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Investigation of *in vitro* Biological Activities of 2-((6-methylpyridin-2-yl)amino)acetic acid

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2-((6-metilpiridin-2-il)amino)asetik asid'in *in vitro* Biyolojik Aktivitesinin Araştırılması

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Abstract

Peptoids (N-substituted glycine oligomers) are a class of synthetic heteropolymers that can mimic the structures of peptides. Due to their potent biological activities, their use as drugs in the medical and health sectors is being investigated. This study investigates the in vitro biological activities and drug potential of the peptoid derivative 2-((6-methylpyridin-2-yl)amino)acetic acid. Antioxidant properties were determined by CUPRAC (Cupric Reducing Antioxidant Capacity), DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) methods. DNA protection and DNA damage analyses were performed to determine its effects on plasmid DNA. Finally, it's in vitro inhibitory activity on the enzyme activities of GST (glutathione s-transferase) and hCA I-II (human carbonic anhydrase I-II), important enzymes of various metabolic pathways, was determined. It was found that the highest antioxidant activity of 2-((6-methylpyridin-2-yl)amino) acetic acid was 0.0372±0.0018 µg trolox eq./µL by the CUPRAC method. While the compound did not cause any damage to DNA in the 0.5-1 mg/mL range, it was found to have a DNA protective effect at a concentration of 0.5 mg/mL. Besides, the best inhibitory effect of the compound was shown to be against hCA I isoenzyme.

Keywords: *Antioxidant, DNA protection, Enzyme inhibition, N-substituted amino acid*

Öz

Peptoidler (N-sübstitüe glisin oligomerleri), peptitlerin yapılarını taklit edebilen sentetik heteropolimer sınıfıdır. Güçlü biyolojik aktivitelerinden dolayı tıp ve sağlık sektöründe ilaç olarak kullanımları araştırılmaktadır. Bu çalışma, peptoid türevi 2-((6-metilpiridin-2-il)amino)asetik asidin in vitro biyolojik aktivitelerini ve ilaç potansiyelini araştırmaktadır. CUPRAC (Bakır (II) indirgeyici antioksidan kapasitesi), DPPH (2,2-difenil-1-pikrilhidrazil) ve FRAP (Demir (III) indirgeyici antioksidan kapasite yöntemleri ile antioksidan özelliği belirlendi. Plazmit DNA üzerindeki etkilerini belirlemek için DNA koruma ve DNA hasar analizleri gerçekleştirildi. Son olarak çeşitli metabolik yolakların önemli enzimleri olan GST (glutatyon s-transferaz) ve hCA I-II (human carbonic anhidraz I-II) enzim aktiviteleri üzerindeki in vitro inhibisyon aktivitesi tespit edildi. Yapılan analizler neticesinde 2-((6-metilpiridin-2-il)amino) asetik asid'in en yüksek antioksidan aktivitesi CUPRAC yöntemiyle 0,0372±0,0018 µg troloks ekv./µL olarak hesaplandı. Bileşik 0,5-1 mg/mL aralığında DNA üzerinde bir hasar oluşturmaz iken 0,5 mg/mL konsantrasyonda DNA koruma etkisine sahip olduğu belirlenmiştir. Ayrıca en iyi inhibitör etkisini hCA I izoenzime karşı göstermiştir.

Anahtar Kelimeler: *Antioksidan, Enzim İnhibisyonu, DNA koruma, N-sübstitüe amino asit*

1. Introduction

Short peptide and nucleic acid oligomers bind to a variety of molecular targets but exhibit poor pharmacokinetic properties. Therefore, peptidomimetics have been developed that approximate the polypeptide structure, retain chemical diversity and a polar backbone, and are not protease-degradable. These structures, consisting of repeating units of N-substituted glycine, are called peptoids. A peptoid is a regioisomer of a peptide (Zuckermann, 2011). In peptoids, the side chain is attached to the nitrogen atom instead of the α-carbon (Robertson et al., 2016)As a potential source of bioactive compounds, they have several advantages over peptides and many other types of peptide-like oligomers, such as increased cell permeability and greater stability against proteases and peptidases (Suwal & Kodadek, 2013). Peptoids are being studied for their use as protein ligands, antibacterial agents, and pharmacophores such as estradiol molecules. Their favorable cytotoxicity profiles make them a promising group of compounds in medicine and healthcare (Elchinger et al., 2017). In addition to these uses, they can also be used to create films or surface coatings (Yoo & Kirshenbaum, 2008)

Reactive oxygen species (ROS) are the oxygen derivatives released from the electron transport system, mostly in characterized by unpaired oxygen electrons. They include

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such free radicals as hydroxyl radicals ($^{\bullet}OH$), superoxide anions ($O_2^{\bullet-}$), alkyl peroxy radicals ($ROO^{\bullet}$), and non-radicals such as ozone (O_3), hydrogen peroxide (H_2O_2) (Xu et al., 2024). As a by product of aerobic metabolism, ROS production, coupled with ROS scavenging by cellular antioxidant mechanisms, occurs continuously in cells to prevent some of the potentially toxic effects of ROS, such as oxidation and damage to DNA, RNA, proteins, and membranes, which is called oxidative stress (Mittler, 2017). Excessive ROS can breakdown various biomolecules, such as lipids, proteins and DNA, and due to increased oxidative stress, can result in in various human diseases such as rheumatoid arthritis, neurodegenerative diseases, diabetes and cataracts, cardiovascular diseases, respiratory diseases, and the aging process (Phaniendra et al., 2015). Various active metal cations like Cu^{2+} and Fe^{2+} react with hydrogen peroxide in biological systems and produce the activating hydroxyl radical in a reaction named the Fenton reaction. DNA damage as a result of ROS groups is associated with DNA base changes or DNA double strand breaks. Therefore, it is important to find ways of inhibiting the Fenton reaction(Hashkavayi et al., 2020).

While nearly all organisms possess antioxidants and various enzyme systems to prevent oxidative damage, these systems are insufficient to eliminate damage. Therefore, antioxidant supplements or antioxidant-rich food are needed to protect against free radicals and alleviate oxidative damage (Shah & Modi, 2015). Synthetic antioxidants are also added to foods to enhance their resistance to various processes and conditions, extend shelf life, and prevent food oxidation (Carocho & Ferreira, 2013).

The process of metabolizing xenobiotics to prevent their toxic effects is called detoxification. It consists of two stages: Phase I and Phase II. The former is catalyzed by enzymes of the cytochrome P450 family. Phase II conjugation reactions include different conjugation reactions such as acetylation, methylation, and glutathione conjugation (Holm et al., 2002). Glutathione S-transferases (GSTs; EC 2.5.1.18) are a multifunctional group of phase II detoxification enzymes that protect against various electrophilic compounds such as reactive lipid peroxidation products. Eight different families of GST gene have been reported in human liver: alpha, kappa, mu, omega, pi, sigma, theta and zeta (Prabhu et al., 2004).

Carbonic anhydrases (CAs; E.C.4.2.1.1) are metalloenzymes that catalyze the reversible hydrolysis of carbon dioxide to bicarbonate and protons (Karioti et al., 2016). CAs participate in different biological processes such as acid-base balance, respiration, carbon dioxide and ion transport, bone resorption, ureogenesis, gluconeogenesis, body fluid production, and lipogenesis (Lehtonen et al., 2004). They are divided into seven groups, viz. α-, β-, γ-, δ-, η-, ζ-, and θ-CAs. Only α-CAs are found in the human body. Of the 16 CA isoenzymes, two of them are of mitochondrial origin (CA VA, VB) while five are cytosolic (CA I, II, III, VII and XIII), four are membrane-associated (CA IV, IX, XII and XIV) and the other one is secreted (secreted from saliva) (CA VI) (Taslimi et al., 2019). CA isoenzymes have important therapeutic purposes, with the potential to be activated/inhibited for the treatment of a number of disorders such as cancer, obesity, osteoporosis, epilepsy and glaucoma. Since many drug molecules are in fact enzyme inhibitors, their development and discovery is a hot topic in biochemistry and pharmacology (Caglayan & Gulcin, 2018).

In recent years, interest in biologically active pharmaceutical agents has increased due to the rise in metabolic diseases, rapid mutations of viral strains, the adverse effects of factors associated with environmental pollution on the human immune system, and the high toxicity of existing drugs. Therefore, chemists are striving to develop new synthetic drugs that exhibit high efficacy, minimal toxicity, and maximum bioavailability at low cost (Askin et al., 2021).

In this study, the antioxidant and enzyme inhibition activity of a synthesized peptoid, 2-((6-methylpyridin-2-yl)amino)acetic acid, and its DNA protection effect via the Fenton Reaction were investigated. The aim was to determine the properties of a synthetic, low-cost, low-toxic new drug candidate by investigating the biological activity of the compound. Based on the structural features of peptoids and the biological importance of the target enzymes, we hypothesized that 2-((6-methylpyridin-2-yl)amino)acetic acid would demonstrate (1) measurable antioxidant capacity, (2) inhibitory activity against human carbonic anhydrase I/II and glutathione S-transferase enzymes, and (3) a protective effect against oxidative DNA damage *in vitro*. This study was designed to test this hypothesis and evaluate the compound's potential as a lead for further drug development.

2. Materials and Methods

2.1. Chemicals and reagents

All reagents and solvents were purchased from Sigma (Sigma-Aldrich, Germany). The 2-((6-methylpyridin-2-yl)amino)acetic acid compound was previously synthesized and characterized in studies conducted by Çalışır et al. (Figure 1) (Çalışır, 2025; Çalışır & Çiçek, 2023).

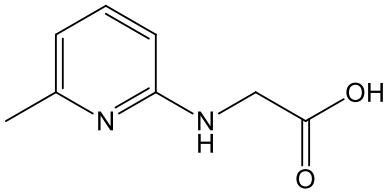


Figure 1 Structure of the 2-((6-methylpyridin-2-yl)amino)acetic acid compound

2.2. Dissolution of 2-((6-methylpyridin-2-yl)amino)acetic acid compound

The 2-((6-methylpyridin-2-yl)amino)acetic acid compound was dissolved in dimethyl sulfoxide (DMSO) to obtain a stock solution at a concentration of 1 mg/mL and subsequently diluted with distilled water to achieve the desired working concentrations.

2.3. Antioxidant studies

In this study, antioxidant analyses were performed to evaluate the antioxidant capacity of the samples. For this purpose, the stock solution was first dissolved in DMSO and then diluted with ultrapure water to obtain a final concentration of 1 mg/mL for use in the analyses. All experiments were carried out in triplicate to ensure the reliability of the results.

2.3.1. Antioxidant Activity Assay by CUPRAC Method

The method established by Apak et al. (2007) (Apak et al., 2007) is used with modifications. This method is based on the reduction of Cu^{2+} to Cu^{+} by antioxidants (reductants) present in the medium. Trolox solution is prepared at a concentration of 0.2 mg/mL as a standard. After a 20-minute incubation, the absorbance values of the samples and standards are measured at a wavelength of 450 nm. A graph of the absorbance of the standard versus concentration was plotted. The antioxidant activity of the sample is determined in terms of Trolox equivalents and as % CUPRAC reducing activity based on the plotted graph.

2.3.2. Antioxidant Activity Assay by DPPH Method

The method proposed by Benvenuti et al. (2004) was used to determine antioxidant activity using the DPPH method (Benvenuti et al., 2004). The method is based on the reduction of DPPH radical, a stable purple compound, as a result of its reaction with antioxidant substances present in the medium. Trolox (0.2 µg/µL) was prepared as a standard. Into the reaction medium, 60 µL of ethanol, 70 µL DPPH (0.5 mM), and 70 µL of sample were pipetted. After a 30-minute incubation in the dark, the absorbance values of the samples and standards are measured at a wavelength of 517 nm. The absorbance graph of the standard versus concentration was plotted. The antioxidant activity results of the samples were determined in terms of Trolox equivalents and as % DPPH removal according to the plotted graph (1).

$$\%\ DPPH\ Removal = \left[\frac{Abs(control) - Abs(sample)}{Abs\ (control)}\right] x100 \qquad (1)$$

Abs$_{(Control)}$: Control absorbance value
Abs$_{(Sample)}$: Sample/standard absorbance value

2.3.3. Antioxidant Activity Assay by FRAP Method

With the FRAP method, antioxidant activity is determined as the reducing power of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) (Benzie, 1996; Benzie & Szeto, 1999). In this method, the Fe^{3+} tripridyltriazine (Fe^{3+}–TPTZ) complex in a low pH environment is reduced to the Fe^{2+}-tripridiltriazin (Fe^{2+} –TPTZ) complex by the effect of antioxidants. The absorbance of this formed complex at 593 nm is measured and the total antioxidant capacity of the sample is determined. 0.3 M Na-Acetate buffer (pH: 3.6), 20 mM $FeCl_3$ and FRAP reagent are added to the reaction medium and the absorbance values are measured at 593 nm wavelength after pipetting and incubation for 10 min. FRAP reagent is prepared by Na-Acetate buffer (0.3 M, 10 volume), TPTZ (10 mM, 1 volume), and $FeCl_3.6H_2O$ solution (20 mM, 1 volume). The absorbance graph of the standard versus concentration is plotted, and the antioxidant activity of the sample is determined in terms of Trolox equivalents and % FRAP Reducing Activity according to the plotted graph (2).

$$\%\ Reduction\ Power = \left[\frac{Abs(sample) - Abs(control)}{Abs\ (sample)}\right] x100 \quad (2)$$

2.4. Enzymatic Studies

2.4.1. Purification of human hCA I and II isoenzymes and GST enzyme

The human erythrocyte carbonic anhydrase isoenzymes (CA I and CA II) used in this study and the glutathione S-transferase (GST) enzyme isolated from Shabut (*Barbus grypus*) fish liver were obtained and characterized in previous studies conducted by us (Camadan et al., 2024; Çiftçi et al., 2025). The enzymes in question were prepared in accordance with the isolation and purification protocols defined in the relevant study and were used in experimental analyzes in the current study.

2.4.2. Determination of inhibitory activities of 2-((6-methylpyridin-2-yl)amino)acetic acid on enzymes

The activities of hCA I and II isozymes were determined according to Verpoorte et al. (1967) (Verpoorte et al., 1967). In this method, 4-nitrophenylacetate (NPA) is converted to 4-nitrophenolate ion and the absorbance of the product formed is read at 348 nm. Reaction medium containing no enzyme solution is used as a blank. The GST

enzyme catalyzes the conjugation of a glutathione molecule with an aromatic electrophile. The most commonly used aromatic electrophile is CDNB (1-chloro-2,4-dinitrobenzene). Using this substrate, dinitrobenzene 5-glutathione (DNB-SG) is formed and the absorbance is read at 340 nm. In our studies, this activity measurement reaction medium contains 0.1 M KH_2PO_4 (pH: 6.5), 1 mM GSH, 0.5 mM CDNB and enzyme solution (Aksoy et al., 2016). The IC_{50} values of the enzymes were measured using five different inhibitor concentrations. Inhibitor free medium was called control and its activity was considered as 100%. By plotting the % activity against enzyme concentration, and IC_{50} values was calculated (Akkemik et al., 2019; Smirnovienė et al., 2017).

2.5. DNA Protection/Fragmentation Assay by Fenton Reaction

To establish the damaging potency of Fenton's solution and the potential defensive capacity of 2-((6-methylpyridin-2-yl)amino)acetic acid on DNA conformation was to utilized by pBR322 plasmid DNA. 2-((6-methylpyridin-2-yl)amino)acetic acid's two different concentrations as 1 and 0.5 mg/mL, negative (only plasmid DNA) and positive control (Fenton + plasmid DNA) were added in the reaction medium (Akkemik et al., 2022; Li et al., 2002). Accordingly, the reaction mixtures to be incubated were prepared as 3 µL of pBR322 plasmid DNA, 5 µL of Fenton's solution (30 mM H_2O_2, 50 mM ascorbic acid, and 80 mM $FeCl_3$) and 5 µL of 2-((6-methylpyridin-2-yl)amino)acetic acid at different concentrations, and the final volume was made up to 20 µL with distilled water. Then, this mixture was incubated at 37°C for 30 minutes. After incubation, 5 µL of 6x loading dye was introduced to the reaction mixture. Ten µL of the mixture was loaded onto a agarose gel (0.8%) prepared with EtBr. The agarose gel was run at 100 volts for 60 minutes. Gel images were then taken with a gel imager.

3. Results and Discussion

The drug potential of 2-((6-methylpyridin-2-yl)amino)acetic acid was investigated by evaluating its enzyme inhibition, antioxidant, and DNA protective activities. GST, hCA I, and hCA II isoenzymes were examined to determine enzyme inhibition activity. Antioxidant activity was assessed using DPPH, FRAP, and CUPRAC assays.

3.1. Enzyme inhibition assay

Five different concentrations of 2-((6-methylpyridin-2-yl)amino)acetic acid were used to determine its effects on GST, hCA I and II isoenzymes and the results are shown in Table 1. The concentration range of 2-((6-methylpyridin-2-yl)amino)acetic acid was determined as 0.001–0.5 µg/mL for GST, 0.005–0.45 µg/mL for hCA I, and 0.05–0.4 µg/mL for hCA II. IC_{50} values were calculated as 0.2208 (mg/mL) for GST and 0.3420 (mg/mL) for hCA I isoenzyme. While it showed an inhibitory effect on hCA I isoenzyme, no regular inhibitory effect was observed on hCA II isoenzyme. In the study conducted by Adem in 2019, it was determined that the hCA I value of the 3'-O-methylhypolaetin 7-O-[6'''-O-acetyl-β-D-allopyranosyl]-6''-O-acetyl-β-D-glucopyranoside compound was 0.1725 mM, while the hCA II inhibition could not be determined (Adem et al., 2019). hCA I and hCA II inhibitions were investigated in different hybrid molecule derivatives containing 1,2,4-triazole and thiazole rings. It was found that hCA I values ranged from 0.135 to 0.523 mM, and some had no effect on hCA II (Camadan et al., 2024). CA inhibitors are widely used in medical and biotechnological applications.

Table 1. The half-maximal inhibition concentration (IC_{50}; mg/mL) of 2-((6-methylpyridin-2-yl)amino)acetic acid against carbonic anhydrase I-II and GST enzymes.

2-((6-methylpyridin-2-yl)amino)acetic acid		
	IC_{50} (mg/mL)	R^{2**}
hCA I	0.3420	0.865
hCA II	ND*	ND*
GST	0.2208	0.916

***ND (Not detected)**

****Coefficient of Determination (R^2)**; Indicates the goodness-of-fit of the linear regression model used to estimate the IC_{50} value. Values closer to 1 signify a stronger linear relationship between inhibitor concentration and enzyme activity, confirming the reliability of the calculated IC_{50}.

CA I inhibitors are used in the treatment of glaucoma, altitude sickness, and epilepsy, while CA II inhibitors are used in the treatment of diseases such as glaucoma, cerebral ischemia, and seizures (Kırboğa & Işık, 2024). Because existing CA inhibitors bind nonspecifically to the enzyme, they can cause undesirable side effects such as nausea and fatigue as a result of off-target inhibition. Since CA II is physiologically the most abundant isoenzyme, it is generally considered an off-target isoenzyme whose inhibition should be avoided. Development of new isoform-specific inhibitors is thought to eliminate these side effects (Lomelino et al., 2016). GSTs facilitate the binding of glutathione to various endogenous and exogenous electrophilic molecules. Overexpression of GSTs have been shown in many different human cancer cell lines. Resistance to many anticancer chemotherapeutic drugs has been found to be directly associated with GST overexpression. Therefore, finding new GST inhibitors to prevent cell resistance to anticancer drugs appears to be important (Wang et al., 2019). Since the inhibitory effect of the compound used in this study was observed to be greater on the hCA I

isoenzyme, it may be considered a candidate for an isoform-specific inhibitor at the concentrations studied. Furthermore, because it also inhibits the GST enzyme, it has promising potential as a compound that can be used to reduce resistance to anticancer drugs.

3.2. Antioxidant Activity

Antioxidant activity analysis was performed using three different methods. By plotting standard regression curves using Trolox as a standard (Figures 2,3 and 4) , the synthesized compound was found to exhibit antioxidant activity according to all three methods. The IC_{50} values and radical scavenging capacities of the compound are shown in Table 2. The highest antioxidant activity was calculated as 0.0372±0.0018 µg Trolox eq./µL by the CUPRAC method. Literature data indicate that the presence of carboxyl groups in natural compounds is responsible for the antioxidant effects of natural compounds (Elchinger et al., 2017). The presence of a carboxyl group in the synthesized compound may also explain the antioxidant activity.

Table 2. IC_{50} values (µg /µL) and % radical scavenging capacity of CUPRAC, DPPH and FRAP

	2-((6-methylpyridin-2-yl)amino)acetic acid		
	IC_{50} (µg /µL)	Reducing Antioxidant Capacity	
	µg Trolox eq./µL± sd	Trolox	Compound
CUPRAC	0.0372±0.0018	97.81%	92.81%
DPPH	0.0131±0.0010	97.54%	41.82%
FRAP	0.0050±0.0001	89.22%	50.39%

3.3. DNA Protection Activity

As shown in Figure 5, the DNA protection activity of the tested compound against oxidative damage generated by the Fenton reagent was evaluated using a plasmid DNA nicking assay. The agarose gel electrophoresis image and the corresponding quantitative analysis were interpreted based on the proportion of Form I (supercoiled DNA) and Form II (open circular DNA) structures. In the negative control (Lane 5) containing only pBR322 DNA, the plasmid DNA was predominantly observed in the supercoiled Form I structure, indicating that the DNA remained intact and confirming that the experimental conditions did not cause spontaneous DNA damage. In contrast, the positive control (Lane 6) containing DNA and Fenton reagent showed a clear conversion of supercoiled DNA to the open circular Form II structure due to oxidative stress. The quantitative analysis indicated that the Form I ratio decreased to 38.93%, while the Form II ratio increased to 71.78%, confirming that the Fenton system induced significant oxidative damage to the plasmid DNA. The protective effect of 2-((6-methylpyridin-2-yl)amino)acetic acid was also evaluated. At a concentration of 1.0 mg/mL (Lane 3), the percentage of Form I DNA was 51.71%, suggesting a moderate protective effect against Fenton-induced oxidative DNA damage. However, at a lower concentration of 0.5 mg/mL (Lane 4), the proportion of Form I DNA decreased to 34.38%, while the amount of Form II increased, indicating that the DNA protection capacity decreased with lower compound concentration.

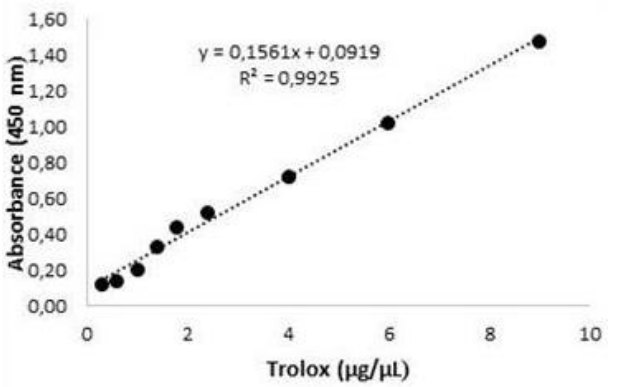


Figure 2. The standard curve of Trolox (0.1-9 µg/µL) for CUPRAC

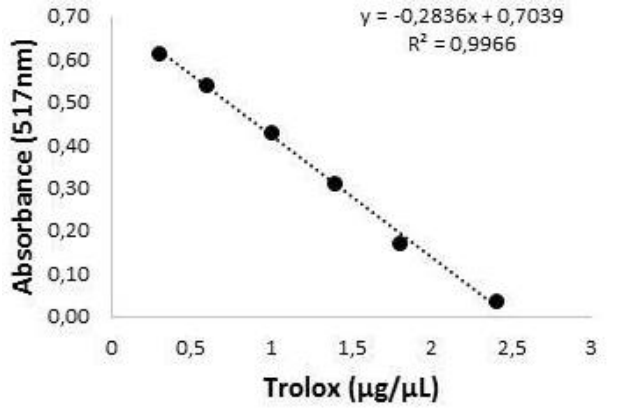


Figure 3. The standard curve of Trolox (0.25-2.5 µg/µL) for DPPH

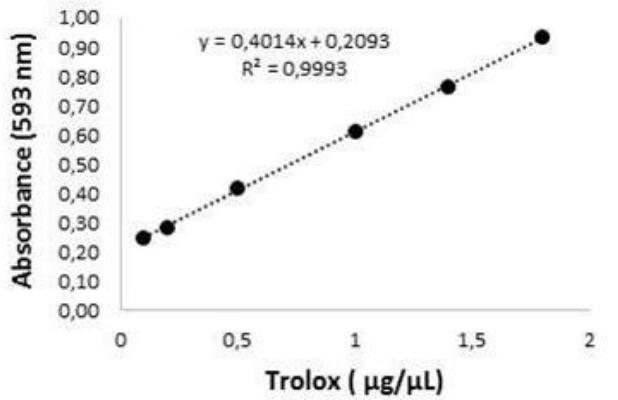
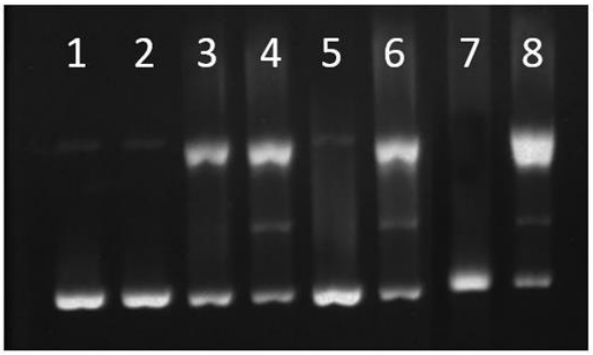
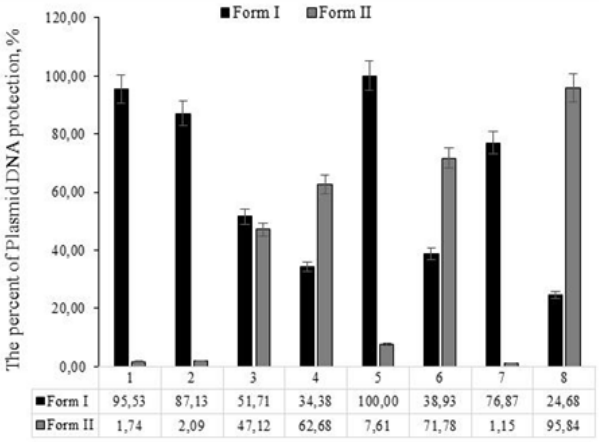


Figure 4. The standard curve of Trolox (0.25-1.75 µg/µL) for FRAP

The results obtained with the standard antioxidant, gallic acid, demonstrated its protective potential. In the DNA + standard group (Lane 7), the Form I ratio was 76.87%, indicating that gallic acid effectively preserved the

supercoiled structure of plasmid DNA. In the DNA + Fenton+standard group (Lane 8), the Form I ratio decreased to 24.68%, while Form II increased to 95.84%, suggesting that although gallic acid exhibited antioxidant activity, it was not able to completely prevent the oxidative damage generated by the Fenton system. Overall, the gel electrophoresis results and quantitative analysis indicate that the tested compound exhibited a moderate protective effect against Fenton-induced oxidative DNA damage, although its activity was hidher than that of the standard antioxidant, gallic acid. In addition, the results suggest that the DNA protection effect of the compound is concentration-dependent.





	1	2	3	4	5	6	7	8
Form I	95,53	87,13	51,71	34,38	100,00	38,93	76,87	24,68
Form II	1,74	2,09	47,12	62,68	7,61	71,78	1,15	95,84

Figure 5. DNA protection gel image. 1. DNA+ 2-((6-methylpyridin-2-yl)amino)acetic acid (1.0 mg/mL), 2. DNA+2-((6-methylpyridin-2-yl)amino)acetic acid (0.5 mg/mL), 3. DNA+Fenton Reagent+ 2-((6-methylpyridin-2-yl)amino)acetic acid (1.0 mg/mL), 4. DNA+Fenton Reagent+ 2-((6-methylpyridin-2-yl)amino)acetic acid (0.5 mg/mL), 5. pBR322 DNA (negative control), 6. DNA+Fenton Reagent (positive control), 7. DNA + std (1 mg/mL), 8. DNA+Fenton Reagent + std (1 mg/mL). The percent of Plasmid DNA protection, % (The negative control was taken as 100%, and the percentages of the other samples were calculated relative to this value. The results were interpreted with respect to Form I.)

4. Conclusions

Peptoids (N-substituted polyglycines) are a new class of synthetic biopolymers. They have become attractive in drug design applications because they are easily and inexpensively synthesized, chemically stable, and possess highly variable side chains. In this study, the antioxidant activity, hCAI-II and GST inhibitory effects, and DNA protection activity of a new peptoid compound, 2-((6-methylpyridin-2-yl)amino)acetic acid, were investigated. While the compound was inactive against hCA II, it exhibited low activity against GST and relatively high activity against hCA I. DNA protection activity was observed. Its antioxidant activity, although not very high, was also revealed. It was determined that the concentration range in which the compound exhibits biological activity is not suitable for drug development. It is preferable for the investigated compounds to exhibit biological activity at low concentrations, such as micromolar or nanomolar levels. The primary reason for this preference is that compounds administered at high doses tend to lose the specificity of their inhibitory and mechanistic actions, leading to the simultaneous modulation of multiple biological pathways. This lack of selectivity may result in inhibition of off-target mechanisms and the emergence of undesirable side effects. In the current drug development process, only approximately one out of every 10,000 chemical compounds ultimately reach patients as a clinically approved drug. Despite this extremely low success rate, tens of thousands of compounds continue to be subjected to biological, biochemical, and pharmacological evaluation in order to identify a single effective and safe therapeutic agent, and such efforts are intensively pursued by researchers. In line with the methods applied within the scope of the study, it can be said that the samples examined are not toxic; However, toxicity tests need to be performed in order to reach a definitive conclusion. The compound holds promise for potential drug candidate status by adding different groups to enhance its activity. These results suggest that the compound cannot currently be considered a strong drug candidate. Instead, the findings should be interpreted as preliminary evidence indicating that this structure may serve as a basic scaffold for future structural optimization. Further chemical modifications and structure–activity relationship (SAR) studies will be necessary to improve the inhibitory potency and evaluate its potential for drug development

Declaration of Ethical Standards

This article is the full-length version of the study entitled "Investigation of the Drug Potential of N-(6-Methylpyridin-2-Yl) Glycine by Examining Its Biological Activities," which was presented as an oral presentation at the 12th International Drug Chemistry Congress held in Antalya, Türkiye, on March 7–10, 2024, and published in the congress abstract book.

The authors declare that they comply with all ethical standards.

Credit Authorship Contribution Statement

Author-1:Methodology / Study design, Software
Author-2:Methodology / Study design, Software
Author-3:Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review and editing, Visualization, Supervision
Author-4:Methodology, Software, Validation, Investigation, Resources, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

The authors declare that the main data supporting the findings of this work are available within the article.
The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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