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Estimation of Antiproliferative Activity of Modified Thio-Nucleosides on Mcf-7 Cells Line with Pure Bacteria Liquid

Sajjad Albasri, Ph.D
Student of environmental medicine faculty, ISEI BSU

Aliaksei G. Sysa
Candidate of science (biochemistry), docent (associate professor); dean of environmental medicine faculty, ISEI BSU

Eugenii I. Kvasyuk
Doctor of science (chemistry), full professor; professor at the department of environmental chemistry and biochemistry, environmental medicine faculty, ISEI BSU

Eugenii R. Gritskevitch
Candidate of science (biology), docent (associate professor); associate professor of immunology department, environmental medicine faculty, ISEI BSU.

Annotation: Thio-nucleosides, characterized by the replacement of an oxygen atom with a sulfur atom in the nucleobase or sugar moiety, exhibit unique chemical and biological properties that make them promising candidates for therapeutic applications. In this study, we investigate the antiproliferative activity of four thio-nitrogen bases and nucleosides, including 2-mercaptopurine, thioguanine, 6-thioguanosine, and 2'-deoxy-6-thioguanosine, as well as Desulfated Aztreonam and 2-(Benzylsulfanyl)-1-hydroxyadenosine, against the MCF-7 human breast adenocarcinoma cell line.

MCF-7 cells were cultured in a controlled environment and treated with the thio-nucleosides for 24 hours. The antiproliferative effects of the compounds were assessed using resazurin assays, and the results were analyzed using R programming. Additionally, we utilized pure Bifidobacterium liquid as a potential delivery method for the thio-nucleosides, following a centrifugation and washing protocol to obtain a concentrated bacterial suspension.

Keywords: MCF-7; anticancer activity; antibacterial activity; thionucleosides.

Introduction

Thio-nucleosides represent a fascinating niche in the field of nucleic acid chemistry, offering unique properties and significant potential in various scientific and medical applications. These modified nucleosides, in which a sulfur atom replaces an oxygen atom in the nucleobase or sugar moiety, exhibit distinctive chemical and biological characteristics compared to their natural counterparts. This sulfur substitution can enhance the stability of nucleic acids, alter their binding affinity, and provide resistance to enzymatic degradation, making thio-nucleosides valuable tools in biochemical research and therapeutic development.

The exploration of thio-nucleosides has opened new avenues in the design of nucleic acid-based drugs and diagnostic tools. For instance, they have been instrumental in the development of antisense oligonucleotides and small interfering RNAs (siRNAs), which are crucial for gene silencing technologies. Additionally, thio-nucleosides are being investigated for their potential in antiviral therapies, given their ability to inhibit viral replication by interfering with enzyme activities and viral genome synthesis.

Moreover, thio-nucleosides serve as powerful probes in structural biology, helping to elucidate the intricate details of nucleic acid interactions and dynamics. Their distinctive spectroscopic properties, such as altered UV absorbance and fluorescence, provide researchers with robust tools to study DNA and RNA structures and functions.

In conclusion, thio-nucleosides are not merely chemical curiosities but pivotal components in advancing our understanding and manipulation of nucleic acids. Their unique properties and versatile applications underscore their importance in scientific research and their potential to drive innovations in medicine and biotechnology.

Write a good introduction about Thio-nucleosides

Thio-nucleosides are a distinctive class of nucleoside analogs characterized by the substitution of a sulfur atom for an oxygen atom within the nucleobase or sugar moiety of the nucleoside structure. This seemingly subtle modification imparts profound changes to the chemical and biological properties of nucleic acids, making thio-nucleosides a subject of intense interest in the fields of biochemistry, molecular biology, and medicinal chemistry.

The introduction of sulfur confers increased nucleic acid stability, resistance to enzymatic degradation, and altered electronic properties, which can influence the binding affinity and specificity of nucleic acid interactions. These attributes render thio-nucleosides invaluable in the development of therapeutic agents, particularly in the realm of nucleic acid-based drugs such as antisense oligonucleotides and small interfering RNAs (siRNAs). These therapeutics leverage the unique properties of thio-nucleosides to enhance efficacy, reduce off-target effects, and improve pharmacokinetic profiles.

Beyond therapeutic applications, thio-nucleosides are pivotal in advancing our understanding of nucleic acid chemistry and biology. Their distinctive spectroscopic characteristics, including altered UV absorbance and fluorescence, enable researchers to probe the structural and dynamic properties of DNA and RNA with greater precision. This has significant implications for the study of genetic regulation, enzyme mechanisms, and nucleic acid-ligand interactions.

In the realm of antiviral research, thio-nucleosides hold promise due to their potential to inhibit viral replication. By interfering with viral polymerases or integrating into viral genomes, these compounds can disrupt the life cycle of various pathogens, offering new avenues for antiviral drug development.

In summary, thio-nucleosides are not merely academic curiosities but key players in the advancement of nucleic acid research and therapeutic innovation. Their unique chemical properties and versatile applications underscore their importance in both basic science and applied medicine, making them a vital focus for ongoing research and development.

In this study the antiproliferative activity of modified thio-nitrogen bases 2-mercaptopurine, thioguanine, and nucleosides 6-thioguanosine and 2’-deoxy-6-thioguanosine and Desulfated_Aztreonam and 2-(Benzylsulfanyl)-1-hydroxyadenosine against MCF-7 cell were studied.

Materials and Methods

Chemicals

Studied compounds 2-mercaptopurine, 6-thioguanine (2-amino-6-mercaptopurine), 6-thioguanosine (2-amino-6-mercaptopurine riboside), 2′-deoxy-6-thioguanosine (6-Thio-2′-Deoxyguanosine) (Figure 1) were purchased at Sigma-Aldrich.

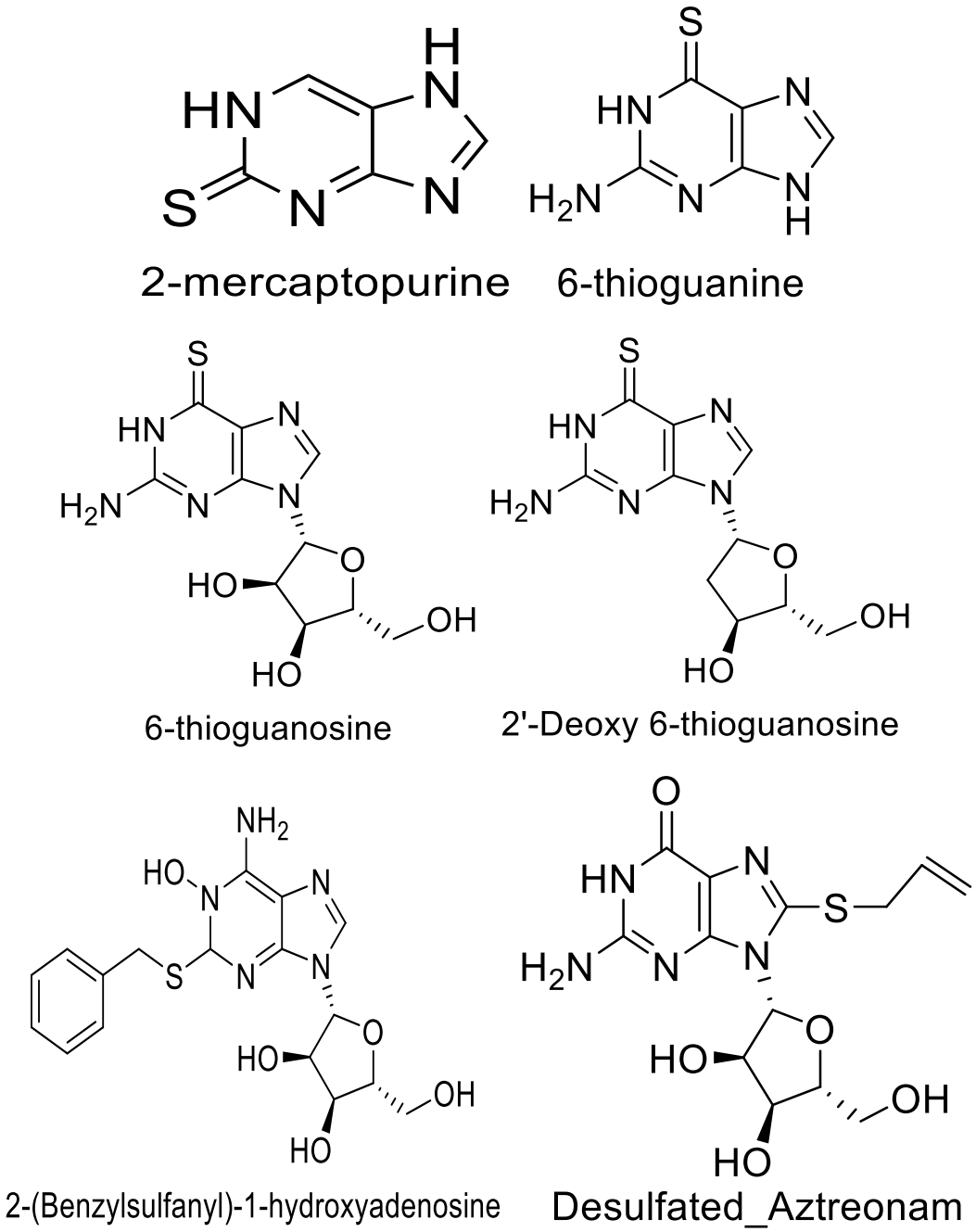


Fig 1. Structures of studied nitrogen bases and nucleosides

Cell Culture

MCF-7 cells, a human breast adenocarcinoma cell line, have been widely used as a model system for studying the biological effects of thio-nucleosides. These cells are well-characterized, easy to culture, and exhibit many characteristics of breast cancer cells, making them an ideal choice for investigating the antiproliferative and cytotoxic effects of thio-nucleosides. MCF-7 cells were obtained from the American Type Culture Collection (ATCC) and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, and 1% penicillin-streptomycin. Cells were maintained at 37°C in a humidified atmosphere of 5% CO2 and 95% air.

After the MCF-7 cells are ready for experiment we add nucleotides in very clean and satirized lab to prevent any contamination and then put it in the incubator for 24 hours in 37 degrees after that we add resazurin and he pure Bactria liquid and wait for another hour for the reactions and make measurements then put data in R program and collect the results.

This direct addition method is straightforward, minimally disruptive to the cells, and does not require specialized equipment or techniques. However, it is essential to consider the solubility, stability, and potential interactions of the compound with the cell culture medium components.

It's worth noting that for compounds with limited solubility or stability in aqueous solutions, alternative delivery methods, such as nanoparticle encapsulation or microinjection, may be necessary to ensure efficient cellular uptake and appropriate compound concentrations.

PURE BACTERIA LIQUID

Bifidobacterium, a genus of gram-positive, anaerobic bacteria, is commonly used in probiotic applications due to its beneficial effects on human health. When working with Bifidobacterium, it is often necessary to centrifuge liquid cultures to separate the bacterial cells from the growth medium.and to make pure bacteria liquid from this Bifidobacterium bacteria we should do

1. After incubation, the liquid culture containing Bifidobacterium is transferred to a centrifuge tube.
2. The tube is placed in a centrifuge and spun at 4,000-6,000 rpm (approximately 2,000-4,000 x g) for 10-15 minutes at 4°C.
3. The resulting pellet contains the Bifidobacterium cells, while the supernatant consists of the growth medium and any soluble components.
4. The supernatant is carefully discarded, and the pellet is washed with a sterile buffer (e.g., phosphate-buffered saline) to remove any remaining growth medium.
5. The pellet is then resuspended in a suitable buffer or medium for further analysis or application.

Resazurin reduction assay

Resazurin reduction is a cell viability assay that measures the ability of cells to reduce the blue dye resazurin to a pink-colored compound called resorufin. This assay is commonly used to determine cell viability, cytotoxicity, and cell proliferation.

The resazurin metabolization experiments were performed in 96-well plates as described [16]. 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. The percentage of survival was established for wells containing nucleosides/nucleotides relative to control wells containing no compounds. The core principle behind the resazurin reduction assay is the metabolic activity of living cells. In viable cells, mitochondrial and cytoplasmic enzymes reduce resazurin to resorufin. The extent of this reduction correlates with the number of metabolically active cells in the culture. Dead or non-metabolically active cells do not reduce resazurin, thus providing a clear distinction between live and dead cells.

Statistical analysis

The trials were repeated until three data sets (in triplicate) had been collected for each answer (n=6). All data are expressed as the median (interquartile range (IQR)) and were analyzed using the Kruskal-Wallis test for comparing more than two independent sets of samples.

When the Kruskal-Wallis test revealed significant differences between groups, the Wilcoxon rank sum post hoc test was performed to identify pairings of groups with statistically significant differences. A p-value of less than 0.05 indicates significance.

All statistical analyses were carried out using the R statistical code. (*ver.* 4.1.2).

Results and discussion

Various concentrations of the modified nitrogen bases 2-mercaptopurine, 6-thioguanine, and nucleosides 6-thioguanosine and 2'-deoxy-6-thioguanosine are assessed for their impact on the viability of MCF-7 cells line.

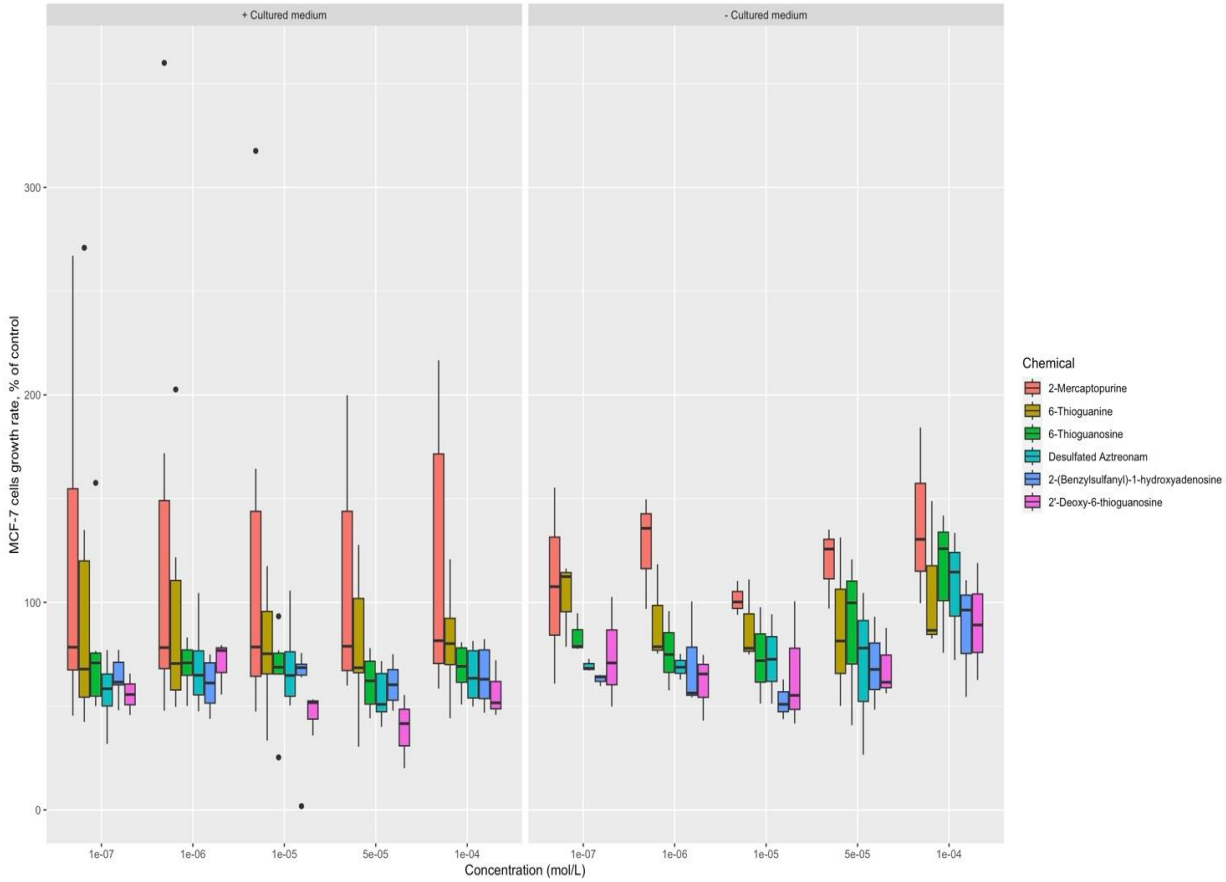


Fig 2. Effect of different concentrations of 2-mercaptopurine, 6-thioguanine, 6-thioguanosine, and 2'-deoxy-6-thioguanosine on the growth of MCF-7 cells

The concentration of nucleotides is critical to the development and proliferation of MCF-7 cells, a commonly utilized breast cancer cell line.

Nucleotides are the structural components of nucleic acids (DNA and RNA) and are required for a variety of biological functions such as DNA replication, transcription, and translation. Cancer cells, especially MCF-7 cells, have a higher requirement for nucleotides due to their fast proliferation and uncontrolled development.

Overall, nucleotide concentration and balance are essential for MCF-7 cell growth and survival, and knowing the link between nucleotide metabolism and cancer cell behavior is fundamental for creating successful treatment options.

In conclusion, these purine analogs (2-mercaptopurine, 6-thioguanine, 6-thioguanosine, and 2'-deoxy-6-thioguanosine) were shown to have dose-dependent inhibitory effects on the proliferation and viability of MCF-7 breast cancer cells. The fundamental processes include disrupting nucleic acid synthesis and inducing cell cycle arrest and death. These findings have significant implications for the compounds' potential therapeutic use in the treatment of breast cancer.

Next pairwise comparisons between group levels with corrections for multiple testing were calculated (Table 1).

Table 1

Pairwise comparisons of the effect of different concentrations of 2-mercaptopurine, 6-thioguanine, 6-thioguanosine, and 2'-deoxy-6-thioguanosine on the viability of life cancer cells using Wilcoxon rank sum test results

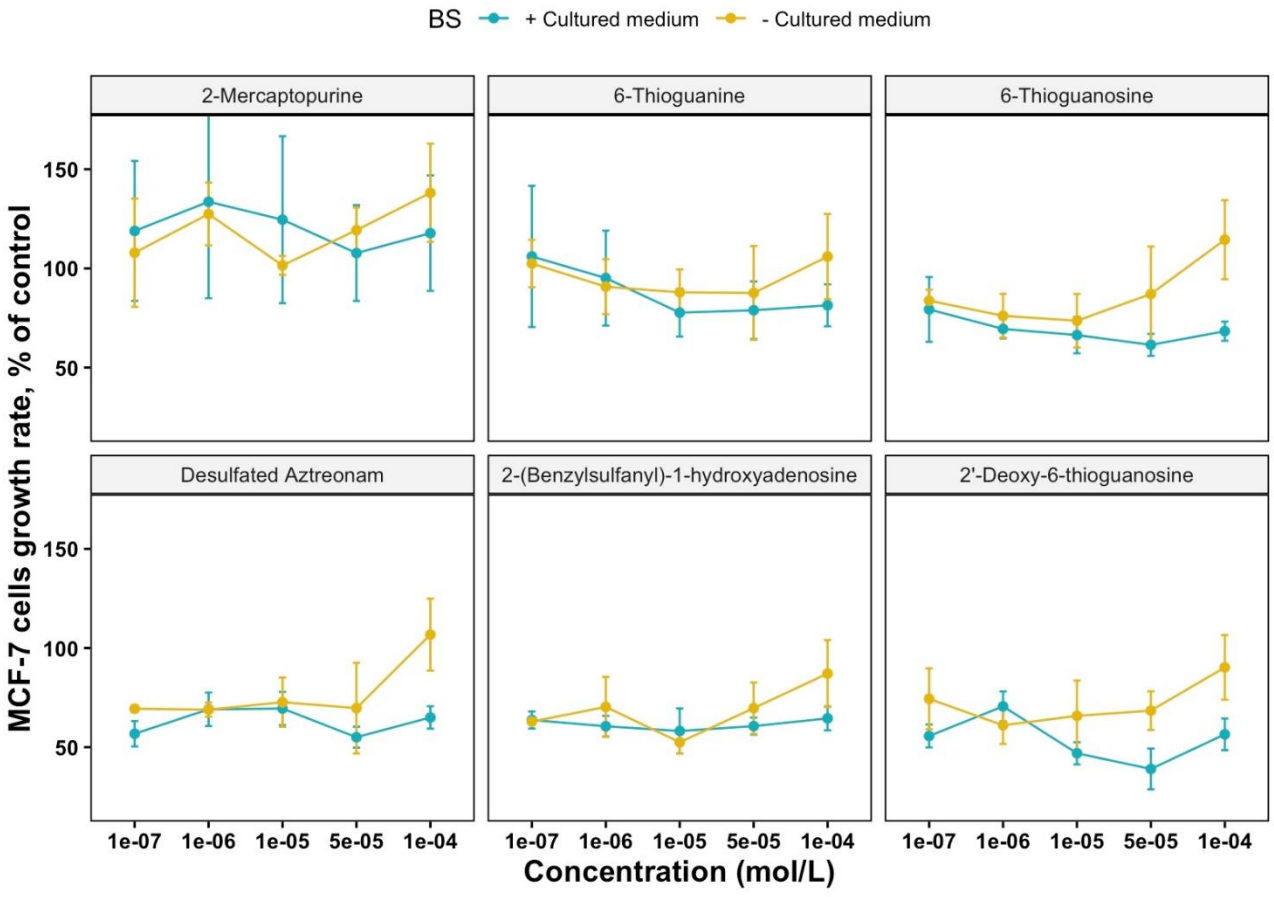
- full.aov2 <- aov(Activity ~ Chemical + BS + Concentration, data = full_data_upd)
- summary(full.aov2)

	Df	Sum Sq	Mean	Sq F value	Pr(>F)
Chemical	5	104507	20901	13.942	5.45e-12 ***
BS	1	5963	5963	3.977	0.0472 *
Concentration	4	6185	1546	1.031	0.3916
Residuals	244	365798	1499		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

From the ANOVA table we can conclude that *Chemical* and *BS* but not *Concentration* are statistically significant. *Chemical* is the most significant factor variable. These results would lead us to believe that adding the cultured media from bacteria or changing the nucleoside, will impact significantly the mean MCF-7 growth rate.

Not the above fitted model is called **additive model**. It makes an assumption that the two factor variables are independent.



As the ANOVA test is significant, we can compute **Tukey HSD** (Tukey Honest Significant Differences, R function: **TukeyHSD**()) for performing multiple pairwise-comparison between the means of groups. The function **TukeyHD**() takes the fitted ANOVA as an argument.

We don't need to perform the test for the "BS" variable because it has only two levels, which have been already proven to be significantly different by ANOVA test.

This study consistently indicated that 24-hour treatment with 6-thioguanine, 6-thioguanosine, and 2'-deoxy-6-thioguanosine, but not 2-mercaptopurine, had no significant harmful effects on cancer cells as measured by resazurin. This study also found that the modified nitrogen bases 2-mercaptopurine, 6-thioguanine, and nucleosides 6-thioguanosine and 2'-deoxy-6-thioguanosine significantly reduced cancer cell proliferation in a concentration-dependent manner, particularly 6-thioguanine.

The mean difference values indicate how much the activity levels vary between the compounds or concentrations being compared. For example, a negative mean difference suggests lower activity levels compared to the reference compound.

The confidence intervals (lwr and upr) provide a range within which the true mean difference is likely to lie. A wider interval indicates more uncertainty in the estimate of the mean difference.

The p adj values are adjusted p-values that take into account multiple comparisons, helping to determine if the observed differences are statistically significant after considering the possibility of random chance.

The active form of 6-thioguanine is structurally similar to mercaptopurine, which inhibits purine metabolism. 6-Thioguanine is currently used to treat inflammatory bowel disease and a variety of lymphoid malignancies, including acute myeloid leukemia (AML), acute lymphoid leukemia (ALL), and chronic myeloid leukemia (CML). D. Chin et colleagues. discovered that 6-thioguanine, a purine analog, reduced S. aureus growth by preventing de novo purine synthesis [22].

The molecular basis for 6-thioguanine's therapeutic effect is most likely due to its ability to suppress purine biosynthesis, which results in decreased ribosome synthesis and toxin formation. Furthermore, 6-thioguanine suppresses the transcription of the global virulence regulator agr, which reduces toxin production. The studies reveal that 6-thioguanine can function as an antivirulence agent in an MCF-7 by preventing de novo purine production.

In this study, a series of potential chemotherapeutic agents were tested for their efficacy in inhibiting the growth of cancer cells. The data suggests variability in the potency and consistency of the agents' effects. For example, Chemical: This represents the effect of the "Chemical" factor on the outcome variable. The high F-value (13.942) and very low p-value (5.45e-12) indicate a statistically significant difference in the outcome variable across different chemical groups.

BS: This represents the effect of the "BS" factor. The F-value (3.977) and p-value (0.0472) indicate a statistically significant difference in the outcome variable across different BS groups.

Concentration: This represents the effect of the "Concentration" factor. The F-value (1.031) and high p-value (0.3916) indicate no statistically significant difference in the outcome variable across different concentration groups.

Residuals: This represents the variation within each group that cannot be explained by the factors being tested.

The difference between "6-Thioguanine-2-Mercaptopurine" and "6-Thioguanosine-2-Mercaptopurine" is -29.7511111. This means that "6-Thioguanine-2-Mercaptopurine" had an outcome value that was 29.7511111 units lower than "6-Thioguanosine-2-Mercaptopurine".

The p-value (0.0043737) is very small, indicating a statistically significant difference

The confidence interval (-53.19856 to -6.303662) suggests that the true difference between the two nucleoside combinations is likely somewhere between -53.19856 and -6.303662.

The vitality of opportunistic gram-positive bacterial cultures of B. cereus and S. aureus is tested at different doses of the modified nitrogen bases 2-mercaptopurine, 6-thioguanine, and the nucleosides 6-thioguanosine and 2'-deoxy-6-thioguanosine. This work consistently established that 24-hour administration of 6-thioguanine, 6-thioguanosine, and 2'-deoxy-6-thioguanosine, but not 2-mercaptopurine, on B. cereus bacterial cells by resazurin reduction test had no substantial harmful effects. The modified nitrogen bases 2-mercaptopurine, 6-thioguanine, and the nucleosides 6-thioguanosine and 2'-deoxy-6-thioguanosine all significantly reduced the growth of S. aureus bacterial cells in a concentration-dependent manner, with 6-thioguanine being the most effective. Our results demonstrated that 6-thioguanine was effective against S. aureus (35.6% growth reduction), but not against B. cereus.

Furthermore, it is crucial to assess the therapeutic implications of these findings. While in vitro effectiveness is an important stage in drug development, in vivo investigations and clinical trials are required to evaluate the pharmacokinetics, pharmacodynamics, toxicity, and overall therapeutic potential of these medicines.

Finally, the facts reported here add to the expanding corpus of research aimed at generating innovative cancer therapies. While certain medicines show promise in terms of effectiveness and consistency, further study is needed to completely comprehend their therapeutic applications. Collaboration between laboratory research and clinical trials will be required to transform these insights into successful cancer medicines.

Conclusion

The results we obtained suggested that treatment with the nitrogen bases 2-mercaptopurine, 6-thioguanine, and the nucleosides 6-thioguanosine and 2'-deoxy-6-thioguanosine might have an antibacterial effect on Bacillus cereus and Staphylococcus aureus. Our findings provide new light on the efficacy of thiopurine cytotoxicity and provide a rationale for using mercaptopurine instead of thioguanine in the treatment of a variety of bacteria-caused diseases.

In this study, we investigated the effects of modified thio-nucleosides on the proliferation and viability of MCF-7 breast cancer cells. The thio-nucleosides tested, including 2-mercaptopurine, 6-thioguanine, 6-thioguanosine, and 2'-deoxy-6-thioguanosine, exhibited concentration-dependent inhibitory effects on MCF-7 cell growth. The results suggest that these compounds disrupt nucleic acid synthesis and induce cell cycle arrest and death, leading to their potential therapeutic use in breast cancer treatment. The findings also highlight the importance of understanding the relationship between nucleotide metabolism and cancer cell behavior for the development of effective treatment strategies.

The statistical analysis revealed that the type of chemical and the presence of bacterial supernatant (BS) had significant impacts on MCF-7 cell growth, while the concentration of the compounds showed no significant effect. The active form of 6-thioguanine, structurally similar to mercaptopurine, inhibits purine metabolism and has therapeutic effects on inflammatory bowel disease and lymphoid malignancies. Additionally, 6-thioguanine has been found to reduce the growth of certain pathogens by disrupting purine synthesis.

Overall, this study contributes valuable insights into the potential of thio-nucleosides as chemotherapeutic agents and underscores the need for further in vivo and clinical investigations to fully realize their therapeutic potential. The findings also emphasize the importance of collaboration between laboratory research and clinical trials to translate these insights into effective cancer treatments.

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