOH in the presence of catalytic amounts of piperidine to give bicyclic products **2** in very good yields (64-91%) [33] (Scheme). A possible mechanism involves the Knoevenagel reaction between the aromatic aldehydes/ketones and malononitrile, followed by Michael addition of a ketosulfone anion to α,β -unsaturated dinitriles **3** and subsequent hetero-Thorpe-Ziegler cyclization of Michael adducts **4**. Using this general approach, we synthesized sulfones **VP-4528**, **VP-4529**, **VP-4530**, **VP-4531**, **VP-4535** and test them for antimicrobial activity.

Biological activity. Antimicrobial screening. The primary screening towards 5 key ESKAPE

pathogens and 2 fungi were performed by the Community for Antimicrobial Drug Discovery (CO-ADD), funded by the Wellcome Trust (UK) and The University of Queensland Australia [28, 34]. All synthesized compounds were evaluated in concentration 32 $\mu\text{g/ml}$ (approx. 100 μM) for their antimicrobial activity towards five pathogenic bacteria, methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC 43300) as Gram-positive bacteria and *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 700603), *Acinetobacter baumannii* (ATCC 19606), and *Pseudomonas aeruginosa* (ATCC 27853) as Gram-negative bacteria, and antifungal activity towards two pathogenic fungal strains *Candida albi-*

Table. The percentage of growth inhibition of studied microorganisms by 2-amino-4,6,7,8-tetrahydrothiopyrano[3,2-*b*]pyran-3-carbonitrile 5,5-dioxides **VP-4528**, **VP-4529**, **VP-4530**, **VP-4531**, **VP-4535**

Compound	<i>S. aureus</i> methicillin-resistant strain	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	<i>A. baumannii</i>	<i>C. albicans</i>	<i>C. neoformans</i>
VP-4535	106.7; 99.9 ^a	-0.4; 0.0	1.7; 9.1	2.2; 8.1	-2.3; 1.2	-0.3; 2.8	-0.5; 6.8
VP-4528	18.8; 33.1	6.6; 7.0	11.0; 6.8	1.3; 6.6	-14.0; -22.0	0.5; 4.7	-1.3; -9.8
VP-4529	-12.5; -4.6	-9.0; -9.8	-0.1; -4.1	-2.5; 0.5	--10.8; 10.1	2.0; 5.4	-6.1; 0.5
VP-4530	-0.0; 2.7	-3.4; -6.2	-2.3; -4.5	-2.0; -4.8	-0.1; 8.0	2.2; 2.7	-6.7; -7.4
VP-4531	-2.9; 11.7	-4.7; 2.8	-4.8; 2.9	1.3; 1.3	-7.0; 1.2	4.9; 9.2	-6.2; -7.8

^aResults of two independent trials

cans (ATCC 90028) and *Cryptococcus neoformans* var. *Grubii* (H99; ATCC 208821). The results of two parallel trials are presented in Table.

Most compounds were inactive towards selected pathogens, but one compound **VP-4535** showed excellent activity and selectivity toward methicillin-resistant *Staphylococcus aureus* (ATCC 43300). The MIC of compound **VP-4535** was 32 µg/ml. In this regard, the compound was further investigated in detail on a strain of non-resistant *Staphylococcus aureus* (ATCC 25923) (Fig. 2) and for comparison on another strain gram-negative bacteria *Pseudomonas aeruginosa* ATCC9027 (Fig. 3).

Studies have shown that compound **VP-4535** was non-active against *S.aureus* ATCC25923, but at the same time has an antibacterial effect toward methicillin-resistant *Staphylococcus aureus*. Based on this we can propose that there is some specific mechanism of the selective action of compound **VP-4535**, which will be studied in further research (Fig. 2).

Also, we studied **VP-4535** toward gram-negative bacteria *Pseudomonas aeruginosa* ATCC9027. Compound passed some antibacterial activity only in high concentration. Noteworthy, that a solvent (DMSO) has a significant effect on growth inhibition of *Pseudomonas aeruginosa* ATCC9027 (Fig. 3).

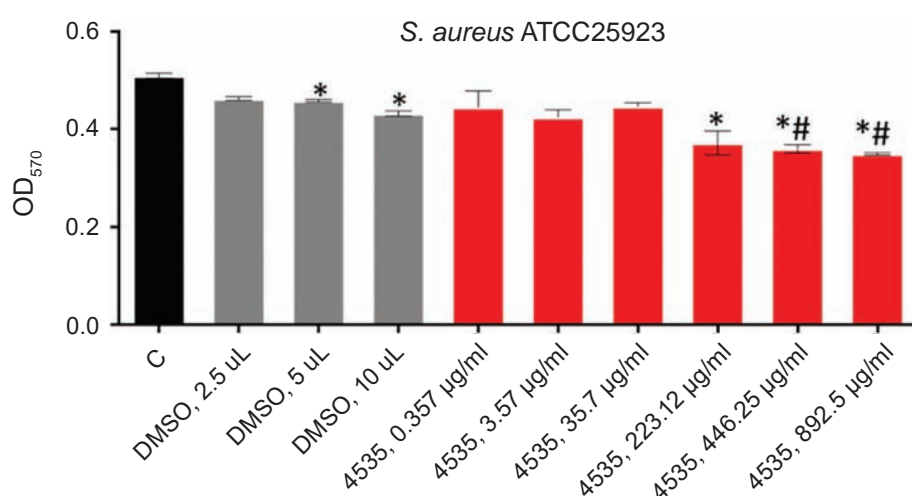


Fig. 2. Antibacterial effect of studied compound **VP-4535** towards *Staphylococcus aureus* ATCC25923. C – control data, *reliable to control, #reliable to DMSO concentration equal concentration of DMSO in which was dissolved tested compound, and added to sample

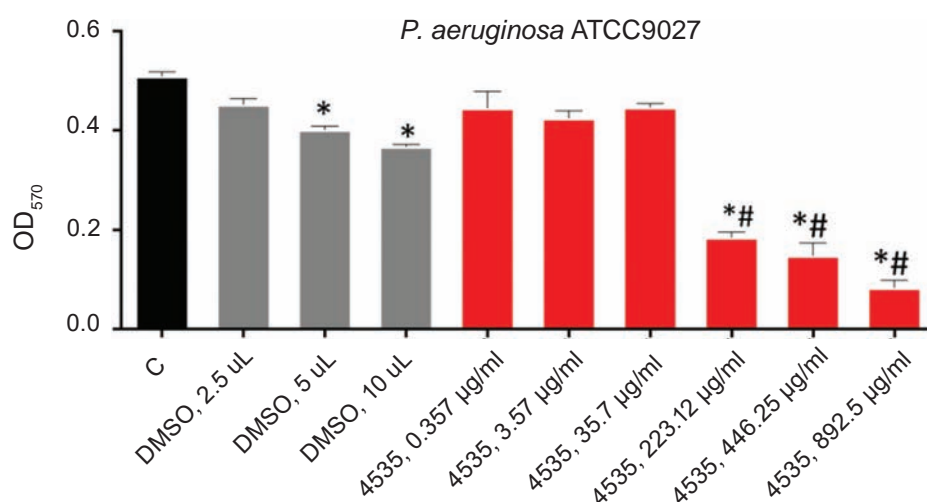


Fig. 3. Antibacterial effect of studied compound **VP-4535** against *Pseudomonas aeruginosa* ATCC9027. C – control data, C – control data, *reliable to control, #reliable to DMSO concentration equal concentration of DMSO in which was dissolved tested compound, and added to sample

Summarizing, we detected, that there is some selectivity mechanism of **VP-4535** towards resistant and non-resistant *Staphylococcus aureus*. This allows us to assume the effective use of **VP-4535** in the treatment of resistant infections, minimally disturbing the balance of the microflora.

Cytotoxicity. Some antimicrobial agents may affect normal red and white blood cells, tissues and organs [35-37]. In this regard, cytotoxicity of the compound **VP-4535** was evaluated towards human embryonic kidney cells (HEK-293) cell line, human keratinocytes (HaCaT), murine fibroblasts (Balb/c 3T3), human lymphocytes and red blood cells. Lead compound was well tolerated to human red blood cells, the HC_{10} was $> 32 \mu\text{g/ml}$. Human mitogen-activated lymphocytes were relatively non-sensitive to the action of compound **VP-4535**. The IC_{50} level of this compound was above $892.5 \mu\text{g/ml}$ (2.5 mM), and one can see 67.5% of alive lymphocytes. While, solvent DMSO was more toxic for lymphocytes, IC_{50} was approximately $892.5 \mu\text{g/ml}$ (Fig. 4).

Studied compounds had low cytotoxic action towards normal cells: human lymphocytes, HaCaT (human keratinocytes), Balb/c 3T3 line (murine fibroblasts) (Fig. 5), and HEK-293 (human embryonic kidney cells). The IC_{50} of **VP-4535** was $> 32 \mu\text{g/ml}$ for HEK-293 cells. Compound did not reach the IC_{50} value for HaCaT and Balb/c 3T3 cells. At the highest dose of $892.5 \mu\text{g/ml}$, compound **VP-4535** inhibited the growth of these cells by 37.7% and 21.7%, respectively. DMSO demonstrated similar toxicity towards HaCaT and Balb/c 3T3 cells (Fig. 5). The doxorubicin IC_{50} value of $0.5 \mu\text{g/ml}$ for HaCaT and

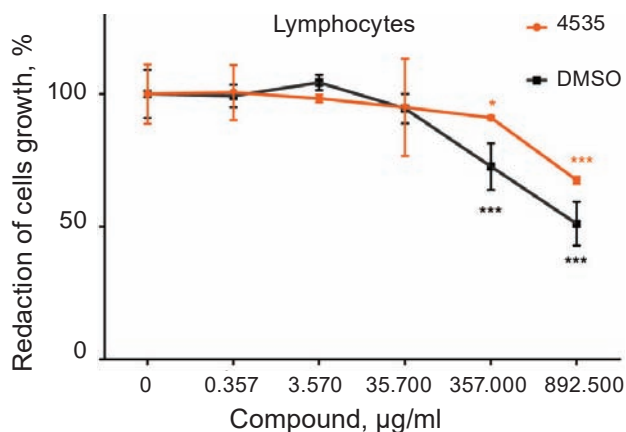


Fig 4. Cytotoxicity of compound **VP-4535** towards mitogen-activated lymphocytes isolated from healthy adult human peripheral blood. The cell vitality was evaluated by the MTT assay on 48 h of compound effect. $*P \leq 0.05$; $***P \leq 0.001$ (difference compared with the not treated control cells)

Balb/c 3T3 cells, and $3.4 \mu\text{g/ml}$ – for human lymphocytes indicated higher cytotoxic effect of this chemotherapeutic drug.

To compare the data of the cytotoxicity of compound **VP-4535** with doxorubicin towards human embryonic kidney cells (HEK-293) and human keratinocytes (HaCaT) please revised our recent works [38-40].

Conclusion. In summary, we discovered the 2-amino-4,6,7,8-tetrahydrothiopyrano[3,2-b]pyran-3-carbonitrile 5,5-dioxide **VP-4535** as a selective agent toward Methicillin-Resistant *Staphylococcus aureus*. Compound **VP-4535** shows quite interesting

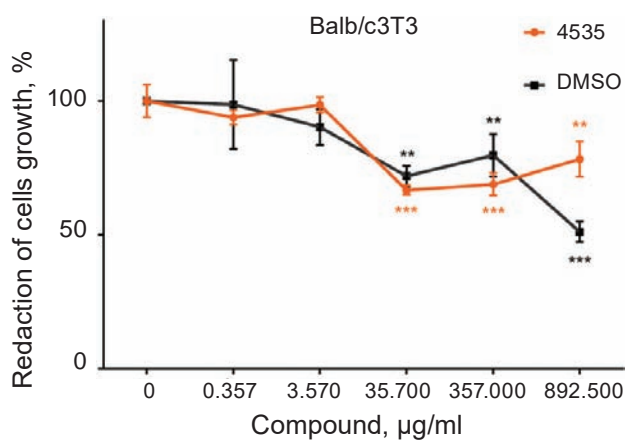
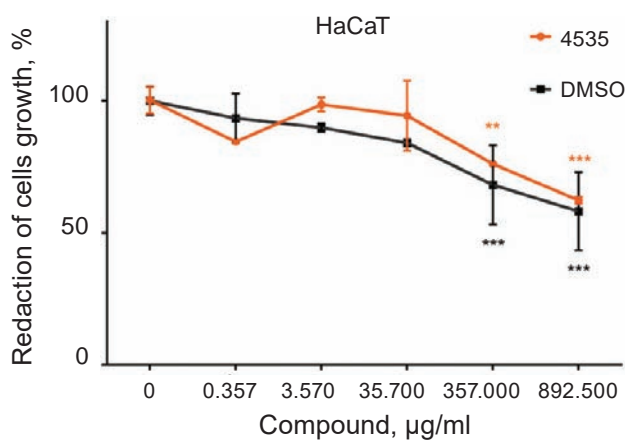


Fig. 5. Cytotoxicity of studied compound **VP-4535** towards human keratinocytes of HaCaT line, Balb/c 3T3 murine fibroblasts. After a total experimental time (72 h), cell vitality was evaluated by the MTT assay. $**P \leq 0.01$; $***P \leq 0.001$ (difference compared with the not treated control cells)

selective activity towards resistant and non-resistant *Staphylococcus aureus* and was safe in low concentrations to human red blood cells, human lymphocytes, HaCaT (human keratinocytes), Balb/c 3T3 line (murine fibroblasts), and HEK-293 (human embryonic kidney cells). This gives a perspective for medical use of compound **VP-4535** in future.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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**2-АМІНО-4,6,7,8-
ТЕТРАГІДРОТІОПІРАНО[3,2-*b*]
ПІРАН-3-КАРБОНІТРИЛ
5,5-ДІОКСИД VP-4535 ЯК
СЕЛЕКТИВНИЙ АНТИМІКРОБНИЙ
АГЕНТ, СЕЛЕКТИВНИЙ ЩОДО
МЕТИЦИЛІН-РЕЗИСТЕНТНОГО
STAPHYLOCOCCUS AUREUS**

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Оцінювали дію 2-аміно-4,6,7,8-тетрагідротіопірано[3,2-*b*]піран-3-карбонітрил 5,5-діоксидів щодо п'яти ключових патогенних бактерій групи ESKAPE: метицилін-резистентного *Staphylococcus aureus* (ATCC 43300), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), *Acinetobacter baumannii* (ATCC 19606), *Pseudomonas*

aeruginosa (ATCC 27853) і патогенних грибів *Candida albicans* (ATCC 90028) та *Cryptococcus neoformans* var. *Grubii* (ATCC 208821). Сполука **VP-4535**, що містить 5-метиліндолін-2-оновий фрагмент, виявила найвищу пригнічувальну активність та відмінну селективність щодо метицилінрезистентного *Staphylococcus aureus*, проте була неактивною щодо нерезистентного штаму *Staphylococcus aureus*. Встановлено, що сполука **VP-4535** у терапевтичній концентрації є безпечною для людських еритроцитів та лімфоцитів, клітин HaCaT, Balb/c 3T3 та HEK-293.

Ключові слова: циклічні сульфи, тіопірано[3,2-*b*]пірани, антимікробні агенти, антибактеріальні агенти, патогенні бактерії групи ESKAPE, цитотоксичність.

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