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Analysis of the antibacterial efficacy of modified purines derivatives

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***Abstract*---**Recently, it was postulated that antimicrobial substances kill bacteria by a common mechanism involving the formation of reactive oxygen species, in addition to particular drug-target interactions (ROS). However, there is a lot of controversy about this mechanism that produces hydroxyl radicals. Different experimental approaches are anticipated to be the root of the inconsistent results because the role of ROS to antibiotic-mediated death most likely varies on the circumstances. In the current work, the bacteria strains *Escherichia coli*, *Sarcina lutea*, *Bacillus cereus*, and *Proteus mirabilis*

were treated with nucleoside-based compounds 2-F-araA, 2-F-araAMP and NH_2-6-Cl-araPur, and the formation of reactive oxygen species (ROS) was measured. The formation of intracellular reactive ROS was shown to be increased over time by the examined modified pyrimidine nucleoside derivatives (validated via DCFA-DA probe assay). For instance, an increase in the ROS was measured in *E. coli* after treatment with NH_2-6-Cl-araPur but not after treatment with 2-F-araA, and2-F-araAMP. Results also vary depending on the species studied and the experimental setup. Despite this, our data strongly imply that using antioxidants as therapeutic agents to treat some infections is a viable option that is starting to be used against bacterial strains.

***Keywords*---**analysis antibacterial efficacy, modified purines derivatives.

Introduction

A natural side effect of aerobic respiration is the production of reactive oxygen species (ROS) [1]. These ROS are generated via successive single-electron reductions and can damage DNA, proteins and lipids, ultimately leading to cell death. To protect themselves against the deleterious effects of ROS, aerobic bacteria are equipped with enzymes (catalases and superoxide dismutases) that can detoxify ROS and regulatory mechanisms (SoxRS, OxyRS, and SOS regulons) to counter the damage [2]. Interestingly, in 2007 Kohanski et al. identified a common mechanism involving the production of hydroxyl radicals by which all bactericidal antibiotics could induce cell death [3]. Currently, a mechanism is proposed in which bacterial membrane disturbance triggers envelope stress and subsequent perturbation of the Arc regulatory system accelerates respiration [4]. Hyperactivation of the electron transport chain induces the formation of superoxide and hydrogen peroxide which damage iron-sulphur clusters, thereby releasing ferrous iron. This iron can then react with hydrogen peroxide in the Fenton reaction and generate hydroxyl radicals which can directly damage DNA, lipids and proteins or oxidize the deoxynucleotide pool and indirectly damage DNA. However, this theory has recently become the subject of much debate [5–8]. Moreover, it was found that protection against ROS has a positive effect on bacterial cell survival not only after treatment with oxidizing agents but also after treatment with antibiotics [2, 13]. Antioxidants have anmultidirectional effect on the efficacy of antibacterial agents in vitro and under experimental bacterial peritonitis. Combination of antibiotics with antibacterial agents should be accompanied by preliminary in vitro studies. The rational combination of antibacterial agents and antioxidants increases the effectiveness of anti-infective chemotherapy and prevents the formation of resistant strains.

Most studies investigating the contribution of ROS in antibiotic-mediated killing have focused on antibiotics, but there are other promising classes of antimicrobial agents like nucleoside-based molecules [7]. Nucleoside analogs can be active antibacterial agents, and the mechanism of their action depends on the type and position of the substituents. The huge interest in the creation of antibacterial

drugs is not limited to the consideration of classical approaches, and as new technologies appear, new approaches are also being developed [1–5]. The emergence of drug resistance in pathogenic microorganisms is a serious problem for humanity due to the widespread, not always justified, use of antibiotics. That is why the development of new antimicrobial agents has been, and remains, relevant [6–9].

The creation of drugs based on natural compounds is a well proven classic approach. To date, about a hundred drugs have been created on the basis of nucleosides, half of which are antiviral and a quarter antitumor [10]. Natural nucleosides have a diverse structure; they are part of nucleotides, DNA, RNA, and coenzymes. More than 140 minor nucleosides were isolated from tRNA, and about 100 disaccharide nucleosides and 200 nucleoside antibiotics, in the structure of which there are additional functional groups and hydrophobic residues, were isolated from various natural sources. The library of natural nucleosides contains about 600 compounds, which serve as the basis for the creation of new biologically active compounds [11].

In connection with the recognition of the universal role of strengthening the processes of free radical oxidation in the development of inflammation of infectious etiology, the additional appointment of antioxidants is pathogenetically justified. Bacterial infection is accompanied by increased generation of reactive oxygen species that damage biomolecules and make a significant contribution to the development of cellular metabolism disorders, tissue and organ dysfunction [6]. At the same time, the bactericidal effect of many antibacterial agents has a common mechanism associated with the generation of OH-radicals and the development of oxidative stress in bacterial cells [8]. In this regard, antioxidants can reduce the effect of such drugs and, accordingly, reduce the effectiveness of treatment. It is also impossible to exclude the possibility of direct chemical interaction of antibacterial agents and antioxidants. The aim of our work is to study the interaction between particular antioxidants (quercetin) and nucleoside–based compounds with promising antibacterial properties (2-F-araA, 2-F-araAMP, and NH_2-6-Cl-araPur) *in vitro.*

Materials and Methods

The used nucleosides and nucleotides were synthetized and characterized as described in our previous articles [Arshed Shihad et.al (2022)]

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 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 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 [Travnickova et al. AMB Expr (2019) 9:183]. Briefly, a volume of 10 µL of each suspension concentration was mixed with 200 µL of resazurin at a concentration of 20 µmol L−1 in phosphate buffered saline (PBS). The fluorescence (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 ±SD 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′-dichlorodihydrofluorescein diacetate (DCFH-DA) (Sigma-Aldrich, UK), which can detect a broad range of ROS including nitric oxide and hydrogen peroxide [12]. 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 a Tecan microtiter plate reader with an excitation wavelength of 485 nm [13]. 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:

$$\varphi(\mathrm{x}) = c + \frac{d - c}{1 + e^{b\,(\log x - \log e)}}$$

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.

Results and Discussion

Killing kinetics was performed to evaluate the effect of different concentrations of modified nucleosides/nucleotides 2-F-araA, 2-F-araAMP, and NH_2-6-Cl-araPur with or without antioxidant against different bacteria strains, e. g. *E. coli*, and *P. mirabilis* (gram-negative, facultative anaerobes), as well as *S. lutea* (gram-positive, obligate aerobe), and *B. cereus* (gram-positive, facultatively anaerobe) bacterial strains for 24 h.

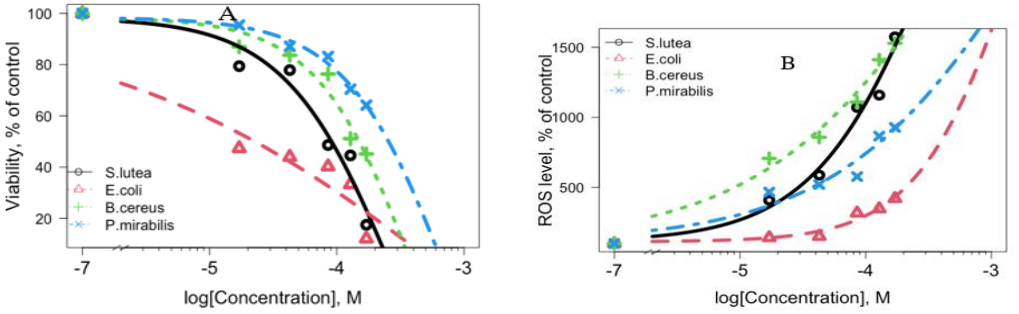

Figure 1. Effect of different concentrations of 2-F-araA without antioxidant against exponential phase *E. coli*, *S. lutea*, *B. cereus*, and *P. mirabilis* (incubated aerobically) at 37 °C for 24 h

As can be seen from the picture (figure 1A), modified nucleoside 2-F-araA achieved the same maximum effect against *S. lutea*, *B. cereus*, and *P. mirabilis* bacteria strains, i.e. it had equal efficacy against that three strains but not against *E. coli* strain. However, 2-F-araA achieved this effect at lower dose in case of action on both gram-positive strains *S. lutea* (ED_{50} = 3.0 * 10^{-4} M), and *B. cereus* (ED_{50} = 4.4 * 10^{-4} M) compared to *P. mirabilis* strain (ED_{50} = 7.9 * 10^{-4} M). The shape of dose-effect curve for *E. coli* bacteria strain differed from all other used in experiments bacteria strains with calculated value of ED_{50} = 9.9 * 10^{-4} M, what was closer to the value for another gram-negative bacteria strain. Next, we found a strong match between efficacy and potency of 2-F-araA to bacteria cells growth inhibition and level of intracellular ROS burst after cells treatment in the same conditions (figure 1B). Indeed, the lowest ROS level growth (500% compared to control without 2-F-araA) was detected in case of the most resistant *E. coli* bacteria strain. In the same time, both the most sensitive gram-positive strains *S.*

lutea, and *B. cereus* showed the 15-fold burst of intracellular ROS after treatment with near-ED_{50} concentrations of 2-F-araA (1.01 * 10^{-4} M and 1.03 * 10^{-4} M, respectively).

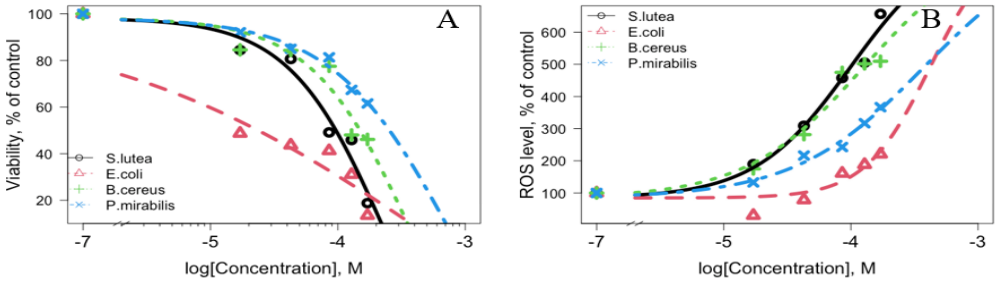

Figure 2. Effect of different concentrations of 2-F-araA with equimolar antioxidant concentrations against exponential phase *E. coli*, *S. lutea*, *B. cereus*, and *P. mirabilis* (incubated aerobically) at 37 °C for 24 h

The presence of antioxidant in the reagent mixture hasn't changed the dose-effect relationships in general, gram-positive bacteria strains remained more sensitive to 2-F-araA then gram-negative ones, but we observed the decrease of ED_{50} values (i.e. increase of efficiency) in case of *S. lutea* and *E. coli* bacteria strains to 2.4 * 10^{-4} M and 5.0 * 10^{-4} M, respectively while the potency of 2-F-araA against *B. cereus* and *P. mirabilis* strains almost hasn't changed (ED_{50} = 4.4 * 10^{-4} M and 7.8 * 10^{-4} M, respectively).

The values of ROS levels in all bacteria strains after antioxidant adding have changed more dramatically. The most resistant bacteria strain *E. coli* showed 2,5-fold decrease of intracellular level after treatment with 2-F-araA combined with quercetin at the highest used concentration 1.9 * 10^{-4} M. The same 2.3-3.0-fold decrease of ROS levels was detected in case of all other bacteria strains, but what more important is that the dose-activity relationships remained the same, i.e. the more sensitive to 2-F-araA bacteria strain was, the higher ROS level at the highest used compound concentration was detected.

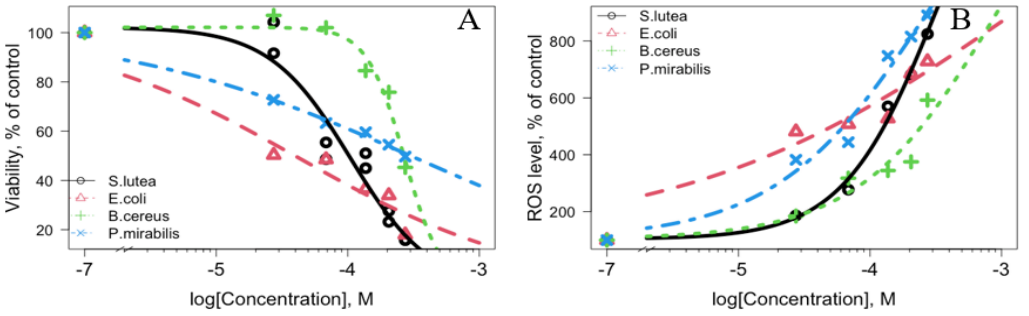

Figure 3. Effect of different concentrations of 2-F-araAMP without antioxidant against exponential phase *E. coli*, *S. lutea*, *B. cereus*, and *P. mirabilis* (incubated aerobically) at 37 °C for 24 h

Figure 3A showed that the modified nucleoside 2-F-araAMP achieved the same maximum effect against gram positive *S. lutea*, and *B. cereus* bacteria strains, and same maximum effect against gram negative *E. coli* , and *P. mirabilis*. 2-F-araAMP had equal efficacy against the gram positive and gram negative strains separetly. However, 2-F-araAMP achieved this effect at lower dose in case of action on both gram-positive strains *S. lutea* (ED_{50} = 1.0 * 10^{-4} M), and *B. cereus* (ED_{50} = 2.0 * 10^{-4} M) compared to both gram-negative strains *P. mirabilis* strain (ED_{50} = 3.0 * 10^{-4} M) and *E. coli* bacteria strain ED_{50} = 4.0 * 10^{-5} M . The shape of dose-effect curve for *gram positives and gram negatives* bacteria strain differed from each other's.Next, we found a strong match between efficacy and potency of 2-F-araAMP to bacteria cells growth inhibition and level of intracellular ROS burst after cells treatment in the same conditions (figure 3B). Indeed, the lowest ROS level growth (600% compared to control without 2-F-araAMP) was detected in case of the most resistant *B. cereus* bacteria strain.

In the same time, both the most sensitive gram-positive strains *S. lutea*, and *P. mirabilis* showed the 8-fold burst of intracellular ROS after treatment with near-ED_{50} concentrations of 2-F-araAMP (1.5 * 10^{-4} M and 1.4 * 10^{-4} M, respectively).

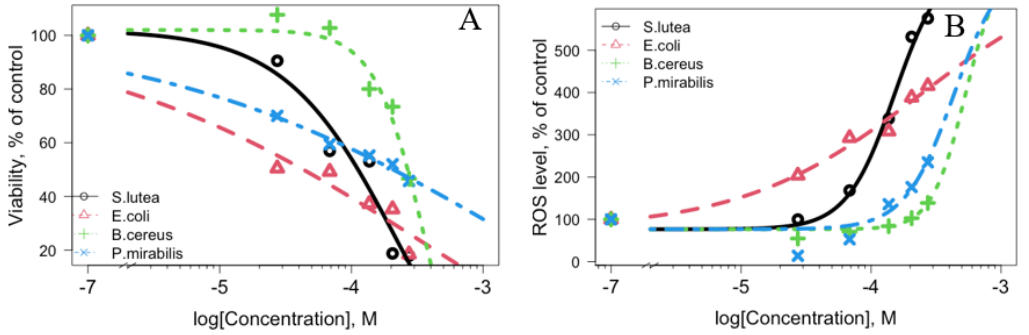

Figure 4. Effect of different concentrations of 2-F-araAMP with equimolar antioxidant concentrations against exponential phase *E. coli*, *S. lutea*, *B. cereus*, and *P. mirabilis* (incubated aerobically) at 37 °C for 24 h

Similar results to what was done previously for the presence of antioxidant in the reagent mixture, where antioxidant hasn't changed the dose-effect relationships in general, gram-negative bacteria strains remained more sensitive to 2-F-araAMP than gram-positive strains, but we also observed the increase of ED_{50} values (i.e. decrease of efficiency) in case of all bacterial strains where *S. lutea* 2.2 * 10^{-4} M, *E. coli* 4.0 * 10^{-4} M, *B. cereus* 3.6*10^{-4} M, and *P. mirabilis* 2 * 10^{-3} M, respectively). The values of ROS levels in all bacteria strains after antioxidant adding have changed more dramatically. The most resistant bacteria strain *B. cereus* showed 6-fold decrease of intracellular level after treatment with 2-F-araAMP combined with quercetin at the highest used concentration 2.7 * 10^{-4} M. The same 3.75-1.75-fold decrease of ROS levels was detected in case of all other bacteria strains, but what more important is that the dose-activity relationships remained the same, i.e. the more sensitive to 2-F-araAMP bacteria strain was, the higher ROS level at the highest used compound concentration was detected.

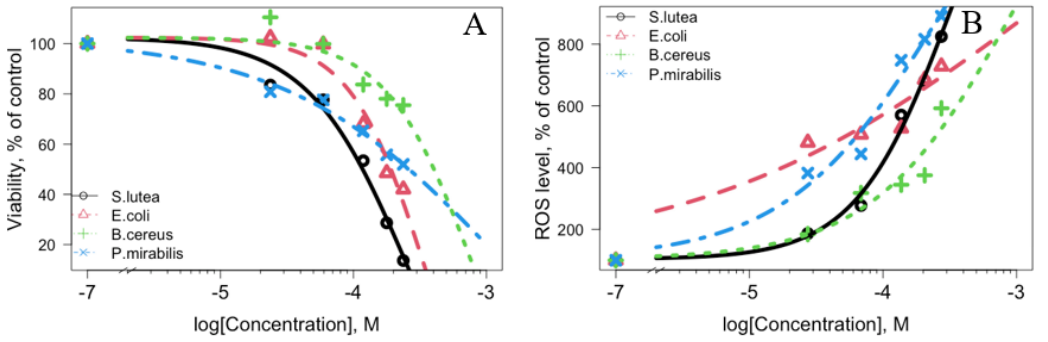

Figure 5. Effect of different concentrations of NH_2-6-Cl-araPur without antioxidant against exponential phase *E. coli*, *S. lutea*, *B. cereus*, and *P. mirabilis* (incubated aerobically) at 37 °C for 24 h

As showed in (figure 5A), modified nucleoside NH_2-6-Cl-araPur achieved the same maximum effect against all strain *S. lutea*, *B. cereus*, *P. mirabilis* and *E. coli* , i.e. it had equal efficacy against that four strains. However, NH_2-6-Cl-araPur achieved this effect at lower dose in case of action on *S. lutea* (ED_{50} = 2.5 * 10^{-4} M) and *E. coli* strain (ED_{50} = 3.4 * 10^{-4} M) compare to *B. cereus* (ED_{50} = 7.9 * 10^{-4} M) and *P. mirabilis* (ED_{50} = 1.4 * 10^{-3} M). The shape of dose-effect curve for *all* bacteria strain were same each other.

Next, we found a strong match between efficacy and potency of NH_2-6-Cl-araPur to bacteria cells growth inhibition and level of intracellular ROS burst after cells treatment in the same conditions (figure 5B). Indeed, the lowest ROS level growth (600% compared to control without NH_2-6-Cl-araPur) was detected in case of the most resistant *S. lutea* bacteria strain. In the same time, both the most sensitive gram-positive strains *E. Coli*, and *P. Mirabilis* showed the 8-fold burst of intracellular ROS after treatment with near-ED_{50} concentrations of NH_2-6-Cl-araPur (1.5 * 10^{-4} M and 1.5 * 10^{-4} M, respectively).

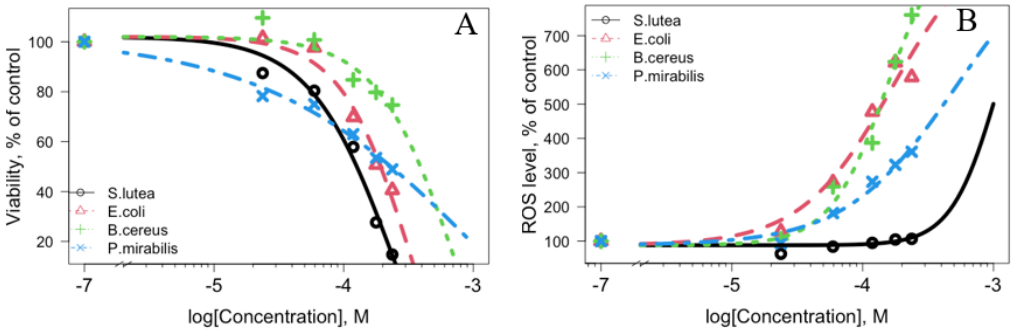

Figure 6. Effect of different concentrations of NH_2-6-Cl-araPur with equimolar antioxidant concentrations against exponential phase *E. coli*, *S. lutea*, *B. cereus*, and *P. mirabilis* (incubated aerobically) at 37 °C for 24 h

In general, the existence of antioxidant in the reagent mixture doesn't changed the dose-effect relationships, gram-positive and gram negative bacteria strains showed the same sensitive to NH_2-6-Cl-araPur. ED_{50} values were exactly the same as what was described in figure 5A. The upsides of ROS levels in all bacteria strains after added antioxidant have changed more decisively. The most resistant bacteria strain *S. lutea* showed 8-fold decrease of intracellular level after treatment with NH_2-6-Cl-araPur combined with quercetin at the highest used concentration 3.3 * 10^{-4} M. The 1.2-3.0-fold decrease of ROS levels was detected in case of all other bacteria strains, but what more important is that the dose-activity relationships remained the same, i.e. the more sensitive to NH_2-6-Cl-araPur bacteria strain was, the higher ROS level at the highest used compound concentration was detected.

Discussion

Infectious diseases may be said to be making a come-returned, due to chronic below-funding in anti-infective drug improvement, decreased attractiveness of vaccines, and the growing spread and severity of drug resistance [12,13]. Most antibacterial, antifungal, and antiparasitic pills are decades vintage, and the ongoing loss of development threatens the treatability of many infectious sicknesses. Even if new healing procedures are proposed they're often developed from present antimicrobial retailers, e.g., new penicillines, new tetracyclines, diamidines, new minor groove binders etc. [14, 15]. At the same time as such strategies can (temporarily) keep away from resistance, it turned into inherent to this approach that resistance to the elegance of compound turned into already massive within the microbial populations centered.

One class of medicine that are critical from a scientific angle is nucleoside analogues, a pharmacologically numerous elegance of drugs that arose from chemically changed natural ribose or 2′-deoxyribose nucleosides [14]. Nucleoside analogues are a few of the most essential pills in the medical putting and are used widely as both anticancer and antiviral marketers [15]. Nucleoside analogues mimic endogenous nucleosides, exploiting mobile metabolism and turning into incorporated into each DNA and RNA. Their structural similarity to nucleosides and nucleotides worried in number one metabolism endows purine or pyrimidine nucleoside antibiotics with particular biochemical houses and talents; accordingly, those herbal merchandise can regularly be extraordinarily influential to the internal workings of dwelling organisms. Now not exceedingly, extensive effort has been directed to developing purine nucleosides natural merchandise and derivatives as pills. Indeed, a number of such compounds have visible clinical use for decades. As an example, carbocyclic nucleoside analogues, compounds wherein a methylene institution replaces the oxygen atom inside the farinose sugar moiety, have a prominent records as anti-infectious sellers, along with the food and Drug management (FDA)-authorized antiviral drugs abacavir, entecavir, and lobucavir, as well as the certainly taking place neplanocin and aristeromycin [16, 17, 18].

It's miles recognized that the primary mechanism of the harmful impact on eukaryotic cells below management of antimetabolites is the immoderate accumulation of reactive oxygen species as a result of activation of microsomal

oxidation, respectively. The outcome of this is harm to the functioning of the antioxidant protection gadget (which include its enzymatic and non-enzymatic links). In this regard, we assessed the level of reactive oxygen species formed inside the bacteria cells under cultivation situations with changed purine nucleosides/nucleotides.

In this work we analyzed the pastime of a few modified purine nucleotides/nucleosides in opposition to one of a kind bacteria lines, 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 section of exponential increase of bacterial culture was used on this work. Exponential section culture consists of actively developing cells which consume comfortably available oxygen and nutrients for growth.

The gram-negative bacterial cells wall lipopolysaccharide coat (LPS) gives a few safety from the toxic consequences of exogenous agents [19]. This capability allows these microorganism to survive in what otherwise must be taken into consideration hostile environments, which include mammalian intestines. The LPS has formerly been shown to provide a bodily or chemical barrier through which ROS generated outside of cells must skip to have interaction with a vital goal, together with membrane or cytoplasmic additives [20]. As an end result, a few traces that fail to supply a big part of the LPS have displayed greater sensitivity to exogenous ROS than do lines that retain this capacity. Most gram-positive bacteria lack a shielding structure analogous to the gram- negative LPS and the outer membrane wherein it's miles anchored. Similarly to likely forming a structural barrier to penetration, this outer membrane may also shape a chemical lure for ROS; it is composed of unsaturated fatty acids and proteins that are compounds known to react chemically with ROS [21]. The outer membrane and LPS of gram-negative microorganism do now not, but, constitute important targets for the lethal movement of ROS, considering those can be removed without killing the cells (spheroplastformation). Because the cellular wall shape of gram-tremendous and gram-bad bacteria represents the fundamental distinction among those cells, as soon as the barrier is crossed with the aid of ROS, the objectives and mechanisms for cell killing for each gram-high quality and gram-bad bacteria can be anticipated to be similar or same.

Carotenoid pigments are acknowledged to physically quench ROS [22] and to defend microorganism towards the deadly outcomes of photosensitization, whether by means of endogenous or exogenous photosensitizers [23]. Mathews-Roth and co-people [24] have correlated the protective results of carotenoids in opposition to photosensitization and singlet oxygen lethality in microorganism. Carotenoids also had been observed to shield Sarcina lutea from killing by using leukocytes, probably by way of quenching singlet oxygen [25]. Administration of the carotenoid, -carotene has also been observed to shield mice from lethal exposure to hematoporphyrin by-product and mild and, in people, to mitigate the photosensitivity related to erythropoietic protoporphyria [26]. We've blanketed for look at a bacteria strain that produces high ranges of carotenoid pigments in order to verify what protective consequences the carotenoids may have against killing of those cells with the aid of publicity to pure exogenous ROS.

Antioxidants are molecules that act to deplete ROS. Molecules that inhibit ROS-generating pathways, molecules that directly scavenge ROS, and molecules that interfere with ROS degrading pathways can act as antioxidants. Common antioxidants are ROS-scavengers, NOX2-inhibitors, inhibitors of various ROS-generating pathways, and nuclear factor (erythroid-derived 2)-like 2 (NRF2)-activators, which are a class of compounds that induce the expression of antioxidant enzymes, thus classified as indirect antioxidants. ROS-scavengers, such as *N*-acetyl-cysteine (NAC), which replenishes glutathione, have been by far the most studied class of antioxidants and became accessible at pharmacies, but recently, NRF2-activators raised much interest. Many NRF2-activators are considered "nutraceuticals," molecules naturally found in foods to which healthy effects have been ascribed: resveratrol (wine), pterostilbene (blueberry), sulforaphane (broccolis), curcumin (turmeric), cafestol (coffee), quercetin (red onion), epigallocatechin-3-gallate (green tea), and carnosol (rosemary) [27]. The food conservation additive tert-butylhydroquinone is also a potent NRF2 activator. Cobalt-protoporphyrin (CoPP) is a drug largely used in experimental research that is capable of inducing heme-oxygenase 1 (HO-1) expression through NRF2 activation. NOX2 inhibitors, however, have attracted less attention, most likely due to their less specific effects on NOX family proteins. Apocynin, the most studied NOX2 inhibitor, derives from vanillin and is nontoxic but has no current use in clinics [28].

The housekeeping production of ROS is generally neutralized by constitutive antioxidant defenses. Oxidative stress ensues when ROS production overwhelms antioxidant defenses. The oxidative hit then promotes the dissociation of kelch-like ECH-associated protein (Keap) from NRF2, allowing NRF2 to translocate to the nucleus where it activates cytoprotective and antioxidant defenses by turning on the transcription of genes that contain antioxidant response element (ARE) motifs in promoters. A subject recently reviewed in the literature is the ability of NRF2 to interact with many other transcription factors [29]. The indirect antioxidants that act by activating NRF2-dependent mechanisms are usually oxidants that promote transient surges of ROS production and some of them can even act as pro-oxidants in large concentrations. Most NRF2 activators fit into the general definition of "hormetic" agents: they induce a low level of stress that activates the antioxidant defenses, and the general outcome is beneficial to the organism [30].

The NRF2-target genes include the following phase II enzymes: HO-1, NAD(P)H quinione oxidoreductase 1, glutathione peroxidase, glutamate cysteine ligase, and glutathione S-transferases. However, not all genes under NRF2 control are enzymes of direct antioxidant action. For instance, H-ferritin and ferroportin (FPN)-1, proteins that regulate the labile iron pool, contain ARE motifs in their promoters but are only indirectly linked to redox regulation. NRF2 acts on tissue regeneration, DNA repair, and lipid metabolism genes. Some promoters that contain ARE motifs, such as that of CD36, also contain peroxisome proliferator-activated receptor (PPAR)-γ-controlled PPARE motifs and can be operated by both factors at a time [31]. Recently, the macrophage phenotype MHem was described to result from a genetic program commanded by activation transcription factor (ATF)-1 and NRF2 simultaneously and to be capable of countering foam cell

formation. This indicates that an NRF2-dependent event does not necessarily activate antioxidant defenses [32].

The current results showed that both gram-negative (*E. coli* and *P. mirabilis*) and gram-positive (*S. lutea* and *B. cereus*) bacteria stains were sensitive to the exposure of such modified purines nucleosides and/or nucleotides derivatives as 2-F-araA, 2-F-araAMP, and NH_2-6-Cl-araPur. Besides that our results consider to set up some structure-function relationships in the range of modified purines nucleosides and/or nucleotides derivatives by the bacteria cell growth inhibition. Gram-negative *E. coli* bacteria stains were less sensitive to the exposure of 2-F-araA and more sensitive to the exposure of 2-F-araAMP and NH_2-6-Cl-araPur compared to other bacterial strians. The most effective cells growth inhibitor for both gram-positive strains and gram negative strian was NH_2-6-Cl-araPur. *Sarcina lutea* appeared to be the most sensitive bacteria strain to the exposure of all studied compounds.

Next it was shown that the ROS production in bacteria strains was enhanced in a dose dependent manner when treated with all studied compounds. Both the most sensitive gram-positive strains *S. lutea*, and *B. cereus* showed the 15-fold burst of intracellular ROS after treatment with near-ED_{50} concentrations of 2-F-araA ($1.01 * 10^{-4}$ M and $1.03 * 10^{-4}$ M, respectively). The lowest ROS level growth was detected with 2-F-araAMP in case of the most resistant *B. cereus* bacteria strain while NH_2-6-Cl-araPur was detected in case of the most resistant *S. lutea* bacteria strain.

The results showed that the existence of antioxidant in the reagent mixture doesn't changed the dose-effect relationships, gram-positive and gram negative bacteria strains showed the same sensitive to all modified purine dervitatives. In case of ROS levels in all bacteria strains after added antioxidant have changed more decisively. The most resistant bacteria strain showed the highest decrease of intracellular level after combined with quercetin at the highest concetrations where: *E. coli* after treatment with $1.9 * 10^{-4}$ M -2-F-araA at, *B. cereus* with $2.7 * 10^{-4}$ M- 2-F-araAMP , and *S. lutea* with $3.3 * 10^{-4}$ M- NH_2-6-Cl-araPur .

Conclusion

1. Thus, 2-F-araA had higher potency against gram-positive strains *S. lutea* ($ED_{50} = 3.0 * 10^{-4}$ M) and *B. cereus* ($ED_{50} = 4.4 * 10^{-4}$ M) than gram-negative *P. mirabilis* strain ($ED_{50} = 7.9 * 10^{-4}$ M). Both gram-positive strains *S. lutea*, and *B. cereus* showed the 15-fold burst of intracellular ROS after treatment with near-ED_{50} concentrations of 2-F-araA ($1.01 * 10^{-4}$ M and $1.03 * 10^{-4}$ M, respectively). The presence of antioxidant in the reagent mixture hasn't changed the dose-effect relationships in general, gram-positive bacteria strains remained more sensitive to 2-F-araA then gram-negative ones even with the increase of efficiency. The 2.3-3.0-fold decrease of ROS level in all bacteria strains was detected, but the dose-activity relationships remained the same, i.e. the more sensitive to 2-F-araA bacteria strain was, the higher ROS level at the highest used compound concentration was detected.

2. F-araAMP had higher potency against gram-positive strains *S. lutea* (ED_{50} = 1.0 * 10^{-4} M) and *B. cereus* (ED_{50} = 2.0 * 10^{-4} M) than gram-negative *P. mirabilis* strain (ED_{50} = 3.0 * 10^{--4} M), and *S. lutea* (ED_{50} = 1.0 * 10^{-4} M). Both gram-positive strains *S. lutea* (ED_{50} = 1.0 * 10^{-4} M), and *B. cereus* (ED_{50} = 2.0 * 10^{-4} M) compared to both gram-negative strains *P. mirabilis* strain (ED_{50} = 3.0 * 10^{-4} M) and *E. coli* bacteria strain ED_{50} = 4.0 * 10^{-5} M .The presence of antioxidant in the reagent mixture hasn't changed the dose-effect relationships in general, gram-positive bacteria strains remained more sensitive to 2-F-araA then gram-negative ones even with the increase of efficiency. The 3.75-1.75-fold decrease of ROS levels was detected in case of all other bacteria strains, but what more important is that the dose-activity relationships remained the same, i.e. the more sensitive to 2-F-araAMP bacteria strain was, the higher ROS level at the highest used compound concentration was detected.
3. Finally, NH_2-6-Cl-araPur had higher potency against all strains *S. lutea* all strain *S. lutea* (ED_{50} = 2.5 * 10^{-4} M), *B. cereus* (ED_{50} = 7.9 * 10^{-4} M), *P. Mirabilis (*ED_{50} = 3.4 * 10^{-4} M), and *E. Coli* (ED_{50} = 7.9 * 10^{-4} M). Both the most sensitive gram-positive strains *E. Coli*, and *P. Mirabilis* showed the 8-fold burst of intracellular ROS after treatment with near-ED_{50} concentrations of NH_2-6-Cl-araPur (1.5 * 10^{-4} M and 1.5 * 10^{-4} M, respectively). The 1.2-3.0-fold decrease of ROS levels was detected in case of all other bacteria strains, but what more important is that the dose-activity relationships remained the same, i.e. the more sensitive to NH_2-6-Cl-araPur bacteria strain was, the higher ROS level at the highest used compound concentration was detected.

References

1. Hawkins C.L., Davies M.J. Detection, identification, and quantification of oxidative protein modifications. *J. Biol. Chem.* 2019;294:19683–19708.
2. Magnani F., Mattevi A. Structure and mechanisms of ROS generation by NADPH oxidases. *Curr. Opin. Struct. Biol.* 2019;59:91–97.
3. Collin F. Chemical basis of reactive oxygen species reactivity and involvement in neurodegenerative diseases. *Int. J. Mol. Sci.* 2019;20:2407.
4. Sies H., Jones D.P. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nat. Rev. Mol. Cell Biol.* 2020;21:363–383.
5. Heid M.E., Keyel P.A., Kamga C., Shiva S., Watkins S.C., Salter R.D. Mitochondrial reactive oxygen species induces NLRP3-dependent lysosomal damage and inflammasome activation. *J. Immunol.* 2013;191:5230–5238.
6. Sousa J.S., D'Imprima E., Vonck J. Mitochondrial Respiratory Chain Complexes. In: Harris J.R., Boekema E.J., editors. *Membrane Protein Complexes: Structure and Function.* Springer; Singapore: 2018. pp. 167–227.
7. Valenta H., Erard M., Dupré-Crochet S., Nüβe O. The NADPH Oxidase and the Phagosome. In: Hallett M.B., editor. *Molecular and Cellular Biology of Phagocytosis.* Springer International Publishing; Cham, Switzerland: 2020. pp. 153–177.
8. Kehrer J.P. The haber–weiss reaction and mechanisms of toxicity. *Toxicology.* 2000;149:43–50.

9. Chen H.-Y. Why the reactive oxygen species of the fenton reaction switches from oxoiron(IV) species to hydroxyl radical in phosphate buffer solutions? A computational rationale. *ACS Omega.* 2019;4:14105–14113..
10. Fujiwara O., Fukuda S., Lopez E., Zeng Y., Niimi Y., DeWitt D.S., Herndon D.N., Prough D.S., Enkhbaatar P. Peroxynitrite decomposition catalyst reduces vasopressin requirement in ovine MRSA sepsis. *Intensive Care Med. Exp.* 2019;7:12
11. Jiang H., Xue A., Wang Z., Xia R., Wang L., Tang Y., Wan P., Chen Y. Electrochemical degradation of lignin by ROS. *Sustain. Chem.* 2020;1:345–360.
12. Arshed Shihad, Maksim Khancheuski, Aliaksei Sysa, Evgeniy Gritskevitch , Evgeniy Kvasyuk, Viktar Lemiasheuski. In vitro Antimicrobial Activity Profile of Modified Pyrimidine Nucleosides Derivatives. Journal of Pharmaceutical Research International 34(38A): 34-45, 2022; Article no.JPRI.86839.
13. Ventola CL. The antibiotic resistance crisis: Part 1: Causes and threats. Physical Therapy, 2015;40(4):277–283.
14. Koszytkowska-Stawińska M., Buchowicz W. Multicomponent reactions in nucleoside chemistry. Beilstein Journal of Organic Chemistry. 2014;10:1706–32.
15. Travnickova E., Mikula P., Oprsal J. [et al.]. Resazurin assay for assessment of antimicrobial properties of electrospun nanofiber filtration membranes. AMB Express. 2019;9:183.
16. Jordheim L. P., Durantel D., Zoulim F., Dumontet C. Advances in the development of nucleoside and nucleotide analogues for cancer and viral diseases. Nature Reviews Drug Discovery. 2013;12(6):447–64.
17. Seley-Radtke KL, Yates MK. The evolution of nucleoside analogue antivirals: a review for chemists and non-chemists. Part 1: early structural modifications to the nucleoside scaffold. Antiviral Research. 2018;154:66–86.
18. Khandazhinskaya AL, Matyugina ES, Solyev PN, Buckheit WM, Karen W, Buckheit RW. [et al.]. Investigation of 5'- Norcarbocyclic Nucleoside Analogues as Antiprotozoal and Antibacterial Agents. Molecules. 2019;24(19):3433.
19. Yates MK, Seley-Radtke KL. The evolution of antiviral nucleoside analogues: A review for chemists and non-chemists. Part II: Complex modifications to the nucleoside scaffold. Antiviral Research. 2019;162:5– 21.
20. Yang Z., Unrine J., Nonaka K., Van Lanen S. G. Fe(II)-Dependent, Uridine-50 – Monophosphate a-Ketoglutarate Dioxygenases in the Synthesis of 50-Modified Nucleosides. Modified Nucleoside. 2012;516:153–168.
21. Xing L, Honda T, Fitz L, Ojima I. Case studies of fluorine in drug discovery. Fluorine in Life Sciences: Pharmaceuticals, Medicinal Diagnostics, and Agrochemicals, Eds. Haufe, G., Leroux, F. R., Academic Press; Cambridge, MA, USA. 2019;181– 211.
22. Sanderson KE, Mac Alister T, Costerton JW, Cheng K-J. Permeability of lipopolysaccharide-deficient (rough) mutants of Salmonella typhimurium to antibiotics, lysozyme, and other agents. Canadian Journal of Microbiology. 1974;20:1135–1145.
23. Dahl TA, Midden WR, Hartman PE. Pure singlet oxygen cytotoxicity for bacteria. Photochemistry and Photobiology. 1987;46:345–352.

24. Breijyeh Z., Jubeh B., Karaman R. Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to Resolve It. Molecules. 2020;25(6):1340.
25. Foote CS, Denny RW. Chemistry of singlet oxygen. VII. Quenching by P-carotene. Journal of the American Chemical Society. 1968;90:6233–6235.
26. Mathews MM, Sistrom WR. The function of carotenoid pigments of Sarcina lutea. Archives of Microbiology. 1960;35:139– 146.
27. Brigelius-Flohe R. and Flohe L. Basic principles and emerging concepts in the redox control of transcription factors. *Antioxid Redox Signal* 15: 2335–2381, 2011.
28. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, and Zychlinsky A. Neutrophil extracellular traps kill bacteria. *Science* 303: 1532–1535, 2004.
29. Buchmeier NA, Lipps CJ, So MY, and Heffron F. Recombination-deficient mutants of *Salmonella typhimurium* are avirulent and sensitive to the oxidative burst of macrophages. *Mol Microbiol* 7: 933–936, 1993.
30. Buommino E, Donnarumma G, Manente L, De Filippis A, Silvestri F, Iaquinto S, Tufano MA, and De Luca A. The *Helicobacter pylori* protein HspB interferes with Nrf2/Keap1 pathway altering the antioxidant response of Ags cells. *Helicobacter* 17: 417–425, 2012.
31. Burke MF, Khera AV, and Rader DJ. Polyphenols and cholesterol efflux: is coffee the next red wine? *Circ Res* 106: 627–629, 2010.
32. Bustamante J, Aksu G, Vogt G, de Beaucoudrey L, Genel F, Chapgier A, Filipe-Santos O, Feinberg J, Emile JF, Kutukculer N, and Casanova JL. BCG-osis and tuberculosis in a child with chronic granulomatous disease. *J Allergy Clin Immunol* 120: 32–38, 2007.
33. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2022). Post-pandemic health and its sustainability: Educational situation. *International Journal of Health Sciences*, *6*(1), i-v. https://doi.org/10.53730/ijhs.v6n1.5949
34. Ratnawati, I. G. A. A., Sutapa, G. N., & Ratini, N. N. (2018). The concentration of radon gas in air-conditioned indoor: Air quality can increase the potential of lung cancer. *International Journal of Physical Sciences and Engineering*, *2*(2), 111–119. https://doi.org/10.29332/ijpse.v2n2.169
35. Susilo, C. B., Jayanto, I., & Kusumawaty, I. (2021). Understanding digital technology trends in healthcare and preventive strategy. *International Journal of Health & Medical Sciences*, *4*(3), 347-354. https://doi.org/10.31295/ijhms.v4n3.1769