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Synthesis, Spectral Characteristics, and Molecular Docking Studies of 2,4-Dichloro-*N*-(2,2,2-trichloro-1-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)amino)ethyl)benzamide †

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Abstract: Derivatives of 1,3,4-thiadiazole are of great interest for scientific and practical human activities as biologically active substances, dyes, components for creating semiconductors, energy accumulators, liquid crystals, polymers, nanomaterials, etc. Here we report the synthesis of 2,4-dichloro-*N*-(2,2,2-trichloro-1-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)amino)ethyl)benzamide based on *N*,*N*′-disubstituted hydrazinecarbothioamide—2,4-dichloro-*N*-(2,2,2-trichloro-1-(2-(phenylcarbamothioyl)-hydrazine-1-carbothioamido)ethyl)benzamide. The method for obtaining the target product is based on the dehydrosulfurization reaction of the starting hydrazinecarbothioamide under the action of a mixture of iodine and triethylamine in a DMF medium. A new derivative of 1,3,4-thiadiazole was obtained in 84% yield, and its structure was confirmed by ^{1}H and ^{13}C NMR spectroscopy data. Molecular docking studies were carried out with the structure of the resulting compound and dihydrofolate reductase (DHFR) in the AutoDock Vina program. The resulting compound is a potential inhibitor of DHFR and surpasses several known analogues in terms of the strength of the complex formed with the active site of this enzyme.

Keywords: synthesis; 1,3,4-thiadiazole; dehydrosulfurization; dihydrofolate reductase; molecular docking

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1. Introduction

Derivatives of 1,3,4-thiadiazole are widely used in medicine, agriculture, materials science, and other areas of science and technology [1]. These compounds are of particular interest as biologically active compounds [2]. Among the derivatives of 1,3,4-thiadiazole, substances with antitumor [3–11], antiviral [11–17], antimicrobial [10,18–30], antioxidant [29,30], neuroprotective [31], antiprotozoal [32]] and anti-inflammatory [33,34] activity. Also, these substances act as inhibitors of acetylcholinesterase [35,36], α-glucosidase [37], and carbonic anhydrase [38]. Substances containing the 1,3,4-thiadiazole ring are also of interest as pesticides [39–41].

Derivatives of 1,3,4-thiadiazoles are widely used in coordination chemistry as ligands [42–44], in the synthesis of polymers [45], and the creation of polymer films [46,47]. The prospects of using 1,3,4-thiadiazoles in optoelectronics [48], in the purification of water from heavy metal ions [49], for the separation of minerals by the flotation method [50], and the creation of corrosion-resistant coatings [51] are discussed in the literature. A large number of works are devoted to the development of dyes and fluorescent markers based on 1,3,4-thiadiazoles [52–54].

In this paper, we report the synthesis of a new member of the class of 1,3,5-thiadiazoles, which contains an alkylamide fragmen—2,4-dichloro-*N*-(2,2,2-trichloro-1-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)amino)ethyl)benzamide, as well as molecular docking investigations of the obtained compound with the enzyme dihydrofolate reductase (DHFR).

2. Materials and Methods

2.1. Chemistry

^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were measured for solutions in DMSO-d_6 on a Varian VXR-400 spectrometer. Elemental analysis was performed on a LECO CHNS-900 instrument. The reaction and purity of the compounds were monitored by TLC on Silufol UV-254 plates using a chloroform/acetone mixture (3:1) as an eluent.

Synthesis of 2,4-dichloro-*N*-(2,2,2-trichloro-1-(2-(phenylcarbamothioyl)hydrazine-1-carbothioamido)ethyl)benzamide (3). An equimolar amount (1.67 g) of *N*-phenylhydrazinecarbothioamide (**2**) [57] was added to 10 mmol (3.78 g) of 2,4-dichloro-*N*-(2,2,2-trichloro-1-isothiocyanatoethyl)benzamide (**1**) [55,56] in 35 mL of acetonitrile. The mixture was refluxed for 1–3 min and then left for 24 h. The precipitate formed was filtered, washed with acetonitrile (2 × 10 mL), and dried. The product was purified by recrystallization from acetonitrile. White solid; yield 87% (4.75 g); mp 198–200 °C (MeCN); R_f = 0.28. ^{1}H NMR: δ 10.28 (brs, 1H, NH), 9.99 (brs, 1H, NH), 9.86 (brs, 1H, NH), 9.49 (brs, 1H, NH), 7.97 (brs, 1H, NH), 7.76–7.31 (m, 8H, arom.), 7.16 (dd, *J* = 6.4, 6.4 Hz, 1H, CH). ^{13}C NMR: δ 182.8 (C=S), 182.3 (C=S), 164.6 (C=O), 138.9, 135.4, 134.1, 131.4, 130.5, 129.5, 128.1, 127.4, 125.2, 122.7 (arom.), 101.1 (CCl_3), 69.9 (CH). Anal. Calcd (%) for $C_{17}H_{14}Cl_5N_5OS_2$ (545.70): C, 37.42; H, 2.59; N, 12.83; S, 11.75. Found: C, 37.45; H, 2.56; N, 12.87; S, 11.79.

Synthesis of 2,4-dichloro-*N*-(2,2,2-trichloro-1-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)amino)ethyl)benzamide (4). 10 mmol (5.46 g) of *N,N′*-disubstituted hydrazinecarbothioamide (**3**) was dissolved in 20 mL of DMF, a solution of 11 mmol (2.79 g) of iodine and 30 mmol (4.2 mL) of triethylamine in 10 mL of DMF was added in portions to the resulting solution with stirring. The reaction mixture was left at room temperature for 1–1.5 h. The precipitated sulfur was filtered. The product was precipitated from the filtrate with 1% aqueous sodium thiosulfate (250 mL). The precipitate formed was filtered, washed with water (2 × 50 mL), and dried. The product was purified by recrystallization from acetonitrile. Beige solid; yield 84% (4.30 g); mp 162–164 °C (MeCN); R_f = 0.38. ^{1}H NMR: δ 9.73 (s, 1H, NH), 9.59 (brs, 1H, NH), 8.12 (d, *J* = 7.8 Hz, 1H, NH), 7.70 (s, 1H, arom.), 7.52–7.50 (m, 3H, arom.), 7.43–7.41 (m, 1H, arom.), 7.30–7.26 (m, 2H, arom.), 6.93–6.89 (m, 1H, arom.), 6.76 (dd, *J* = 9.3, 7.8 Hz, 1H, CH). ^{13}C NMR: δ 165.4 (C=O), 157.9 (C=N), 156.6 (C=N), 141.2, 134.9, 134.6, 131.3, 130.4, 129.1, 128.9, 127.2, 120.9, 116.6 (arom.), 101.0 (CCl_3), 69.7 (CH). Anal. Calcd (%) for $C_{17}H_{12}Cl_5N_5OS$ (511.63): C, 39.91; H, 2.36; N, 13.69; S, 6.27. Found: C, 39.88; H, 2.33; N, 13.72; S, 6.30.

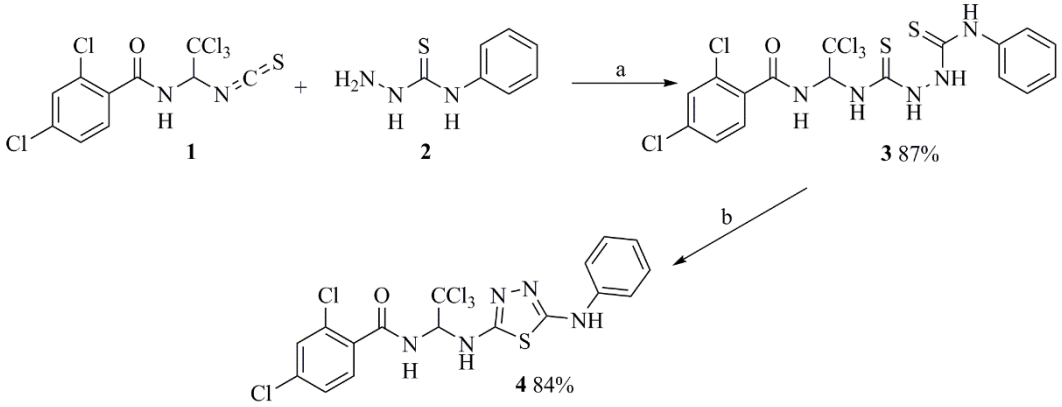
2.2. Molecular Docking Studies

The dihydrofolate reductase enzyme, whose structure was downloaded from the Protein Data Bank (PDB ID: 1DLS) [58], was used as a potential biological target for molecular docking. The preparation of the enzyme structure for docking was carried out using the Chimera 1.14 program [59], while water and Methotrexate molecules were removed. The ligand structures were constructed and optimized by the PM3 method [60] in the ArgusLab 4.0.1 program [61–65]. Molecular docking was performed using the AutoDock Vina program [66] implemented in PyRx 0.8. During the docking procedure, the center of the grid, whose coordinates were: X = 23.4 Å, Y = 16.7 Å, and Z = 1.7 Å, was centered on the amino acids Ile 5, Ala 6, Ala 7, Asp 27, Leu 28, Phe 31, Lys 32, Ser 49, Ile 50, Arg 52, Leu 54, Arg 57, Ile 94, Tyr 100 and Thr 113 [67]. The grid dimensions were 25.0 × 25.0 × 25.0 Å.

3. Results and Discussion

3.1. Chemistry

The starting 2,4-dichloro-*N*-(2,2,2-trichloro-1-(2-(phenylcarbamothioyl)hydrazine-1-carbothioamido)ethyl)benzamide (**3**) was obtained by the addition reaction of *N*-phenylhydrazinecarbothioamide (**2**) [57] to 2,4-dichloro-*N*-(2,2,2-trichloro-1-isothiocyanatoethyl)benzamide (**1**) [55,56] (Scheme 1). The reaction was carried out in an acetonitrile medium, bringing the reaction mass to a boil and then leaving it for 24 h. Hydrazinecarbothioamide (**3**) precipitated quantitatively from the reaction mass. The yield of the product purified by recrystallization from acetonitrile was 87%. Under the action of iodine on hydrazinecarbothioamide (**3**) in a DMF medium, sulfur was eliminated, HI was formed, and the target 1,3,4-thiadiazole cycle was closed. The resulting sulfur precipitated, and HI bound to the corresponding salt with triethylamine. After removing the precipitated sulfur by filtration, the target product, 2,4-dichloro-*N*-(2,2,2-trichloro-1-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)amino)ethyl)benzamide (**4**), was precipitated from the filtrate with 1% aqueous sodium thiosulfate solution. The target derivative of 1,3,4-thiadiazole was quantitatively precipitated with water, and after recrystallization from acetonitrile, the yield was 84%.



Scheme 1. Synthesis of 2,4-dichloro-*N*-(2,2,2-trichloro-1-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)amino)ethyl)benzamide (**4**). Reagents and conditions: (a) CH_3CN, reflux 1–3 min, r.t. 24 h; (b) I_2, Et_3N, DMF, r.t. 1–1.5 h.

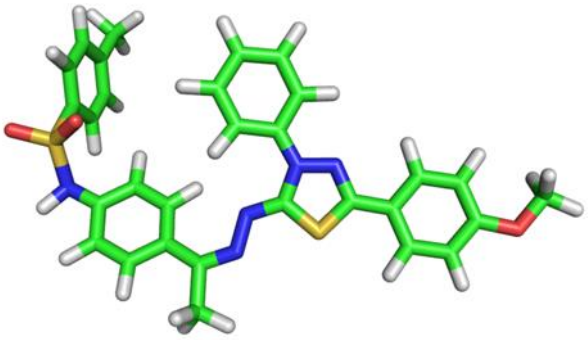
The ^1H NMR spectrum of the starting hydrazinecarbothioamide (**3**) showed five broadened singlet NH proton signals at 10.28–7.97 ppm (*see supporting information*), while the spectrum of compound **4** showed three NH proton signals, a singlet at 9.73 ppm, a broadened singlet at 9.59 ppm and a doublet at 8.12 ppm. The ^{13}C NMR spectrum of compound **3** was characterized by two closely located C=S carbon signals at 182.8 and 182.3 ppm. In turn, in the spectrum of compound **4,** there were no C=S carbon signals, but two C=N carbon signals could be observed at 157.9 and 156.6 ppm. All of the above points to the formal elimination of H_2S and the closure of the 1,3,4-thiadiazole ring.

3.2. Molecular Docking Studies

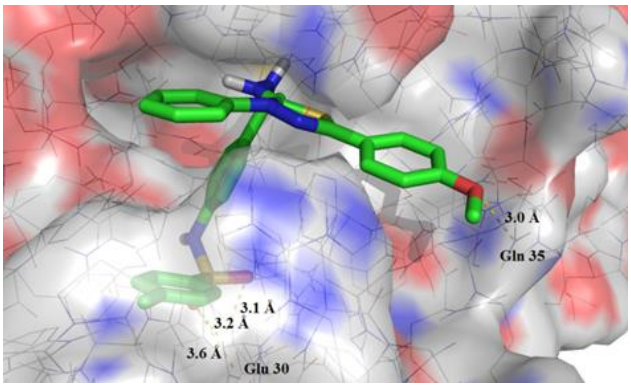
Recently, a large number of works have appeared devoted to the inhibition of dihydrofolate reductase (DHFR) enzyme by 1,3,4-thiadiazole derivatives, which makes these compounds potential agents for combating malignant tumors [67–71]. Therefore, we chose this enzyme as a potential biological target for molecular docking. We took *N*-(4-((*Z*)-1-(((*Z*)-5-(4-methoxyphenyl)-3-phenyl-1,3,4-thiadiazol-2(3*H*)-ylidene)hydrazono)ethyl)phenyl)-4-methylbenzenesulfonamide (Figure 1a) [67] and (*E*)-5-benzylidene-1-(5-(3,5-dinitrophenyl)-1,3,4-thiadiazol-2-yl)-3-phenyl-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione (Figure 1c) [71]. According to the results of molecular docking, *N*-(4-((*Z*)-1-(((*Z*)-5-(4-methoxyphenyl)-3-phenyl-1,3,4-thiadiazol-

2(3*H*)-ylidene)hydrazono)ethyl)phenyl)-4-methylbenzenesulfonamide formed a complex with the DHFR active site with a ΔG value of -8.2 kcal/mol. The inhibitor molecule was effectively fixed in the cavity of the active site due to four intermolecular hydrogen bonds, three of which were formed with the participation of the sulfamide group and the amino acid Glu 30 (bond lengths—3.1, 3.2, and 3.6 Å), and one more with the participation of the methoxy group and Gln 35 (bond length—3.0 Å) (Figure 1b). In turn, the molecule (*E*)-5-benzylidene-1-(5-(3,5-dinitrophenyl)-1,3,4-thiadiazol-2-yl)-3-phenyl-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione formed six intermolecular hydrogen bonds with the DHFR active site (Figure 1d), three of which involved the thiopyrimidinone fragment and amino acids Leu 28 and Gln 35 (bond lengths—2.7, 2.7, and 3.2 Å), and the remaining three hydrogen bonds were formed by the nitro group with the amino acids Asp 21 and Ser 59 (bond lengths—2.9, 3.1, and 3.3 Å). The value of ΔG was −8.4 kcal/mol.

Based on the results of molecular docking, the resulting 2,4-dichloro-*N*-(2,2,2-trichloro-1-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)amino)ethyl)benzamide (**4**) (Figure 1e) surpasses the reference compounds in the strength of the complex formed with DHFR (ΔG = -9.0 kcal/mol). The compound **4** molecule is fixed in the active site of the enzyme via three intermolecular hydrogen bonds, two of which are formed with the participation of the thiadiazole ring and the amino acids Asp 21 and Ser 59 (bond lengths are 3.1 and 2.8 Å, respectively) and the third hydrogen bond 3.2 Å long is formed by the secondary amino group and Tyr 22 (Figure 1f).

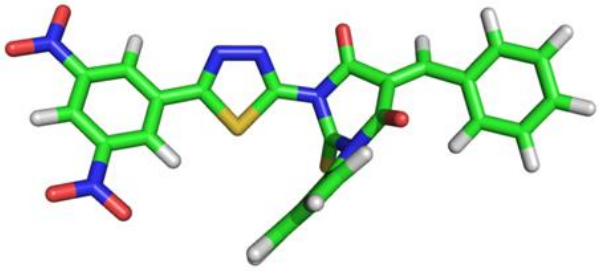


(**a**)

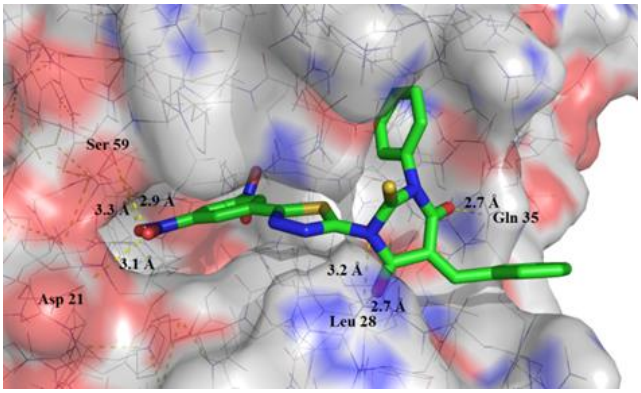


IC_{50} = 0.07 μM [67], ΔG = -8.2 kcal/mol

(**b**)

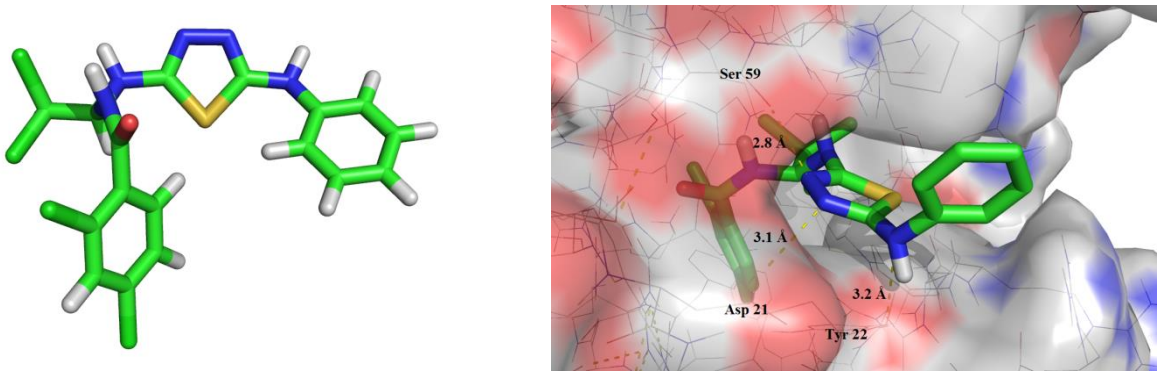


(**c**)



IC_{50} = 0.04 μM [71], ΔG = -8.4 kcal/mol

(**d**)



ΔG = -9.0 kcal/mol

(**e**) (**f**)

Figure 1. Results of molecular modeling investigations of reference substances and the resulting 1,3,4-thiadiazole derivative: (**a**) geometry optimization of *N*-(4-((*Z*)-1-(((*Z*)-5-(4-methoxyphenyl)-3-phenyl-1,3,4-thiadiazol-2(3*H*)-ylidene)hydrazono)ethyl)phenyl)-4-methylbenzenesulfonamide and its position in the DHFR active site **(b)**; (**c**) geometry optimization of (*E*)-5-benzylidene-1-(5-(3,5-dinitrophenyl)-1,3,4-thiadiazol-2-yl)-3-phenyl-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione and its position in the DHFR active site (**d**); (**e**) geometry optimization of 2,4-dichloro-*N*-(2,2,2-trichloro-1-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)amino)ethyl)benzamide and its position in the DHFR active site (**f**).

According to the results of molecular docking, the resulting 1,3,4-thiadiazole derivative is a potential inhibitor of DHFR and can be recommended for further in vitro investigations. Further work in the development of DHFR inhibitors based on 1,3,4-thiadiazole derivatives containing an alkylamide fragment seems to be very promising.

4. Conclusions

In this work, we have obtained a new representative of the series of 1,3,4-thiadiazoles -2,4-dichloro-*N*-(2,2,2-trichloro-1-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)amino)ethyl)benzamide based on 2,4-dichloro-*N*-(2,2,2-trichloro-1-(2-(phenylcarbamothioyl)hydrazine-1-carbothioamido)ethyl)benzamide. The structure of the target and starting compounds has reliably been confirmed by ^{1}H and ^{13}C NMR spectroscopy data. The obtained 1,3,4-thiadiazole derivative is promising as a potential inhibitor of dihydrofolate reductase.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: ^{1}H NMR spectra of 2,4-dichloro-*N*-(2,2,2-trichloro-1-(2-(phenylcarbamothioyl)hydrazine-1-carbothioamido)ethyl)benzamide (**3**); Figure S2: ^{13}C NMR spectra of 2,4-dichloro-*N*-(2,2,2-trichloro-1-(2-(phenylcarbamothioyl)hydrazine-1-carbothioamido)ethyl)benzamide (**3**); Figure S3: ^{1}H NMR spectra of 2,4-dichloro-*N*-(2,2,2-trichloro-1-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)amino)ethyl)benzamide (**4**); Figure S4: ^{13}C NMR spectra of 2,4-dichloro-*N*-(2,2,2-trichloro-1-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)amino)ethyl)benzamide (**4**).

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