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ESTIMATION OF THE ACTIVITY OF MODIFIED PYRIMIDINE NUCLEOSIDE DERIVATIVES ON BACTERIA CELLS

Abstract. The increase in prevalence of antimicrobial-resistant bacteria (ARB) is currently a serious threat, thus there is a need for new classes antimicrobial compounds to combat infections caused by these ARB. The growth inhibition ability of derivatives of the components of nucleic acids has been well-characterized but not for its antimicrobial characteristics. It was found that modified nucleosides arabinofuranosylcytosine (cytarabine, ara-C), [1-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)-4-(1,2,4-triazol-1-yl)]uracil (TTU), and nucleotides cytarabine-5′-monophosphate (ara-CMP), and O^2,2′-cyclocytidine-5′-monophosphate (cyclocytidine monophosphate, cyclo-CMP) were able to inhibit *Escherichia coli*, *Sarcina lutea*, *Bacillus cereus*, and *Proteus mirabilis* strains in a time and dose dependent manner via killing kinetics assay. It was demonstrated that studied modified pyrimidine nucleosides derivatives enhanced the production of intracellular reactive oxygen species (ROS) over time (validated via DCFA-DA probe assay). This study has revealed the mechanism of action of cytarabine, cyclocytidine monophosphate, and TTU as an antimicrobial agent for the first time, and has shown that these pyrimidine derivatives enhanced might be able to combat infections caused by *E. coli*, *S. lutea*, *B. cereus*, and *P. mirabilis* in the future.

Keywords: antibacterial activity, modified nucleosides, cytarabine, viability, oxidative stress, ROS

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ОЦЕНКА ВЛИЯНИЯ МОДИФИЦИРОВАННЫХ ПИРИМИДИНОВЫХ ПРОИЗВОДНЫХ НУКЛЕИНОВЫХ КИСЛОТ НА БАКТЕРИАЛЬНЫЕ КЛЕТКИ

Аннотация. Широкое применение антибиотиков привело к возникновению и быстрому распространению резистентности у микроорганизмов, что обусловливает необходимость поиска новых классов антибактериальных препаратов. Хорошо известна способность производных компонентов нуклеиновых кислот ингибировать рост эукариотических клеток, однако их антимикробные свойства изучены недостаточно. Нами обнаружено, что модифицированные нуклеозиды арабинофуранозилцитозин (цитарабин, ara-C), [1-(2′,3′,5′-три-О-ацетил-β-D-рибофуранозил)-4-(1,2,4-триазол-1-ил)]урацил (TTU) и нуклеотиды цитарабин-5′-монофосфат (ara-CMP) и О2,2′-циклоцитидин-5′-монофосфат (циклоцитидинмонофосфат, цикло-CMP) способны ингибировать рост штаммов *Escherichia coli*, *Sarcina lutea*, *Bacillus cereus* и *Proteus mirabilis*. Показано, что грамотрицательные бактериальные штаммы (*E. coli* и *P. mirabilis*) более чувствительны к воздействию TTU и цикло-CMP и менее чувствительны к воздействию ara-C и ara-CMP по сравнению с грамположительными. Наиболее эффективным ингибитором роста клеток грамположительных штаммов (*S. lutea*, *B. cereus*) оказался ара-CMP с $ED_{50} = 5{,}2–10^{-5}$ и $ED_{50} = 3{,}1 \cdot 10^{-4}$ М соответственно. *S. lutea* оказалась наиболее чувствительным штаммом бактерий к воздействию всех изученных соединений. Установлено, что изученные модифицированные производные пиримидиновых нуклеозидов усиливают выработку внутриклеточных активных форм кислорода (АФК). Наибольшее повышение уровня АФК при культивировании клеток обнаружено в случае грамотрицательного штамма *E. coli* в присутствии TTU, а также цикло-CMP, что сильно коррелирует с эффектом ингибирования роста клеток. Обнаружена сильная корреляция между уровнем АФК и жизнеспособностью штамма *B. cereus* после культивирования с ara-CMP.

Ключевые слова: антибактериальная активность, модифицированные нуклеотиды, цитарабин, жизнеспособность, окислительный стресс, АФК

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Introduction. The development of antibiotics into clinical practice heralded a new era in medicine. However, less than a century later, due to the rise in pathogenic microorganism resistance, the useful adequacy of anti-infection drugs is waning. Antibiotic resistance in microorganisms has emerged and spread quickly as a result of its widespread usage. These days, more and more well-known and novel bacterial strains are developing resistance to the medications being utilized. According to some, society is moving into a post-antibiotic period where even ordinary diseases or minor wounds might be fatal [1, 2]. According to the World Health Organization's 2020 report, there is a considerable increase in the prevalence of resistant microorganisms, which makes it much more difficult to treat diseases brought on not only by bacteria, but also by fungi, parasites and viruses [3]. Every year, about 700 thousand people die from infections caused by drug-resistant bacteria, and this number may rise to 10 million by 2050 [4].

A number of mechanisms can cause an microorganism to become resistant to an antibiotic, including changes in the receptor's structure, inactivation or degradation of the antibiotic by an enzyme (the oldest mechanism effective against β-lactams), inhibition of absorption, or active removal of the antibiotic from the cell. There might be more, different mechanisms [1]. The majority of resistance genes are plasmid-localized, which allows for their heredity and horizontal transmission to other bacteria. No of how an antibiotic works, there are currently cases of resistance for every class of antibiotics [5–7].

Before the 1970s of the previous century, the majority of the classes of antibiotics that are currently in use were discovered [8]. Due to the high time and financial requirements for bringing a medicine to market as well as the lack of effective methodologies for finding leading compounds, there has been a very low activity in the search for new antimicrobial compounds [9]. The vast majority of antibiotics now in use also have high cytotoxicity, which restricts the circumstances in which they can be used. It is clear that new classes of antibiotics must be quickly developed in order to tackle resistant strains of microbes and act on new targets.

Nucleic acid derivatives, such as nucleosides, nucleotides, and their analogs, are among the most promising groups of antibacterial substances. Numerous biological processes, such as the storage of genetic information, gene expression, energy consumption, and cell signaling, require these molecules. All living things, including bacteria, depend on these mechanisms to survive. One of the most significant types of medications used in clinics are nucleoside analogues. Antiviral and anticancer medications are the two most often used nucleoside analogues [10]. However, information about their efficiency against microbes has been accumulating recently. Currently, nucleosides isolated from natural sources and their synthesized equivalents have both shown inhibitory action [11–13]. Additionally, known nucleosides that have been or are now being utilized to treat various diseases have been revealed to possess antimicrobial characteristics [2, 14]. Clinical trials of nucleosides and/or nucletides as antibacterial medicines are not well documented. Their discovery could be a crucial first step toward employing them as full-fledged antibiotics in this regard.

Materials and research methods. The used nucleosides and nucleotides were synthetized and characterized as described in our previous articles [15, 16].

Bacteria strains and culture. The bacterial strains used in the study were *Escherichia coli*, *Sarcina lutea*, *Bacillus cereus*, and *Proteus mirabilis*. The bacterial colonies of different strains were transferred under aseptic conditions into a 10 mL Mueller-Hinton Broth (MHB) containing capped conical flask and incubated overnight at 37 °C. After 18–24 h of incubation, cells were centrifuged at 6000 rpm for 5 min, supernatant was discarded and cell pellet was resuspended in phosphate buffer solution (PBS) followed by centrifugation. This removed debris and a clean bacterial suspension was obtained followed by suspending cells in MHB. The absorbance of the bacterial suspension prepared was recorded by UV-Visible spectrophotometer at 600 nm (OD_{600}). The cells were adjusted in the range of 0.15 to 0.2 OD_{600} which was considered to have cells at a concentration of 10^8 cells/ml. This suspension was further diluted to obtain a concentration of 10^7 cells/ml for testing nucleosides/nucleotides activity.

Resazurin reduction assay. The resazurin metabolization experiments were performed in 96-well plates as described [17]. Briefly, a volume of 10 µl of each suspension concentration was mixed with 200 µl of resazurin at a concentration of 20 µmol/l in phosphate buffered saline (PBS). The fluorescence (relative fluorescence units, RFU) of microbial-generated resorufin was recorded at λ_{ex} = 520 nm/λ_{em} = 590 nm after in 60 min using a multi-detection microplate reader Synergy 4 (BioTek Instruments Inc.,

USA). Each concentration level was measured in hexaplicate and the mean ± standard deviation was calculated. The percentage of survival was established for wells containing nucleosides/nucleotides relative to control wells containing no compounds.

Detection of reactive oxygen species (ROS). The production of ROS by bacterial strains after treatment with modified nucleosides/nucletides was evaluated using indicator 2′-7′-dichlorodihydrofluor escein diacetate (DCFH-DA) (Sigma-Aldrich, UK), which can detect a broad range of ROS including nitric oxide and hydrogen peroxide. The adjusted bacterial culture (0.5 McFarland exponential phase bacteria culture) were treated with different concentrations of studied compounds in presence of DCFH-DA at a final concentration of 5 μM in 0.85 % saline and incubated at 37 °C aerobically for 24 h. Untreated bacterial culture was served as a negative control. The fluorescence emission of DCFH-DA was measured at 525 nm using CLARIOstar Plus (BMG Labtech, Germany) plate reader with an excitation wavelength of 485 nm. The background fluorescence of 0.85 % saline and auto fluorescence of the bacterial cells incubated without the probe was measured to calculate the net fluorescence emitted from the assay itself. Experiment was conducted in triplicate.

Statistical analysis. Bacterial survival data and associated nucleosides/nucleotides concentrations from resazurin and plating were then fit to a a log-logistic model with four parameters (*b*, *c*, *d*, *e*) LL.4 using R (GraphPad Software, Inc.), affording the dose-response curves:

$$\varphi(x) = c + (d - c)/(1 + e^{b(\log x - \log e)}).$$

The estimated parameters of the models have a definite physical meaning. In particular, for the log-logistic model, the parameters *c* and *d* determine the lower and upper horizontal asymptotes of the sigmoid curve, *e* corresponds to the position of the inflection point, and *d* – to the angle of inclination in the transition region. Fitting of model parameters to the analyzed empirical data was carried out using the generalized method of minimizing the sum of squares of deviations of model forecasts from the observed values, taking into account specially selected weight coefficients.

Statistical analysis of the estimated parameters was carried out using Student's *t*-test, which tested the hypothesis of the equality of each coefficient to zero and calculated *p*-values that determine the achieved level of significance. The statistical significance of the model as a whole was verified by comparing it with a simple regression with a zero slope coefficient (the horizontal regression line corresponds to the absence of dose-effect dependence) by ANOVA.

Research results. ***EC_{50} and killing kinetics studies of modified pyrimidine nucleosides/nucleotides.*** Killing kinetics was performed to evaluate the effect of different concentrations of modified nucleosides/nucleotides ara-C, ara-CMP, cyclo-CMP, and TTU on four bacterial strains for 24 h.

All studied modified pyrimidine nucleosides/nucleotides inhibit growth of exponential phase of all used bacterial strains in a dose and time dependent manner (Fig. 1–4).

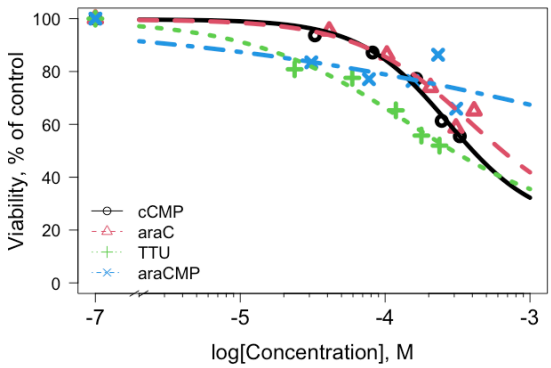

Fig. 1. Effect of different concentrations of modified pyrimidine nucleosides and nucleotides against exponential phase *P. mirabilis* (incubated aerobically) at 37 °C for 24 h

P. mirabilis culture treated with $2.3 \cdot 10^{-4}$ M of TTU achieved 48 % reduction of bacteria cells growth after 24 h; while the other compounds treated with the same bacterial strain achieved : 45 % with $3.2 \cdot 10^{-4}$ M cCMP, 35 % of both $4.2 \cdot 10^{-4}$ M araC and $3.1 \cdot 10^{-4}$ M araCMP separately (Fig. 1). ED_{50} of the

compounds after cultivation with *P. mirabilis* consisted the minimal value of $1.4 \cdot 10^{-4}$ M for TTU, while the maximal ED_{50} value was calculated with $3.3 \cdot 10^{-4}$ M for araCMP. The effectiveness with respect to cCMP and araC were equal to $2.7 \cdot 10^{-4}$ M and $3.8 \cdot 10^{-4}$ M individually.

However, a different scenario was observed when *E.coli* was treated with the current compounds where the reduction achieved 80 % $3.2 \cdot 10^{-4}$ M cCMP, followed by 58 % reduction with $2.3 \cdot 10^{-4}$ M of TTU, then 75 % with $3.1 \cdot 10^{-4}$ M araCMP, and finally the reduction achieved 55 % with $4.2 \cdot 10^{-4}$ M araC (Fig. 2). So, ED_{50} value after *E. coli* cultivation was calculated as $1.6 \cdot 10^{-4}$ M cCMP, $1.5 \cdot 10^{-4}$ M TTU, and $2 \cdot 10^{-4}$ M for both araC and araCMP.

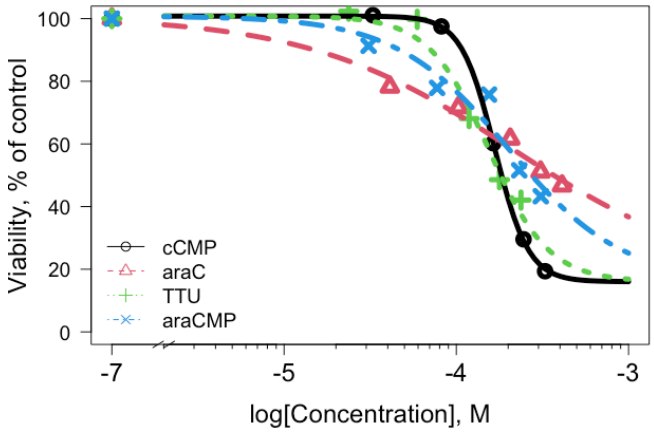

Fig. 2. Effect of different concentrations of modified pyrimidine nucleosides and nucleotides against exponential phase *E. coli* (incubated aerobically) at 37 °C for 24 h

S. lutea culture treated with the compounds achieved the highest percent cultivation from others bacterial strains where *S. lutea* culture treated with $3.1 \cdot 10^{-4}$ M araCMP achieved 91 % reduction of bacteria cells growth after 24 h; while the other compounds treated with the same bacterial strain achieved: 90 % with $4.2 \cdot 10^{-4}$ M araC, 86 % of $2.3 \cdot 10^{-4}$ M of TTU, and 84 % of $3.2 \cdot 10^{-4}$ M cCMP separately (Fig. 3). ED_{50} of the compounds after cultivation with *S. lutea* consisted the minimal value of $5.6 \cdot 10^{-4}$ M for araCMP, while the maximal ED_{50} value was calculated with $1.6 \cdot 10^{-4}$ M for both araCMP and cCMP. The effectiveness with respect to TTU were equal to $1.1 \cdot 10^{-4}$ M.

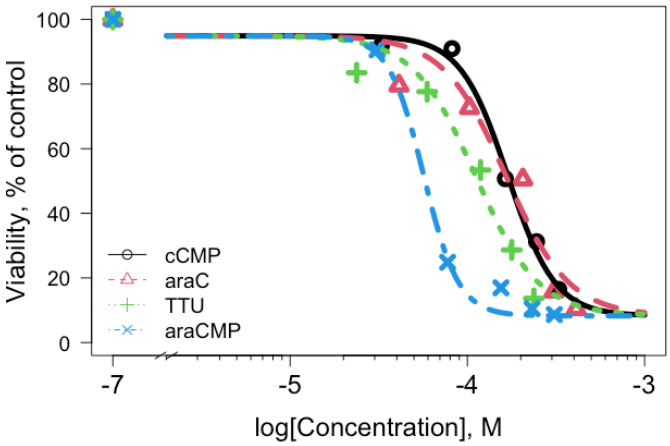

Fig. 3. Effect of different concentrations of modified pyrimidine nucleosides and nucleotides against exponential phase *S. lutea* (incubated aerobically) at 37 °C for 24 h

B. cereus culture treated with the compounds achieved the lowest percent cultivation from others bacterial strains where *B. cereus* culture treated with $3.1 \cdot 10^{-4}$ M araCMP achieved 59 % reduction of bacteria cells growth after 24 h; while the other compounds treated with the same bacterial strain

achieved: 47 % with $4.2 \cdot 10^{-4}$ M araC, 35 % of $3.2 \cdot 10^{-4}$ M cCMP , and 25 % of $2.3 \cdot 10^{-4}$ M of TTU respecvielty (Fig. 4). ED_{50} of the compounds after cultivation with *B. cereus* consisted the minimal value of $2.5 \cdot 10^{-4}$ M for araCMP, while the maximal ED_{50} value was calculated with $8.1 \cdot 10^{-4}$ M for both araC. The effectiveness with respect to TTU and Ccmp were equal to $4.8 \cdot 10^{-4}$ M and $7.1 \cdot 10^{-4}$ M individually.

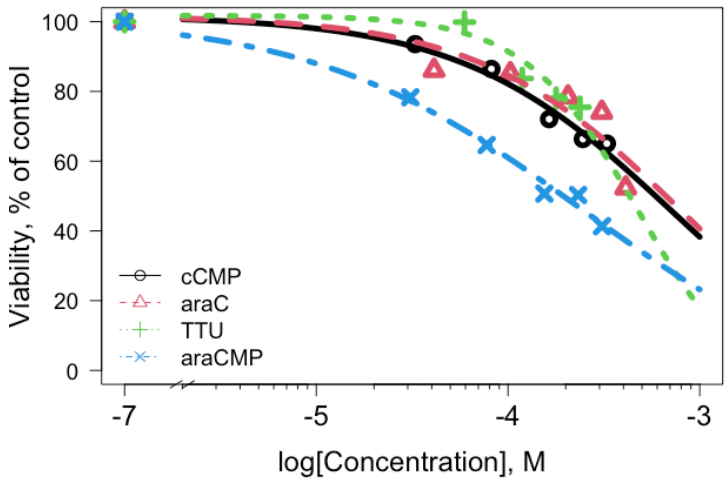

Fig. 4. Effect of different concentrations of modified pyrimidine nucleosides and nucleotides against exponential phase *B. cereus* (incubated aerobically) at 37 °C for 24 h

Effect of modified pyrimidine nucleosides/nucleotides on the enhancement of ROS production. It was hypothesized that in presence of modified pyrimidine nucleosides/nucleotides, the formation of ROS was enhanced in *E. coli*, *S. lutea*, *B. cereus*, and *P. mirabilis* which can damage the iron-sulphur clusters, thereby releasing ferrous ion. This iron can react with hydrogen peroxide in the Fenton reaction, causing a chain reaction, generating hydroxyl radicals which can directly damage intracellular DNA, lipids and proteins. Hence to validate the hypothesis, the intracellular ROS in all used bacteria strains was quantified prior and after modified pyrimidine nucleosides/nucleotides treatment in the subsequent experiments.

The production of ROS in healthy untreated bacterial cells is a natural side effect of aerobic respiration. These ROS can damage the RNA/DNA pool and also oxidizes lipid contents. Thus to protect themselves against the detrimental effect of ROS, bacteria are capable of producing enzymes (catalase and superoxide dismutase) to detoxify the ROS and having regulatory mechanisms (SoxRS, OxyRS and SOS regulons) to counteract the damage. To determine the effect of modified pyrimidine nucleosides/nucleotides on the enhancement of ROS production, *E. coli*, *S. lutea*, *B. cereus*, and *P. mirabilis* was treated with the same concentrations of studied compounds in presence of DCFH-DA, an unspecific probe for ROS. It was shown that the ROS production in bacteria strains was enhanced in a dose dependent manner when treated with all studied compounds.

The highest ROS level increase after cultivating with *P. mirabilis* was araC that is highly correlated with the growth inhibition effect (Fig. 5, *a*). There is a strong correspondence between ROS level and viability of *E. coli* after cultivation with cCMP. Indeed, the lowest rates of both the ROS level and the growth inhibition effect were detected in our experiments (Fig. 5, *b*). Cultivating of *S. lutea* with araC at the ED50 concentration leaded to the ROS burst (13- and 10-fold, respectively), what again correlates with the cell growth inhibition capacity of cyclic modified nucleoside (Fig. 5, *c*). while *B. cereus got* the highest ROS level increase after cultivating with araCMP that is exceptionally corresponded with the development hindrance impact (Fig. 5, *d*).

This recommends that the upgraded creation of ROS by implication affects the development of bacteria strains.

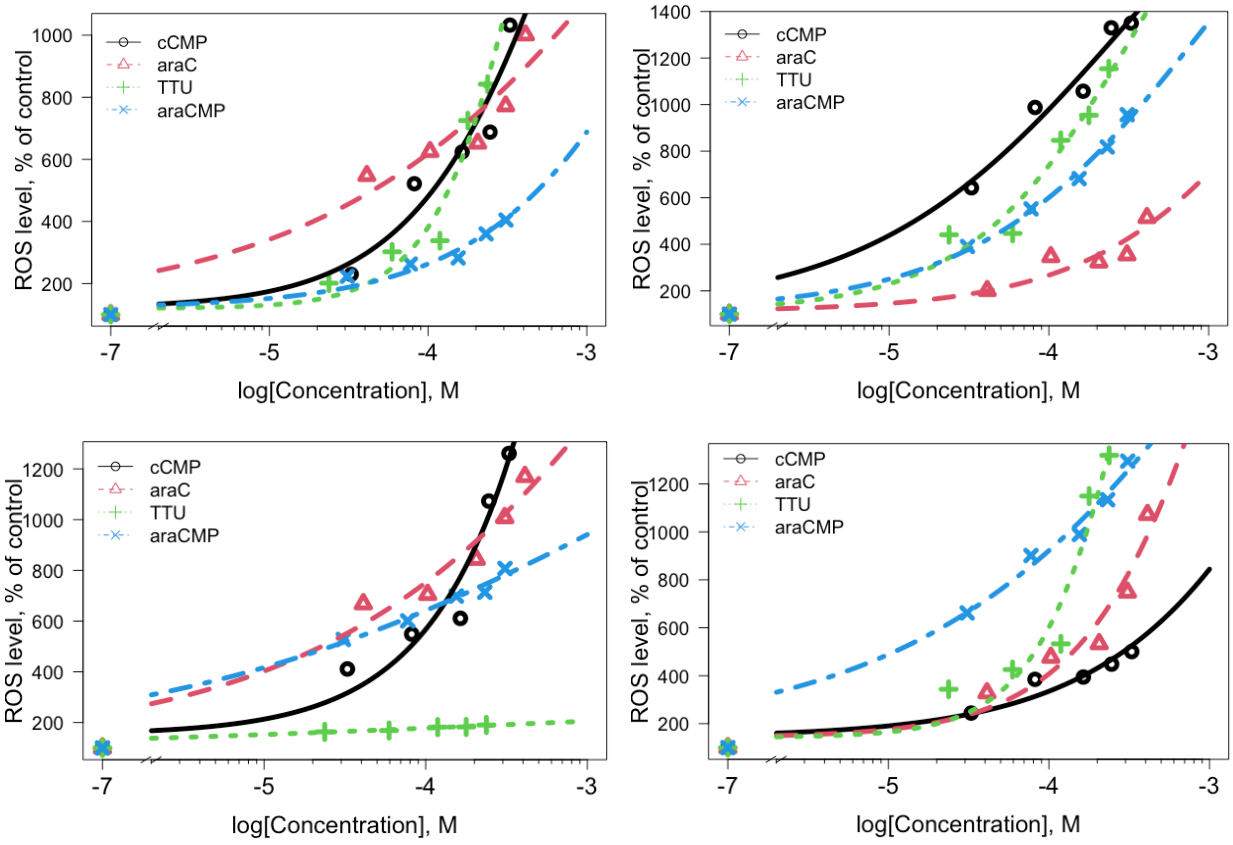

Fig. 5. Quantitation of intracellular ROS production by *P. mirabilis* (*a*), *E. coli* (*b*), *S. lutea* (*c*), and *B. cereus* (*d*) after 24 h treatment with different concentrations of modified pyrimidine nucleosides and nucleotides using the DCFA-DA probe

Discussion. Due to persistent underinvestment in the development of anti-infective drugs, decreased uptake of vaccines, and the growing prevalence and severity of treatment resistance, infectious illnesses could be said to be making a comeback [18, 19]. The majority of antibacterial, antifungal, and antiparasitic medications have been on the market for many years, and the lack of new developments threatens the capacity to treat many infectious diseases. Even when new medicines are suggested, they are frequently created from antimicrobial compounds that are already on the market, such as new penicillins, tetracyclines, diamidines, minor groove binders, etc. [20, 21]. Although such tactics can (temporarily) get around resistance, it was a requirement of this strategy that resistance to the class of compound in the microbial populations targeted was already common.

Nucleoside analogues, a pharmacologically varied class of pharmaceuticals that originated from chemically modified natural ribose or 2′-deoxyribose nucleosides, are one drug class that is significant from a clinical standpoint [19]. In the clinical setting, nucleoside analogues are among the most significant medications and are frequently employed as antiviral and anticancer agents [20]. By taking advantage of cellular metabolism, nucleoside analogues resemble native nucleosides and are integrated into both DNA and RNA. Purine or pyrimidine nucleoside antibiotics have distinct biochemical properties and capabilities due to their structural similarity to nucleosides and nucleotides involved in primary metabolism; consequently, these natural products can frequently have a significant impact on the internal processes of living organisms. Unsurprisingly, a lot of work has gone into creating pyrimidine nucleoside natural compounds and derivatives that can be used as medications. In fact, a lot of these substances have been used in medicine for a long time. Abacavir, entecavir, and lobucavir, as well as the naturally occurring neplanocin and aristeromycin, are examples of carbocyclic nucleoside analogues, compounds in which a methylene group replaces the oxygen atom in the furanose sugar moiety, that have a distinguished history as anti-infectious agents [22–24].

A high-carbon sugar nucleoside that is putatively produced via C-5′-modification of the canonical nucleoside is present in a number of nucleoside antibiotics from different actinomycetes. The 5′-C-car-

bamoyluridine and 5′-C-glycyluridine-containing nucleoside families are two notable examples. These families were found during searches for inhibitors of the bacterial translocase I, which is essential in the construction of the bacterial peptidoglycan cell wall [25]. The lead compound of a new class of antibiotics that targets iron acquisition through inhibition of aryl acid adenylating enzymes (AAAEs) in several pathogenic bacteria and is particularly effective against *M. tuberculosis* is the nucleoside antibiotic 5′-O-[N-(salicyl)sulfamoyl]adenosine (SAL-AMS) [26].

The overabundance of reactive oxygen species that results from the activation of microsomal oxidation is known to be the primary mechanism of the harmful action of antimetabolites on eukaryotic cells. Damage to the antioxidant defense system's functionality results as a result (including its enzymatic and non-enzymatic links). In this context, using modified pyrimidine nucleosides and nucleotides, we evaluated the level of reactive oxygen species produced in the bacterial cells during cultivation.

In this research, we assessed the efficacy of some modified pyrimidine nucleotides/nucleosides against various bacterial strains, e.g. *E. coli* (*gram-negative, facultative anaerobe*), *S. lutea* (*gram-positive*, *obligate aerobe*), *B. cereus* (*gram-positive*, *facultatively anaerobe*), and *P. mirabilis* (*gram-negative*, *facultative anaerobe*). The phase of exponential growth of bacterial culture was used in this work. Exponential phase culture consists of actively growing cells which consume readily available oxygen and nutrients for growth.

The lipopolysaccharide coat (LPS) on gram-negative bacteria's cell walls provides some defense against the toxicity of external substances [27]. These bacteria can thrive in places that would normally be regarded as unfriendly, such the intestines of mammals, thanks to their ability. It has been demonstrated in the past that the LPS acts as a physical or chemical barrier that prevents ROS produced outside of cells from interacting with important targets like membrane or cytoplasmic components [28]. As a result, certain strains that are unable to produce a significant amount of LPS have shown higher sensitivity to exogenous ROS than strains that are still able to do so. A protective structure like the gram-negative LPS and the outer membrane in which it is embedded does not exist in the majority of gram-positive bacteria. This outer membrane, which is made up of proteins and unsaturated fatty acids, which are substances known to chemically react with ROS, may operate as a structural barrier to penetration as well as a chemical trap for ROS [29]. However, since they can be eliminated without killing the cells, the outer membrane and LPS of gram-negative bacteria do not represent essential targets for the fatal impact of ROS (spheroplastformation). Once the barrier is crossed by ROS, the targets and mechanisms for cell killing for both gram-positive and gram-negative bacteria may be expected to be similar or identical because the cell wall structure of gram-positive and gram-negative bacteria represents the fundamental difference between these cells.

Whether caused by endogenous or exogenous photosensitizers, carotenoid pigments are known to physically quench ROS [30] and shield bacteria from the deadly effects of photosensitization. The protective effects of carotenoids against photosensitization and singlet oxygen mortality in bacteria have been linked, according to Mathews-Roth and colleagues [31]. Additionally, it has been discovered that carotenoids shield *S. lutea* from leukocyte-caused death, probably by quenching singlet oxygen. The carotenoid -carotene has also been reported to lessen the photosensitivity related to erythropoietic protoporphyria in humans and to protect mice from lethal exposure to hematoporphyrin derivative and light. In order to investigate any potential protective benefits that carotenoids might have against the death of these cells caused by exposure to pure exogenous ROS, we have included for investigation a bacteria strain that produces large levels of carotenoid pigments.

Our tests demonstrated that gram-positive (*S. lutea* and *B. cereus*) and gram-negative (*E. coli* and *P. mirabilis*) bacteria stains were both susceptible to the exposure of modified pyrimidine nucleosides and/or derivatives of nucleotides such as ara-C, TTU, ara-CMP, and cyclo-CMP. In addition, our findings suggest certain structure-function connections in the class of modified pyrimidine nucleosides and/or nucleotide derivatives caused by the inhibition of bacterial cell growth. In comparison to gram-positive bacteria, gram-negative ones (*E. coli* and *P. mirabilis*) were more sensitive to the exposure of TTU and cyclo-CMP and less sensitive to the exposure of ara-C and ara-CMP. The most effective cells growth inhibitor for gram-positive strains (*S. lutea*, *B. cereus*) was ara-CMP. *S. lutea* appeared to be the most sensitive bacteria strain to the exposure of all studied compounds.

Next, it was demonstrated that all of the tested chemicals increased the ROS production in bacteria strains in a dose-dependent way. The cultivation of the gram-negative strain of *E. coli* revealed the largest ROS level increase after TTU and after cyclo-CMP, which is strongly connected with the effect of cell growth inhibition. After cultivation with ara-CMP, there was a significant correlation between the ROS level and the viability of the *B. cereus* strain.

Conclusion. Modified pyrimidine nucleosides and/or nucleotides derivatives like ara-C, TTU, ara-CMP and cyclo-CMP were found to be effective in inhibiting the growth of gram-negative (*E. coli* and *P. mirabilis*) and gram-positive (*S. lutea* and *B. cereus*) bacteria stains. Ara-C, TTU, ara-CMP and cyclo-CMP are able to enhance the production of intracellular ROS, moreover the more effective a pyrimidine derivative in the growth inhibition the more ROS species were caused to burst. This study has provided an insight that modified nucleosides and/or nucleotides might potentially be useful in treating infections caused by ARB.

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