Journal of Biology and Genetic Research Vol. 10 No. 1 February 2024 E-ISSN 2545-5710
P-ISSN 2695-222X www.iiardjournals.org Online Version

Estimation of Antiproliferative Activity of Modified Thio-Nucleosides on MCF-7 Cells Line

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DOI: 10.56201/jbgr.v10.no1.2024.pg32.41

Abstract

The MCF-7 cell line, a well-researched human breast cancer model, has significantly contributed to advancing our knowledge of breast cancer biology and innovating treatment approaches. A notable trait of MCF-7 cells is their responsiveness to estrogen. Research involving MCF-7 cells has yielded significant knowledge regarding hormone receptor signaling and the modes of operation of anti-estrogen treatments like tamoxifen. Furthermore, MCF-7 cells have served as a platform for investigating drug resistance and for identifying prospective anti-cancer agents[1]. Modified nitrogen bases 2-mercaptopurine, thioguanine, and nucleosides 6-thioguanosine and 2'-deoxy-6-thioguanosine, along with Desulfated_Aztreonam and 2-(Benzylsulfanyl)-1-hydroxyadenosine, were evaluated for their potential anticancer properties. The antiproliferative assay was utilized to examine the characteristics of MCF-7 cells. The findings indicated that 2-mercaptopurine exhibited notable efficacy against MCF-7 cells, resulting in a 40% inhibition of growth[4]. Treatment with nitrogen bases 2-mercaptopurine and 6-thioguanine, along with nucleosides 6-thioguanosine and 2'-deoxy-6-thioguanosine, may exhibit antibacterial properties against MCF-7. Our findings offer fresh perspectives on the cytotoxic efficacy of thiopurines and propose a justification for opting for mercaptopurine over thioguanine in addressing various bacterial-induced illnesses.

Key words: *MCF-7; anticancer activity; antibacterial activity; thionucleosides.*

Introduction

Thio-nucleosides, a class of compounds obtained from nucleosides through the substitution of the oxygen atom with a sulfur atom, have attracted considerable interest in oncology investigations because of their encouraging anticancer characteristics. The distinctive chemical configuration of thio-nucleosides confers upon them specific biological functions that position them as plausible contenders for cancer therapy[2].

Thio-nucleosides have demonstrated notable cytotoxic impacts on different cancer cell lines, rendering them a topic of interest for additional exploration in both preclinical and clinical investigations. Their capacity to intervene with nucleic acid metabolism and disturb DNA replication and repair mechanisms within cancerous cells has established them as plausible chemotherapeutic substances[1].

Furthermore, thio-nucleosides have indicated the capability to surmount drug resistance, a significant obstacle in cancer therapy, thereby presenting novel pathways for the enhancement of potent anticancer treatments. Indeed, the potential of thio-nucleos ide analogs in the field of oncology is vast , and ongoing research in this area continues to reveal new possibilities for their application[8][9].

For instance, recent studies have shown that certain thio-nucleoside analogs can selectively target and inhibit the activity of specific kinases , which are enzymes that play a crucial role in the regulation of cell division and signal transduction pathways , thereby offering a promising approach to tackle cancer cell prolifer ation and survival[11]. This ability to target specific kinases sets thio-nucleoside analogs apart from many traditional cancer treatments, which often target rapidly dividing cells indiscriminately, leading to significant side effects ; indeed, this targeted approach can help reduce the impact of treatment on healthy cells, thereby minimizing side effects and improving the overall quality of life for patients undergoing treatment.

Furthermore, this targeted therapy can also increase the effectiveness of the cancer treatment, as it specifically inhibits the kinases that are driving the uncontrolled growth and survival of cancer cells. Thus, thio-nucleoside analogs represent a promising advancement in the field of oncology; they offer a more precise and targeted way to attack cancer , all while minimizing the harm to healthy cells[6].

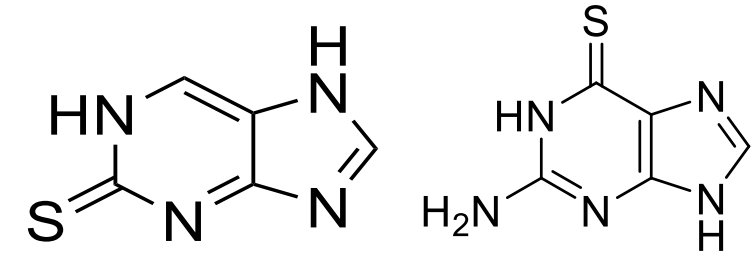
Thio-nucleoside analogs, a class of antimetabolite drugs, have been widely used in the treatment of various types of cancer due to their ability to interfere with DNA synthesis in cancer cells, thereby inhibiting their growth and proliferation[17][18]. Thio-nucleoside analogs used in cancer treatment can lead to severe side effects like nausea, vomiting, and myelosuppression, impacting patients' quality of life and treatment adherence. Managing these side effects effectively is crucial to ensure patients complete their treatment successfully. Healthcare providers can enhance patients' treatment experiences and outcomes by addressing potential side effects proactively and offering suitable interventions[15].

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.



2-mercaptopurine 6-thioguanine

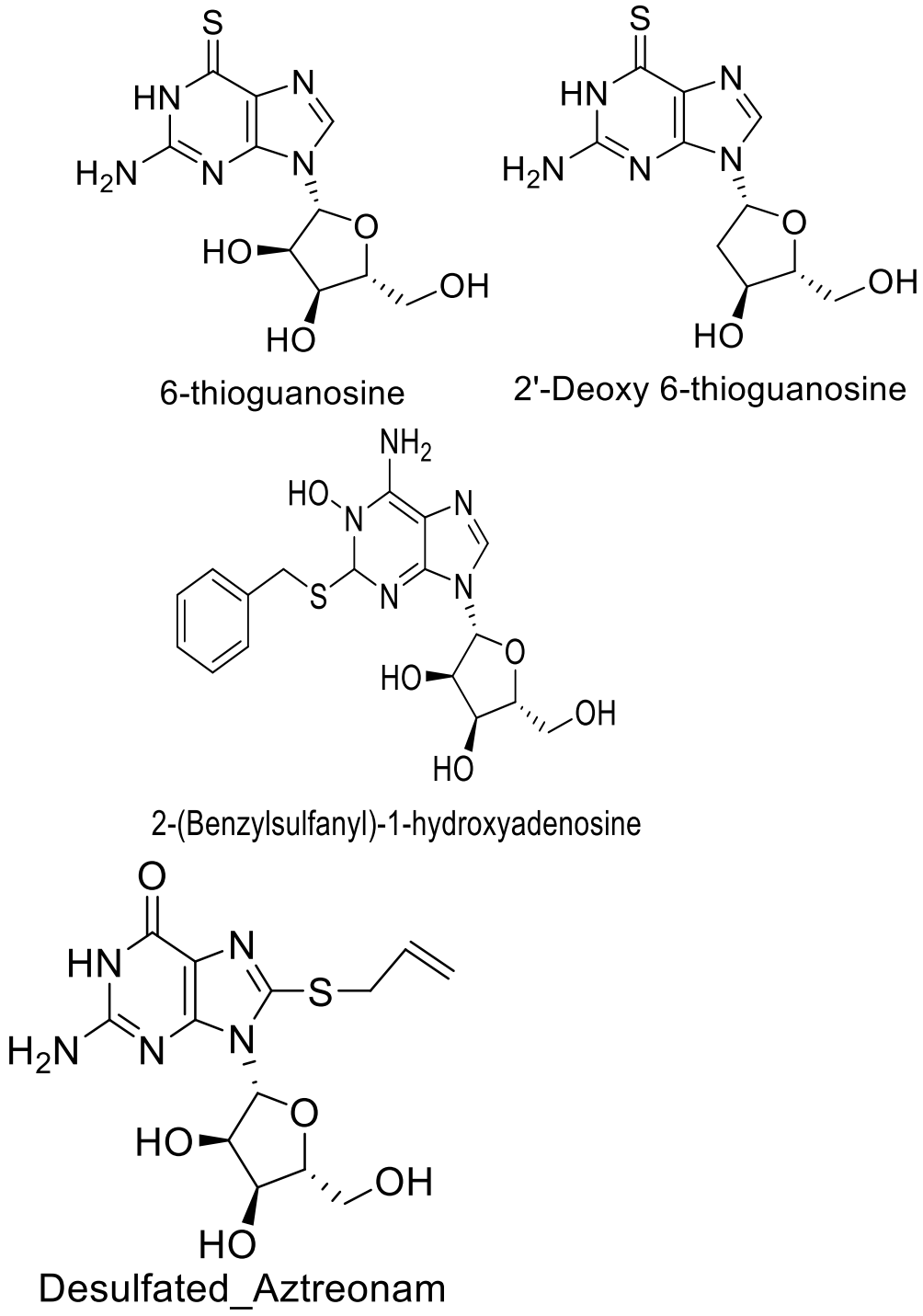


Fig 1. Structures of studied nitrogen bases and nucleosides

Cell Culture

Cultivation conditions for cells, often referred to as cell culture conditions, can vary dramatically based on the type of cells being grown (e.g., bacterial, mammalian, plant cells) and the specific objectives of the cultivation (e.g., protein production, research, drug discovery).

These conditions are highly general and can vary significantly depending on the specific requirements of the cell type and the goals of the cultivation. It's also important to note that cultivating cells requires careful planning and attention to detail to ensure the success and reproducibility of experiments or production processes.

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 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.

Resazurin reduction assay

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.

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.

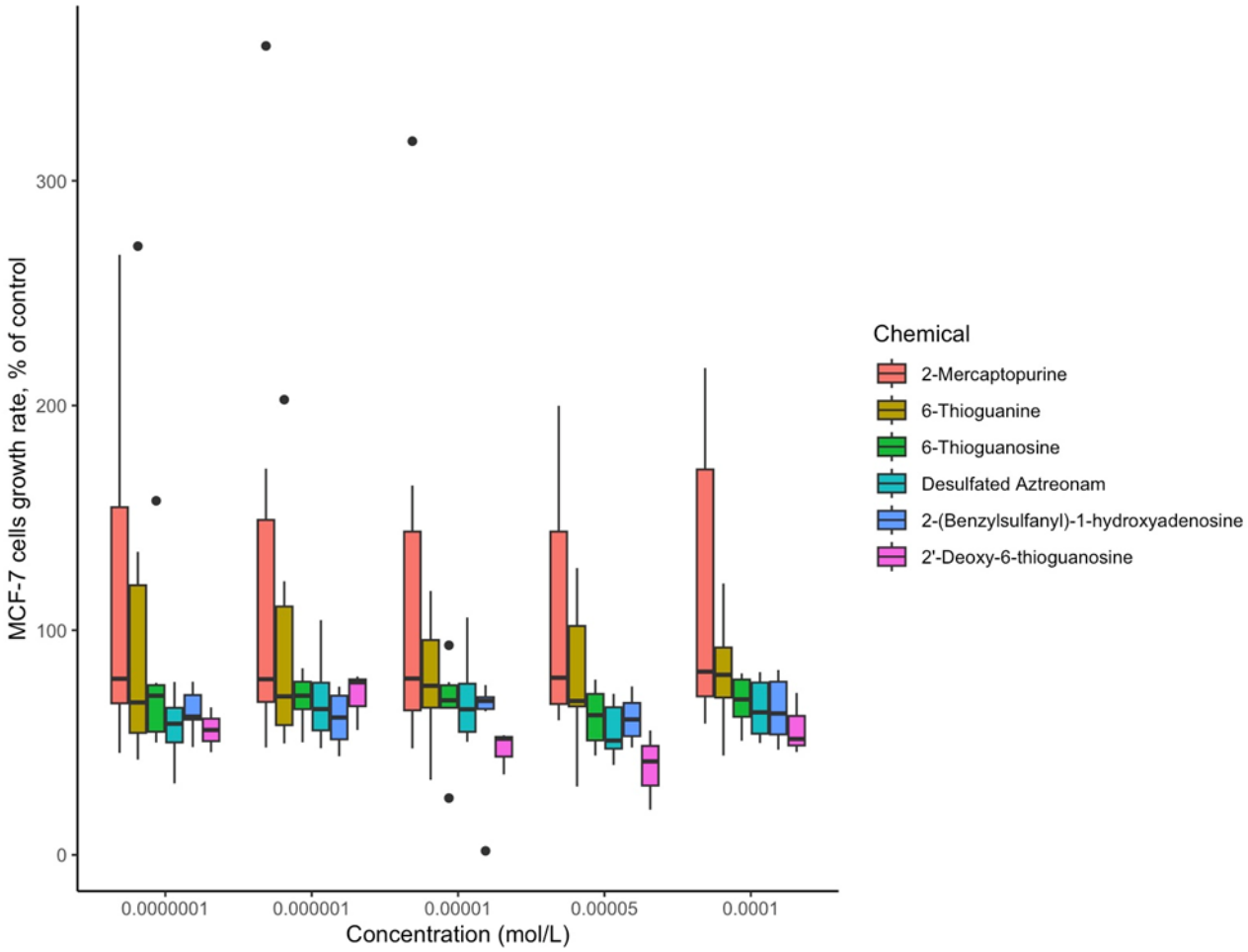


Fig 2. Effect of different concentrations of 2-mercaptopurine, 6-thioguanine, 6-thioguanosine, and 2'-deoxy-6-thioguanosine on the growth of MCF-7 cells

The concentration of nucleotides plays a crucial role in the growth and proliferation of MCF-7 cells, which are a widely used breast cancer cell line.

Nucleotides are the building blocks of nucleic acids (DNA and RNA), and they are essential for various cellular processes, including DNA replication, transcription, and translation. In cancer cells, including MCF-7 cells, there is an increased demand for nucleotides due to their rapid proliferation and uncontrolled growth.

Overall, the concentration and balance of nucleotides are critical for the growth and survival of MCF-7 cells, and understanding the relationship between nucleotide metabolism and cancer cell behavior is important for developing effective therapeutic strategies.

In summary, these purine analogs (2-mercaptopurine, 6-thioguanine, 6-thioguanosine, and 2'-deoxy-6-thioguanosine) have been shown to exhibit dose-dependent inhibitory effects on the growth and viability of MCF-7 breast cancer cells. The underlying mechanisms involve the disruption of nucleic acid synthesis and the induction of cell cycle arrest and apoptosis. These findings have important implications for the potential therapeutic application of these compounds 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

	2-Mercaptopurine	6-Thioguanine	6-Thioguanosine	Desulfated Aztreonam	2-(Benzylsulfanyl)-1-hydroxy-adenosine
6-Thioguanine	0.2143	-	-	-	-
6-Thioguanosine	0.0309	0.2355	-	-	-
Desulfated Aztreonam	0.0035	0.0494	0.2143	-	-
2-(Benzylsulfanyl)-1-hydroxy-adenosine	0.0035	0.0494	0.2143	0.8187	-
2'-Deoxy-6-thioguanosine	0.0020	0.0182	0.0443	0.2143	0.1456

The Kruskal-Wallis rank sum test is a non-parametric method used to determine if there are statistically significant differences between the medians of three or more independent groups. This result indicates that there are statistically significant differences among the groups. With a chi-squared value of 26.233 and 5 degrees of freedom, the p-value of 0.00008042 is well below the common alpha level of 0.05, suggesting that at least one group is different from the others in terms of median activity.

After determining that differences exist, pairwise comparisons can help identify exactly where these differences lie between specific chemicals. The pairwise comparisons are provided with their p-values, indicating the probability of observing the data if there were truly no difference between the groups.

2-Mercaptopurine vs Others:

- Significant differences with Desulfated Aztreonam and 2-(Benzylsulfanyl)-1-hydroxy-adenosine (p-values: 0.0035), and with 2'-Deoxy-6-thioguanosine (p=0.0020), indicating that the activity level of 2-Mercaptopurine is significantly different from these chemicals.

- Not significantly different from 6-Thioguanine (p=0.2143) and 6-Thioguanosine (p=0.0309, may be considered significant depending on the alpha).

6-Thioguanine vs Others:

- Shows significant differences with 2'-Deoxy-6-thioguanosine (p=0.0182).

- Other comparisons show no significant difference within the common alpha level (although close with Desulfated Aztreonam and 2-(Benzylsulfanyl)-1-hydroxy-adenosine, p=0.0494).

6-Thioguanosine vs Others:

- No comparisons result in significant differences at a strict alpha, but close to significant with Desulfated Aztreonam (p=0.2143).

-Desulfated Aztreonam and 2-(Benzylsulfanyl)-1-hydroxy-adenosine:

- Both have significant differences with 2-Mercaptopurine and non-significant with each other (p=0.8187), suggesting similar activity levels between them but significantly different from 2-Mercaptopurine.

- 2'-Deoxy-6-thioguanosine:

- Demonstrates significant differences with most others, especially with 2-Mercaptopurine (p=0.0020) and 6-Thioguanine (p=0.0182), underlining its distinct activity profile.

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.

Thiopurine antimetabolites, such as 6-mercaptopurine and 6-thioguanine, are structural purine analogues that operate as anti-cytotoxic agents and are commonly employed as cancer treatments and acute leukemia treatments. Thioguanine nucleotides are often supplied orally to patients and undergo intestinal and hepatic metabolism before being integrated into DNA to replace the endogenous purine guanine. 6-Mercaptopurine is a purine nucleoside that acts as an antibacterial agent. It inhibits the enzyme adenosine deaminase, which converts adenosine to inosine and so prevents the formation of purines. In animal studies, 6-Mercaptopurine was found to be beneficial against inflammatory bowel illness and bowel disease. At high dosages, it has been demonstrated to suppress subcutaneous cancers in mice. 6-Mercaptopurine also affects the metabolism of some medications, such as phenytoin and rifampicin, thus, thereby increasing their plasma concentration.

The active form of 6-thioguanine is structurally identical to mercaptopurine and inhibits purine metabolism. 6-Thioguanine is now used to treat inflammatory bowel illness and many lymphoid cancers, including acute myeloid leukemia (AML), acute lymphoid leukemia (ALL), and chronic myeloid leukemia (CML). Recently, D. Chin et al. reported that 6-thioguanine, a purine analog, inhibited S. aureus growth by blocking de novo purine production [24].

The molecular foundation for 6-thioguanine's therapeutic impact is most likely owing to its ability to inhibit purine biosynthesis, resulting in reduced ribosome synthesis and toxin generation. Furthermore, 6-thioguanine inhibits transcription of the global virulence regulator agr, resulting in decreased toxin output. The data demonstrate that 6-thioguanine can operate as an antivirulence agent in a MCF-7 by blocking de novo purine manufacture,

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, 2-Mercaptopurine shows the highest mean inhibitory effect at 121, with a relatively high standard deviation of 84.4, indicating a wide range in the responses. This could be due to differences in the cell lines used, the experimental conditions, or inherent properties of the chemical itself. The high mean suggests that 2-Mercaptopurine has a strong potential as a therapeutic agent, but the high variability warrants further investigation to understand the factors that influence its efficacy.

In contrast, 6-Thioguanine and 6-Thioguanosine present lower mean inhibitory effects of 87.8 and 69.0, respectively. The notably lower standard deviation in the response to 6-Thioguanosine implies more consistent performance across different conditions or cell lines. This consistency can be advantageous in a clinical setting, where predictability of a drug’s effect is crucial.

Desulfated Aztreonam and 2-(Benzylsulfanyl)-1-hydroxyadenosine exhibit similar mean inhibitory effects, at 63.1 and 61.5, respectively. These agents also have relatively low standard deviations, suggesting consistent responses. The median and IQR for these agents closely align with their mean values, further confirming this consistency.

The agent with the least number of observations, 2'-Deoxy-6-thioguanosine, has a mean inhibitory effect of 53.7 with a standard deviation of 15.6, based on only 15 measurements. The limited data points for this compound indicate that conclusions regarding its efficacy and consistency are less reliable and that further testing is needed to ascertain its potential as a cancer therapeutic.

In the context of precision medicine and immunotherapy, the efficacy of these agents could be further delineated by stratifying the data based on specific genetic mutations or immune profiles. Understanding the molecular interactions between these chemicals and the cancer cells can provide insights into the mechanisms of action and help identify which patient populations are most likely to benefit from these treatments.

Furthermore, it is critical to consider the clinical applicability of these findings. While in vitro efficacy is a crucial step in drug development, in vivo studies and clinical trials are necessary to evaluate the pharmacokinetics, pharmacodynamics, toxicity, and overall therapeutic potential of these agents.

In conclusion, the data presented here contributes to the growing body of research focused on developing novel cancer treatments. While some agents show promise in terms of efficacy and consistency, further research is required to fully understand their potential for clinical use. Collaboration between laboratory research and clinical studies will be essential to translate these findings into effective cancer therapies.

Conclusion

The study findings demonstrated that the nitrogenous bases 2-mercaptopurine and 6-thioguanine, along with the nucleosides 6-thioguanosine and 2’-deoxy-6-thioguanosine, exhibit antiproliferative effects on the MCF-7 cell line. These results offer novel perspectives on the cytotoxic efficacy of thiopurines and propose a justification for selecting mercaptopurine over thioguanine in cancer therapy.

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