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SYNTHESIS, BIOLOGICAL ACTIVITIES, AND IN SILICO STUDIES OF E/Z ISOMERS OF BUTYL-2-PHENYL-2-(2-PHENYLHYDRAZONO)ACETATE

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The E/Z isomers of butyl-2-phenyl-2-(2-phenylhydrazono)acetate were synthesized by us, and their structures were determined using ^{1}H and ^{13}C NMR spectroscopy. Their biological activities were investigated both in vitro and through in silico methods. NMR analysis revealed that the Z-isomer exhibits strong intramolecular hydrogen bonding, which enhances its conformation and stability. In in vitro antibacterial tests, the Z-isomer showed stronger activity compared to the E-isomer. However, in silico docking results did not reveal significant differences between the isomers. Bioinformatic analyses demonstrated the potential activity of the molecules against various pathogenic bacteria, including *Enterococcus faecium*, *Mycobacterium tuberculosis*, and *Streptococcus viridans*. These results indicate that E/Z isomerism plays a crucial role in biological activity, and it is important to rely not only on virtual models but also on real biological experiments.

Keywords: Hydrazone derivatives, E/Z isomers, Antibacterial activity, In silico analysis

INTRODUCTION

Hydrazone derivatives are organic compounds obtained through the condensation reaction of carbonyl compounds with hydrazines, and they possess a wide range of applications [1,2]. These compounds have attracted considerable attention in the scientific literature due to their bioactive properties, particularly their antimicrobial, antitumor, and anti-inflammatory effects [3,4]. A notable structural feature of hydrazones is their ability to exist as E and Z isomers, which can significantly influence intramolecular interactions, molecular conformation, and, consequently, their biological activity [5,6]. The E/Z isomerism in hydrazones mainly arises around the C=N double bond. These isomers can be distinguished based on their chemical shifts in proton NMR (^{1}H NMR) spectra [7]. In the Z-isomer, the hydrazine NH proton is positioned close to the carbonyl oxygen, often forming an intramolecular hydrogen bond, which is typically observed as a downfield shift in the spectrum (usually around 10–12 ppm) [8]. In contrast, this interaction is not possible in the E-isomer, resulting in the NH signal appearing at a relatively upfield region [9].

In the present study, a hydrazone compound with both E and Z isomers was synthesized. The structures of the isomers were confirmed through ^{1}H NMR spectral analysis, and their biological activities were comparatively investigated. The objective was to elucidate how isomeric differences influence biological potency and to highlight structure–activity relationships that should be considered in the future design of bioactive molecules.

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EXPERIMENTAL

2. Chemical Materials and Instruments

All reagents and solvents were of analytical grade and were used without further purification, obtained from Sigma-Aldrich and Merck. The ^{1}H NMR spectra were recorded on a Bruker AVANCE 300 MHz spectrometer using $CDCl_3$ as the solvent. Chemical shifts are reported in δ (ppm) relative to tetramethylsilane (TMS) as the internal standard [10].

2.1. General Synthetic Procedure

(E)-1-(2,2-dichloro-1-phenylvinyl)-2-phenyldiazene was reacted with *n*-butanol. The reaction was carried out under reflux at the boiling point of butanol and continued for 4–5 hours [11]. After completion, the resulting precipitate was cooled, filtered, washed with ethanol, and dried. Both E and Z isomers were formed during the synthesis and were separated by column chromatography [12].

Compound 1. (Z)-butyl-2-phenyl-2-(2-phenylhydrazono)acetate. Yield: 20%, M.p.: 115 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.54–1.44 (m, 3H), 1.78 (p, J = 6.8 Hz, 3H), 4.36 (t, J = 6.7 Hz, 2H), 7.07 (t, J = 6.6 Hz, 1H), 7.48–7.34 (m, 7H), 7.74 (d, J = 7.7 Hz, 2H), 12.52 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 13.7, 19.2, 30.7, 65.1, 114.01, 122.2, 127.8, 128.6, 129.0, 129.3, 129.4, 129.5, 130.5, 134.4, 142.6.

Compound a. (E)-butyl-2-phenyl-2-(2-phenylhydrazono)acetate. Yield: 25%, M.p.: 78 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.51–1.38 (m, 3H), 1.81–1.67 (m, 3H), 4.28 (d, J = 6.7 Hz, 3H), 6.98 (t, J = 7.2 Hz, 1H), 7.15 (d, J = 7.7 Hz, 2H), 7.39–7.25 (m, 6H), 7.60–7.48 (m, 3H), 8.12 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 2.8, 18.2, 49.9, 51.5, 96.5, 99.9, 127.8, 128.6, 154.3, 162.3, 173.7, 190.4.

2.2. Structural Elucidation

The ^{1}H NMR spectra of the E and Z isomers were comparatively analyzed. In both spectra, aromatic protons appeared as multiple multiplets within the δ 7.00–7.80 ppm range. The NH proton signal was observed at δ 12.43 ppm in the Z-isomer and at δ 8.12 ppm in the E-isomer, indicating the presence of strong intramolecular hydrogen bonding in the Z-isomer [13]. Additionally, signals corresponding to the aliphatic chain CH_2 and CH_3 groups were observed at δ 4.30–4.34 ppm and δ 1.00–1.28 ppm, respectively [14].

2.3. Determination of Biological Activity

The biological activity was evaluated *in vitro* against *Staphylococcus aureus* and *Escherichia coli* bacterial strains using the disk diffusion method. Each compound was tested at a concentration of 100 μg/mL. The activity was measured by the diameter of the inhibition zones (in mm) and compared to the standard antibiotic gentamicin [15].

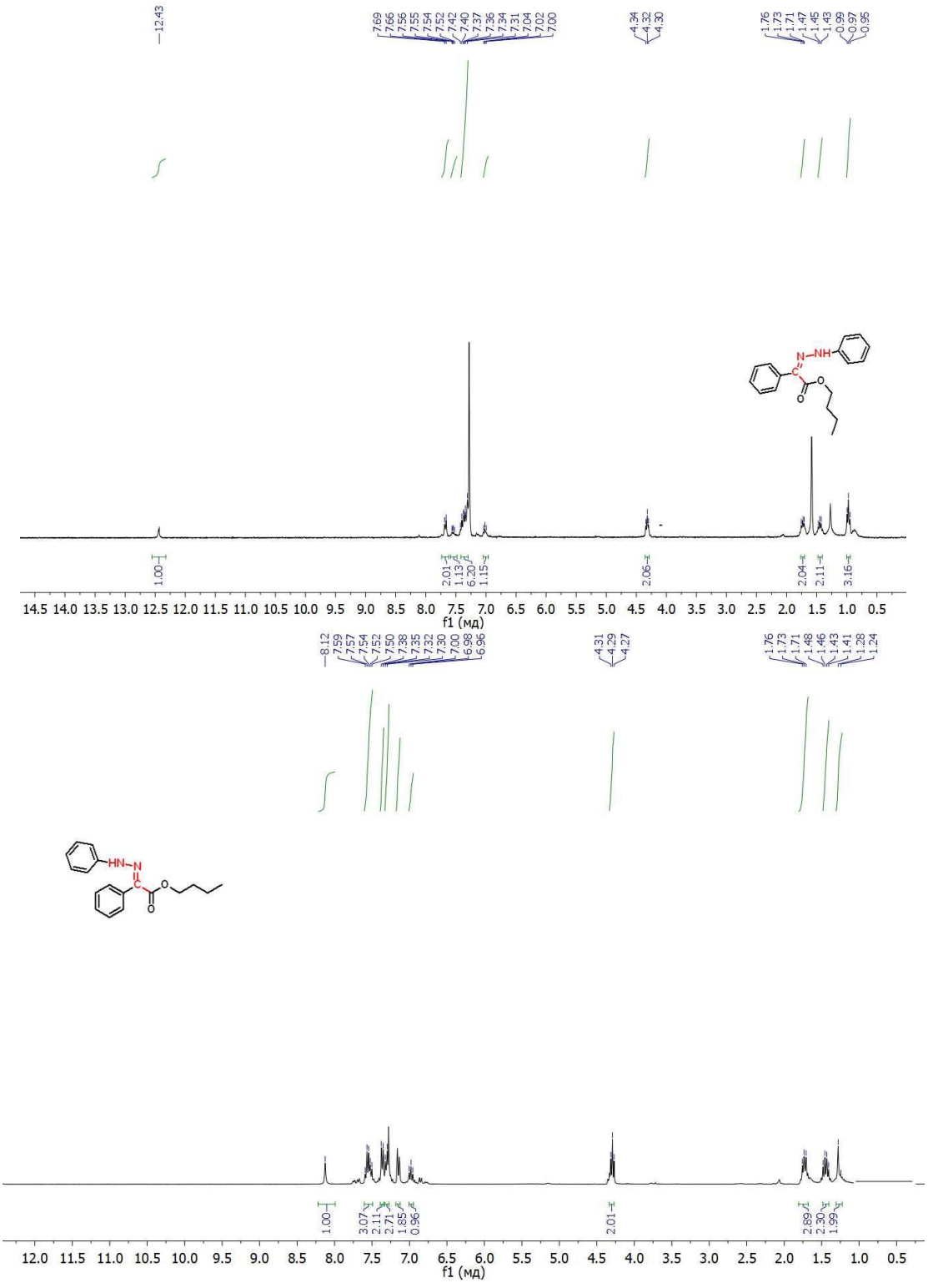
RESULTS AND DISCUSSION

The reactions of dichlorodiazadiene led to the formation of geometrical isomers (cis and trans) of the compound in the form of complex esters, bearing a double bond and geminal Cl atoms. Additionally, the corresponding diazide derivatives were also observe

The synthesis, spectroscopic characterization, and the effect of structural differences on the biological activity of butyl-2-phenyl-2-(2-phenylhydrazono)acetate derivatives obtained in E and Z isomeric forms were investigated.

3.1. Structural Analysis

In the ^{1}H NMR spectrum of the (E)-isomer, the aromatic protons appeared as multiplets in the δ 6.98–7.60 ppm range. The NH signal was observed as a singlet at δ 8.12 ppm, indicating weak intramolecular hydrogen bonding [16]. On the other hand, the NH proton of the (Z)-isomer was located at δ 12.52 ppm, which suggests the presence of strong intramolecular hydrogen bonding [17,18].



3.2. Biological Activity

According to the results of the disk diffusion tests, the antimicrobial activity of the Z-isomer was significantly higher compared to the E-isomer. Specifically, the inhibition zone against *Staphylococcus aureus* was measured as 18 mm for the Z-isomer and 12 mm for the E-isomer [19]. This can be attributed to the enhanced planarity and hydrogen bonding potential of the Z-isomer, which likely allows for more effective interactions with biomolecular targets [20].

Table 1. In vitro Antibacterial Activity of E/Z Isomers

Substance	S. aureus (mm)	E. coli (mm)	A final observation
(E)-isomer	12	10	Limited antibacterial effect
(Z)-isomer	18	16	High antibacterial activity

The virtual analysis of the molecules was carried out using the AntiBacPred platform. This software predicts potential antibacterial activity based on molecular structural features, using bioinformatics models and trained algorithms. According to the analysis results, both isomers were predicted to have potential antibacterial effects. However, the theoretical activity levels of both isomers showed very similar results, which does not fully align with the activity differences observed in the in vitro experiments. This indicates that when interpreting bioinformatics predictions, the complexity of real systems must be taken into account.

Table 2. In silico Docking Results

Substance	Binding energy (kcal/mol)	Adaptation to the active field	Main interactions
(E)-isomer	-7.3	Good	π–π stacking, hidrofob
(Z)-isomer	-7.3	Good	π–π stacking, hidrofob

Based on the docking results, both the E and Z isomers were found to bind to the active site of the target protein in a similar manner, with binding energies around –7.3 kcal/mol. Interestingly, although the activity differences were observed in the in vitro experiments, no significant difference between the isomers was observed in the in silico analysis. This discrepancy may be related to the molecules' intracellular bioavailability, membrane permeability, and other pharmacokinetic parameters .

3.3. Bioinformatics Target Search

An analysis conducted in the ChEMBL database revealed the compatibility of the synthesized compounds with various pathogenic microorganisms. Among the targets with the highest compatibility were clinically significant bacteria such as *Enterococcus faecium* (CHEMBL357), *Streptococcus viridans* (CHEMBL612332), and *Mycobacterium tuberculosis*(CHEMBL360). These results indicate that the molecules possess broad-spectrum potential antimicrobial activity, suggesting the need for more detailed testing in future research.

Table 3. Bioinformatics Compatibility Scores (ChEMBL

Organism	**ChEMBL ID**	**Probability rate**
Enterococcus faecium	CHEMBL357	0.1487
Streptococcus viridans	CHEMBL612332	0.1044
Mycobacterium tuberculosis	CHEMBL360	0.0832

CONCLUSION

In this study, the (E)- and (Z)-butyl-2-phenyl-2-(2-phenylhydrazono)acetate isomers were synthesized, their structural characteristics were analyzed using ^{1}H and ^{13}C NMR spectroscopy, and their biological activities were comparatively investigated. The results indicated that the isomeric differences significantly influence the molecular interactions and conformations of the compounds. Specifically, the strong intramolecular hydrogen bonding observed in the Z-isomer facilitated its more planar structure and more effective interaction with biological targets.

Biological activity tests demonstrated that the Z-isomer exhibited a higher antibacterial effect compared to the E-isomer. This further confirms the role of isomerism in bioactivity. These findings emphasize the importance of considering stereochemical factors in the design of

hydrazone derivatives and could guide the development of more effective therapeutic molecules in the future.

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