

# Examining the Inhibitory Impact of Pyrimidine and Certain Derivatives on the Enzyme Glutathione Reductase



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## Abstract

Glutathione Reductase (GR, EC 1.6.4.2), a member of the oxidoreductase family of enzymes, is a NADPH dependent enzyme. In addition to neutralizing free radicals and reactive oxygen species (ROS), GR acts by maintaining the GSH / GSSG ratio in the regulation of many vital cellular functions, such as protein and DNA synthesis. GR inhibitors are known to be agents used in the treatment of cancer and malaria. The aim of this research is to investigate the in vitro inhibition effects of pyrimidine and some derivative compounds; pyrimidine(a), 4-amino-2-chloropyrimidine(b), 4-amino-6-chloropyrimidine(c) and 4-amino-2,6-dichloropyrimidine (d) on the GR enzyme. The activity measurement of the GR enzyme was conducted according to the Carlberg and Mannervik method, and the effects of pyrimidine and its derivatives on this enzyme were examined through inhibition studies. The obtained results were used to plot Lineweaver-Burk curves were drawn for each derivative compound to examine the inhibition effects of pyrimidine derivatives on the GR enzyme. The types of inhibition and KI values were determined with the help of these graphs. KI values were found between  $0.979 \pm 0.23$  -  $2.984 \pm 0.83$   $\mu\text{M}$ . 4-amino-2,6-dichloropyrimidine (c) has been shown to be the most effective inhibitory property with its noncompetitive inhibition type. This study reports an effective protocol for pyrimidines. The inhibition effects of a total of four compounds, pyrimidine and three different derivatives, were investigated. These compounds were monitored in vitro for their inhibitory potential against the GR enzyme. It is reported that these compounds, which have potential inhibitory properties for GR, reduce the enzyme activity at low concentrations. Finally, all derivatives in this series can be evaluated as outstanding multi-target inhibitors for further investigation in the treatments of various diseases caused by free radicals.

**Keywords:** Artificial Intelligence; Biotechnology; Dentistry; Deep Learning algorithms; Diagnostics

**Abbreviations:** GR: Glutathione Reductase; NADPH:  $\beta$ -Nicotinamide adenine dinucleotide 2'-phosphate; ROS: Reactive Oxygen Species; GSH: L-Glutathione reduced; GSSG: L-Glutathione oxidized; KI: Inhibition Constant; IC50: Concentration of inhibitor that cuts half of the activity of the enzyme;  $\mu\text{M}$ : Micromolar.

## Introduction

Cellular metabolism encompasses the series of reactions necessary to facilitate the movements of living organisms. All of these reaction series occurring within the cell are referred to as metabolic pathways. Metabolic pathways are the functional units of the metabolic network present in an organism [1]. From this perspective, any disruption in a pathway leads to an increasing domino effect throughout metabolism. Nearly all metabolic reactions that occur in metabolism are catalyzed by various enzymes to ensure the rapid and efficient execution of reactions. Enzyme deficiency, inhibition, or activation affects not only the metabolic pathway performed by the enzyme but also the entire metabolic system associated with it. Therefore, studies on enzyme-drug interactions are becoming increasingly important.

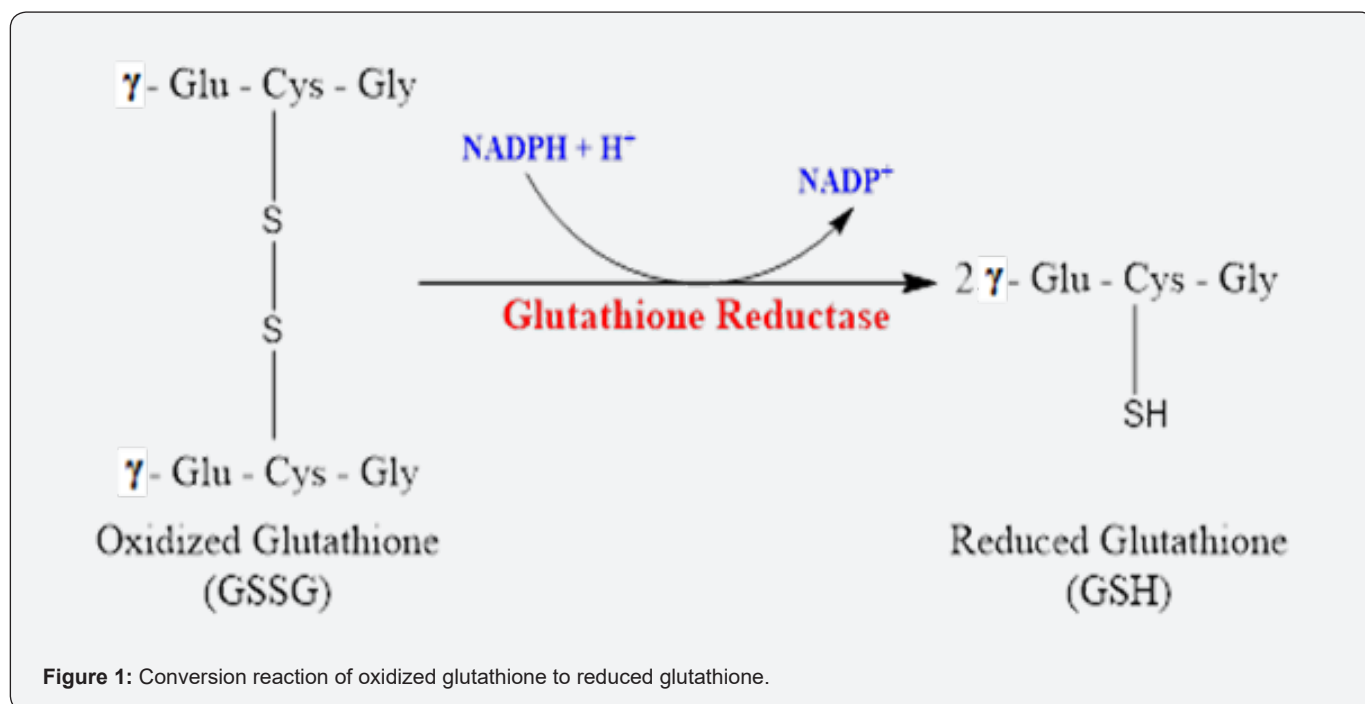
The mechanisms of drugs, chemicals, or their metabolites are carried out by affecting enzyme activities within metabolism because enzymes are directly or indirectly affected by xenobiotics [2].

Free radicals carry many electrons in molecular orbitals. Free radicals such as alkoxy, hydroxy, superoxide, peroxy, nitrogen dioxide, and nitric oxide are derived from oxygen [3]. These compounds are formed in organisms through external influences or metabolic pathways and irreversibly bind to DNA and proteins [4]. Additionally, they cause the degradation of structural coenzymes, nucleotides, and DNA in tissues and cell [5,6]. Moreover, they can covalently bind to enzymes, proteins, and lipids; disrupt cell membranes; damage transport systems;

and alter enzyme activities, leading to the emergence of various metabolic disorders [7,8].

Glutathione Reductase (GR, EC 1.6.4.2) is an important antioxidant enzyme involved in protecting the cell against oxidative stress [2]. Glutathione is believed to be a major contributor to oxidative stress-related diseases. Glutathione serves as a major regulator of intracellular redox reactions. Oxidized and reduced glutathione are the best indicators of oxidative stress.

In conditions of increased oxidative stress, reduced glutathione (GSH) decreases while oxidized glutathione (GSSG) increases. GSH is responsible for reducing reactive oxygen species (ROS) that cause oxidative damage. GR converts oxidized glutathione into its reduced form using NADPH obtained through the hexose monophosphate pathway and/or the NADP<sup>+</sup> dependent malate dehydrogenase enzyme involved in the transport of acetyl CoA from the mitochondria to the cytosol (Figure 1) [9,10].



Throughout history, cancer has remained one of the most significant causes of death worldwide, with limited progress made in reducing its causative factors. Natural products have been utilized to treat various illnesses since ancient times. As a result, numerous important anticancer agents have been derived from natural sources. These anticancer agents can be obtained directly from plants or through the modification of natural compounds, offering not only inspiring new compounds but also modifications of existing ones. Despite the discovery of many anticancer agents from natural sources, the exploration of new agents remains a necessity. This is crucial for the improvement and advancement of cancer treatment, highlighting the importance of ongoing research in this field [11].

Reactive oxygen species (ROS) are physiologically generated by aerobic cells. Their production increases during cell necrosis. Physiological levels of ROS serve as mediators in critical intracellular signaling pathways and are essential for cell survival. However, excessive ROS production can cause damage to cells and lead to cell death. It has been known for many years that oxidative stress plays a significant role in the development and progression of various cancer types. Therefore, antioxidant therapy is considered a potential avenue for cancer prevention [12].

Increased levels of GSH are observed in various tumor types, making neoplastic tissues more resistant to chemotherapy. Consequently, GSH system has become one of the most important targets in medical intervention, both in preventing cancer progression and combating chemotherapy resistance. Therefore, the goal in cancer treatment research is to reduce GSH levels by inhibiting GSH biosynthesis. Inhibition of GR leads to the accumulation of oxidized glutathione while depleting reduced glutathione. As a result, inhibition of GR, an antioxidant enzyme, has played a significant role in the development of anticancer and antimalarial drugs in recent years [13].

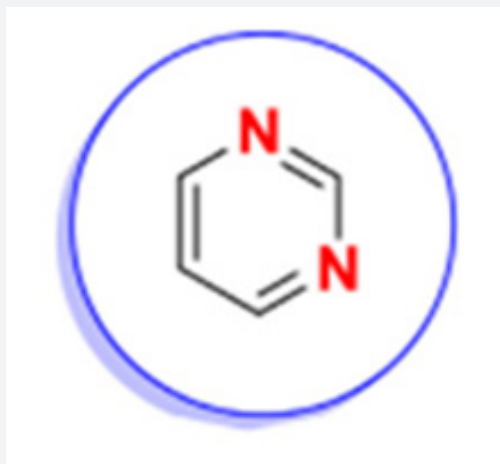
Heterocyclic compounds play a significant role in medicinal chemistry, agricultural chemicals, polymer materials, and various industrial products. Nitrogen-containing heterocycles are abundant in nature and are found in many natural products such as alkaloids, vitamin derivatives, antibiotics, and hormones. About 60% of approved drugs contain nitrogen-containing heterocycles in their structure. Pyrimidine and its analogs have garnered considerable attention due to their diverse biological activities. Pyrimidine is a crucial class of heterocyclic compounds, valued for its medicinal properties and its role as the fundamental molecule in the pyrimidine base, which comprises uracil, cytosine, and

thymine, forming the structures of DNA and RNA [14].

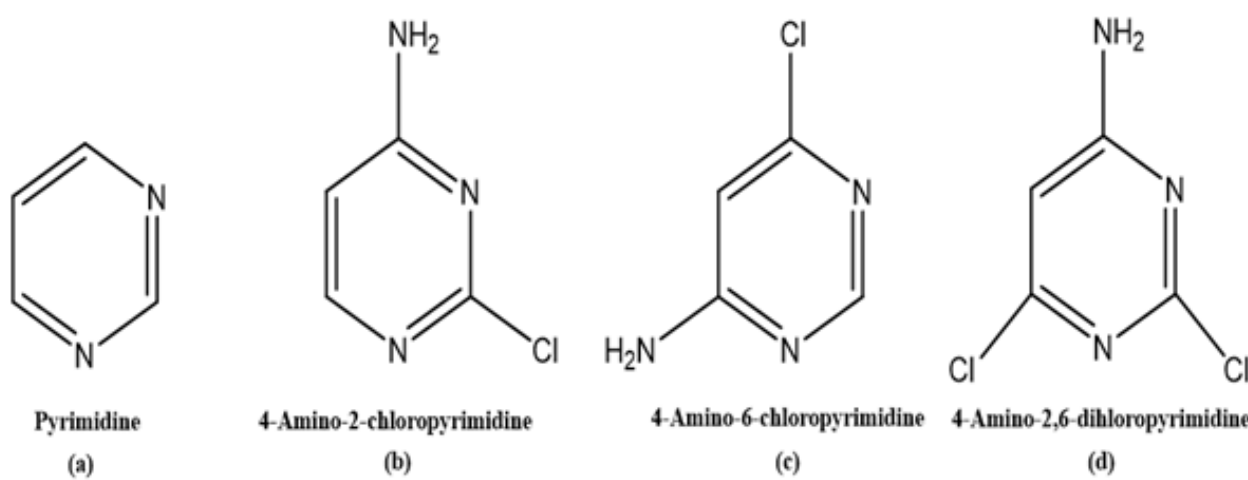
Pyrimidines are indeed vital constituents of all cells, essential for the functioning of biological organisms. These molecules, depicted in Figure 2, belong to the class of heterocyclic compounds and represent a crucial group known for their significant

pharmacological activities [15,16].

In this study, the effects of pyrimidine and some derivative compounds (Figure 3) on GR enzyme activity have been investigated.



**Figure 2:** Structure of pyrimidine.



**Figure 3:** The pyrimidine and some derivative compounds used in the inhibition study.

## Materials and Methods

### Chemicals

All chemicals used in the study were obtained from Sigma-Aldrich.

### GR Enzyme Activity Measurement

GR enzyme activity measurement was performed according to the Carlberg and Mannervik method [17].

### In vitro Inhibition Studies

The inhibitory effects of pyrimidine (a), 4-amino-2-chloropyrimidine (b), 4-amino-6-chloropyrimidine (c) and 4-amino-2,6-dichloropyrimidine (d) compounds on the GR enzyme were investigated. Enzyme activity was measured in the presence of different GSSH concentrations. The control activity was assumed to be 100% in the absence of inhibitors. Percentage activity graphs against inhibitor concentration were

plotted for each inhibitor. Subsequently, Lineweaver-Burk [18] curves for the GR enzyme were obtained using three different concentrations of pyrimidine derivative compounds and five

different concentrations of substrate (GSSG). Thus, KI values and inhibition types for each inhibitor were determined for the GR enzyme.

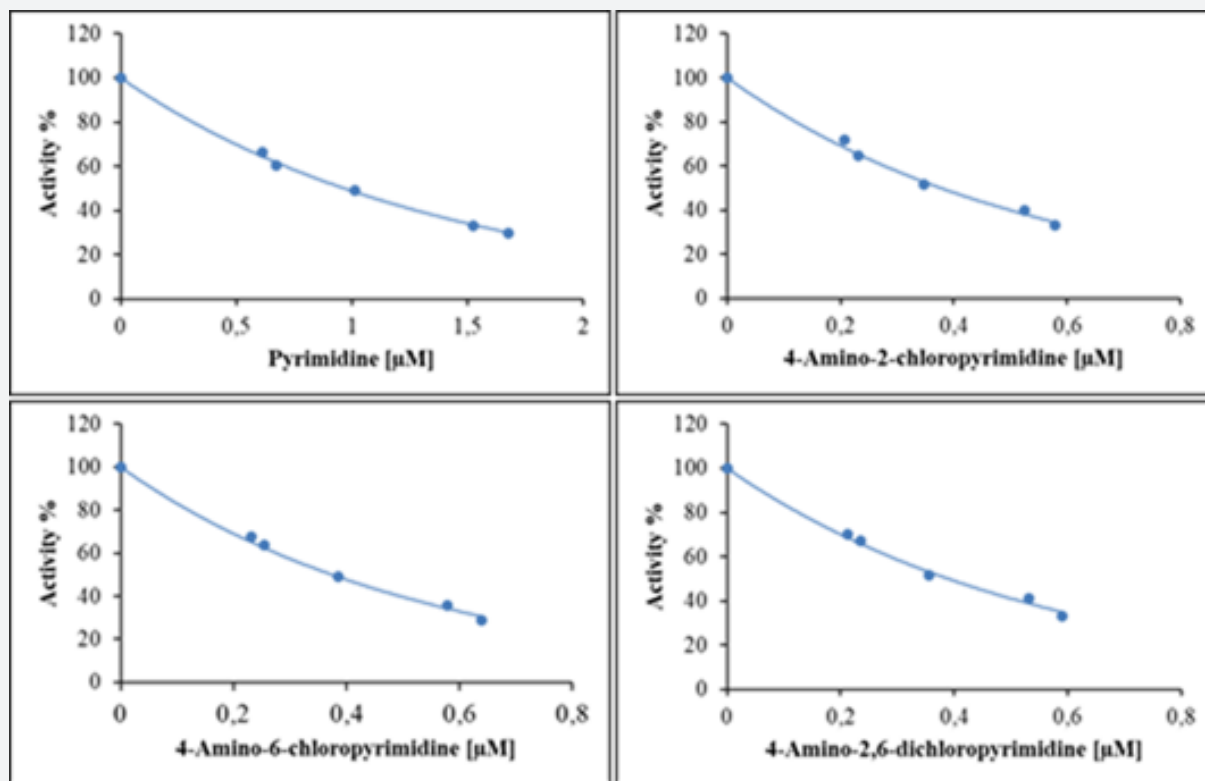


Figure 4: The IC50 graphs of pyrimidine and some derivatives on GR.

## Results

The IC50 values for pyrimidine (a), 4-amino-2-chloropyrimidine (b), 4-amino-6-chloropyrimidine (c) and 4-amino-2,6-dichloropyrimidine (d) were found to be 0.968 µM, 0.377 µM, 0.374 µM, and 0.390 µM, respectively (Figure 4). According to the results of the inhibition studies, 4-amino-2,6-dichloropyrimidine (d) (KI: 0.979 µM) exhibited a high level of inhibition on the GR enzyme.

It is observed that the amino and chloro groups present in 4-amino-2,6-dichloropyrimidine contribute to an effective inhibitory effect. Despite the chemical structures of the pyrimidine and derivative compounds being entirely the same apart from the groups and positions dependent on the ring structure, significantly different inhibition rates were observed. When comparing based on IC<sub>50</sub> values, while 4-amino-2-chloropyrimidine showed three times more inhibition than pyrimidine (a), 4-amino-6-chloropyrimidine (c) and 4-amino-2,6-dichloropyrimidine (d) exhibited similar levels of inhibition. Therefore, it can be concluded that the presence of amino and chloro groups attached

to the pyrimidine ring provides effective inhibition for the GR enzyme.

In the study, KI values were determined for each inhibitor. The KI values were found to be as follows: pyrimidine (a) (2.984 µM) > 4-amino-6-chloropyrimidine (c) (1.269 µM) > 4-amino-2-chloropyrimidine (b) (1.847 µM) > 4-amino-2,6-dichloropyrimidine (d) (0.979 µM) (Figure 5).

These compounds, showing potential inhibitor properties for GR, were reported to decrease enzyme activity at low concentrations (Table 1).

## Discussion

The excessive production of free radicals results in oxidative stress. Oxidants disrupt homeostasis in the body, leading to a significant imbalance. This condition arises from various lifestyle or disease conditions such as chronic hyperglycemia or ketoacidosis attacks, excessive nutrient intake, and sleep disorders [19].

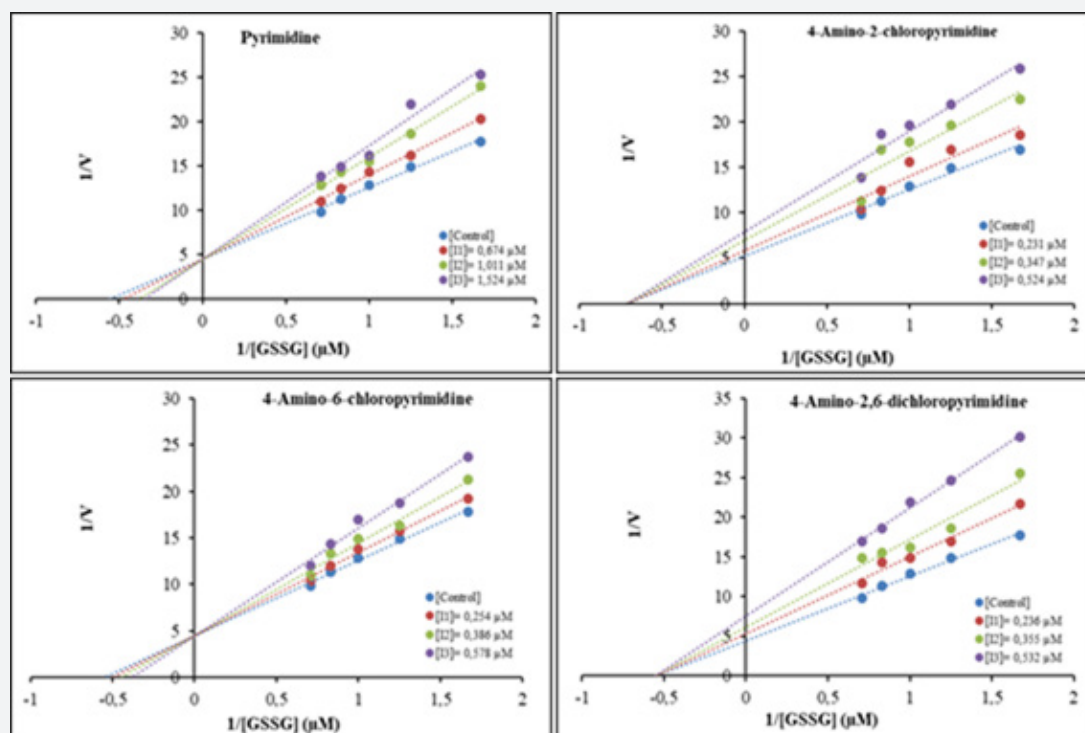


Figure 5: The Lineweaver-Burk plots of pyrimidine and some derivatives on the GR enzyme.

Table 1: IC<sub>50</sub> and K<sub>i</sub> values and inhibition types of pyrimidine derivatives on GR enzyme.

Pyrimidine and Derivatives	IC <sub>50</sub> (μM)	R <sup>2</sup>	K <sub>i</sub> (μM)	R <sup>2</sup>	Inhibition Type
Pyrimidine	0,968 ± 0,21	0.9915	2,984 ± 0,83	0.9904	Competitive
4-amino-2-chloropyrimidine	0,377 ± 0,13	0.9925	1,269 ± 0,46	0.9913	Noncompetitive
4-amino-6-chloropyrimidine	0,374 ± 0,12	0.9909	1,847 ± 0,59	0.9934	Competitive
4-amino-2,6-dichloropyrimidine	0,390 ± 0,27	0.9987	0,979 ± 0,23	0.999	Noncompetitive

The imbalance between antioxidants and oxidants affects lipids, DNA, and proteins, leading to cellular necrosis and impaired physiological function of cells. Additionally, oxidative stress is believed to play a significant role in cardiovascular diseases, as well as neurodegenerative diseases such as Alzheimer’s and epilepsy [20-22].

Glutathione reductase (GR), glutathione S-transferase (GST), catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) are a series of enzyme systems that constitute the enzymatic defense system, which are DNA repair enzymes [23]. GR catalyzes the reduction of oxidized glutathione in the presence of NADPH. GSH is utilized in preserving the thiol groups of intracellular proteins, preventing oxidative events, detoxifying xenobiotics, and neutralizing H<sub>2</sub>O<sub>2</sub> and other organic peroxides [24].

Metabolic events occurring in living organisms are associated with the catalytic activity of enzymes involved in metabolism. Changes in enzyme activities are linked to various diseases. Therefore, compounds that inhibit enzyme activity are used as beneficial therapeutic agents [25]. Hence, in this study, the in vitro inhibition effects of pyrimidine and some derivatives on GR enzyme activity have been investigated.

Various drugs and chemicals have been studied for their effects on the GR enzyme in different studies. For example, in a study conducted by Şentürk et al. [26], the GR enzyme was purified from human erythrocytes, and the inhibitory effects of some antibiotics were investigated. Sulfanilamide, rifamycin, imipenem, ceftazidime, ceftriaxone, chloramphenicol, vancomycin, ornidazole, and cefuroxime showed inhibitory effects on the enzyme, while lincomycin, amikacin, clindamycin, and amoxicillin exhibited activation effects on enzyme activity. According to this



study, imipenem showed the most potent inhibitory effect [26].

In another study, the inhibitory effects of methotrexate, dacarbazine, pantoprazole sodium, 5-fluorouracil thiocolchicoside, and olanzapine on GR activity were investigated, and dacarbazine exhibited the most potent inhibition effect on the enzyme [27]. A group of researchers has investigated the effects of Schiff base derivatives on baker's yeast GR. These compounds exhibited effective inhibitory properties [28]. In a study conducted by Demir [21], the effects of the anti-epileptic drugs phenytoin, gabapentin, and primidone on the GR enzyme were investigated. Phenytoin exhibited the most effective inhibitory property with competitive inhibition type [2]. Çalışkan et al. [29] investigated the effects of brimonidine and proparacaine on the GR enzyme. They found the KI values for brimonidine and proparacaine to be  $144.10 \pm 2.01$  and  $1,654.00 \pm 26.29$   $\mu\text{M}$ , respectively. They stated that proparacaine inhibited the GR enzyme at high concentrations [29].

With the increasing level of knowledge in fields such as medicine, genetics, and proteins, we are approaching a new era in understanding the mechanisms of various diseases, making accurate diagnoses, and developing necessary treatment approaches. This subject has been researched by scientists for many years. Currently, numerous studies have been conducted and are ongoing on antibodies, vaccines, natural interferons, and various metabolic enzymes, which are used in the diagnosis and treatment of many diseases. These products are utilized in various fields, especially in medicine [30].

### Conclusion

This study presents a highly effective protocol for pyrimidines. The inhibitory effects of pyrimidine and its derivatives on GR enzyme activity, which is known as an important target in the treatment of cancer, were investigated. According to inhibition, in studies conducted, KI values for GR were found to be in the range of  $0.979 \pm 0.23$  -  $2.984 \pm 0.83$   $\mu\text{M}$ . 4-amino-2,6-dichloropyrimidine exhibited the most effective inhibition on GR enzyme activity. The findings obtained from this study contribute significantly to the literature. This work focuses on new approaches to assessing cancer progression by GR, which may then open new, exciting drug discovery opportunities and target identification opportunities. The compounds investigated in the study and their inhibitory effects on the GR enzyme could shed light on further biological research.

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