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Proceeding Paper

Absorption of Free Radicals of New S-Derivatives (1,2,4-triazole-3(2H)-yl)methyl)thiopyrimidines †

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Abstract: At the current stage of organic chemistry development, various fundamental synthetic approaches have been developed for the synthesis of 1,2,4-triazole and pyrimidine scaffolds, which exhibit a wide range of biological activities. The relevance of this research lies in the combination of two pharmacophore fragments in one molecule—a pyrimidine and an azole heterocycle—connected by a thiomethylene bridge, which is expected to improve solubility and enhance known biological properties, as well as introduce new ones. This study presents the synthesis of compounds and investigates their antiradical activity using the DPPH free radical test. Three compounds demonstrate greater activity than the reference drug, the natural antioxidant ascorbic acid.

Keywords: pyrimidine-2-thiol; 1,2,4-triazole; free radicals; DPPH

1. Introduction

Antioxidants are substances that prevent and mitigate damage caused by free radicals by transferring electrons from the antioxidants to these reactive species [1]. They also convert free radicals into waste products that are then excreted from the body. Therefore, evaluating such properties remains an interesting and valuable task, particularly in the search for promising synthetic antioxidants derived from azole heterocycles.

Pyrimidine is an aromatic heterocyclic compound containing nitrogen atoms at the 1st and 3rd positions and plays a crucial role in forming the backbone of various biologically active compounds. It is a structural unit of DNA and RNA and is essential in various biological processes. Common pyrimidines include uracil, cytosine, and thymine. Pyrimidines exhibit a range of biological activities [2], including antiviral, antitumor, antimicrobial [3], anti-inflammatory, analgesic [4], antioxidant [5], and antimalarial properties.

Pyrimidine is used as a starting material for synthesizing a wide range of heterocyclic compounds and for producing new molecules. It has been established that pyrimidine ring complexes with various heterocyclic fragments are integral components of pharmaceutical and veterinary preparations.

The 1,2,4-triazole nucleus is a highly promising azole heterocycle, and compounds derived from its chemical transformations have various biological, pharmaceutical, and clinical applications [6]. It is known that modifying azole heterocycles can enhance their efficacy and reduce toxicity.

An increase in solubility, enhancement of known biological properties, and the emergence of new biological activities can be achieved by combining two pharmacophoric fragments in one molecule—a pyrimidine and an azole heterocycle—connected by a thiomethylene bridge. Therefore, we selected new S-derivatives of (1,2,4-triazol-3(2*H*)-yl)methyl)thiopyrimidines for research. Derivatives of (1,2,4-triazol-3(2*H*)-yl)methyl)thiopyrimidines are known to exhibit anticonvulsant effects and to treat nervous system disorders [7].

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2. Materials and Methods

Chemistry. The ^{1}H spectra were recorded on a Bruker AC-400 spectrometer (400 MHz, respectively) in DMSO-d_6, the internal standard was TMS (Agilent Technologies, Santa Clara, CA, USA). LC-MS were recorded on a high-performance liquid chromatograph Agilent 1260 Infinity HPLC System and with the help of a diode array detector with proton ionization. Elemental analysis (C, H, N, S) is made on ELEMENTAR vario EL cube (standard—sulfanilamide). The melting points are determined by the capillary method in «Stanford Research Systems Melting Point Apparatus 100» (SRS, Sand-Hills, SC, USA). Used reagents were purchased from Sigma-Aldrich (Merck), Steinheim, Germany.

Compounds were synthesized using the well-known method [8] 3 with constants corresponding to literature data.

Obtaining of 4-methyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazole-3-thiol **1** (general methods). A mixture of 10 mmol of 2-(pyrimidin-2-ylthio)acetohydrazide, 10 mmol of sodium hydroxide, and 50 mL of purified water is boiled for 2 h. After cooling completely, 2 mL of concentrated acetic acid is added to the filtrate. The resulting precipitate is filtered and washed with purified water. For analysis, the product is purified by recrystallization from DMF. It appears as a light yellow powder, soluble in aqueous alkali solutions, DMF, and 1,4-dioxane.

4-Methyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazole-3-thiol (**1**). Yield 72%, light yellow powder, mp 266 °C (DMF). ^{1}H NMR, δ, ppm. (J, Hz): 3.55 (s, 3H, -N-CH_3), 4.43 (s, 2H, -CH_2-), 7.19 (t, J = 4.4 Hz, 1H, Ar), 8.53 (d, J = 4.4 Hz, 2H, Ar), 12.83 (s, 1H, -SH). Mass spectrum, *m*/*z* (I_{rel}, %) 240 $[M + H]^+$ (100). Anal. calcd. for $C_8H_9N_5S_2$: C: 40.15%; H: 3.79%; N: 29.26%; S: 26.79%; Found: C: 40.11%; H: 3.82%; N: 29.35%; S: 26.71%.

Obtaining of S-alkyl derivatives 4-methyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazole-3-thiols **2**–**4** (general methods). A mixture of 5 mmol of 4-methyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazole-3-thiol and 5 mmol of sodium hydroxide dissolved in 10 mL of propan-2-ol is prepared. To this, 5 mmol of the halogen derivative is added. The mixture is heated for 2 h, then cooled, and the sediment is filtered and washed with purified water. The product is crystallized from methanol for analysis. The crystalline substances (2-4) are yellow or brown, insoluble in water, and soluble in organic solvents.

2-((4-Methyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazol-3-yl)thio)acetic acid (**2**). Yield 1.16 g (78%), white powder, mp 184 °C (MeOH). ^{1}H NMR, δ, ppm. (J, Hz): 3.59 (s, 3H, -N-CH_3), 4.08 (s, 2H, -CH_2-COO), 4.54 (s, 2H, -CH_2-), 7.20 (t, J = 3.7 Hz, 1H, Ar), 8.52 (d, J = 3.7 Hz, 2H, Ar), 11.36 (s, 1H, -COOH). Mass spectrum, *m*/*z* (Irel, %) 298 $[M + H]^+$ (100). Anal. calcd. for $C_{10}H_{11}N_5O_2S_2$: C: 40.39%; H: 3.73%; N: 23.55%; S: 21.56%. Found: C: 40.32%; H: 3.76%; N: 23.58%; S: 21.52%.

Methyl 2-((4-methyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazol-3-yl)thio)acetate (**3**). Yield 1.13 g (73%), white powder, mp 169 °C (MeOH). ^{1}H NMR, δ, ppm. (J, Hz): 3.72 (s, 3H, -N-CH_3), 4.07 (s, 2H, -CH_2-COO), 4.54 (s, 2H, -CH_2-), 7.20 (t, J = 3.7 Hz, 1H, Ar), 8.52 (d, J = 3.7 Hz, 2H, Ar). Mass spectrum, *m*/*z* (I_{rel}, %) 312 $[M + H]^+$ (100). Anal. calcd. for $C_{11}H_{13}N_5O_2S_2$: C: 42.43%; H: 4.21%; N: 22.49%; S: 20.59%. Found: C: 42.18%; H: 4.20%; N: 22.54%; S: 20.67%.

2-((4-Methyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazol-3-yl)thio)acetamide (**4**). Yield 1.11g (75%), white powder, mp 197 °C (MeOH). ^{1}H NMR, δ, ppm. (J, Hz): 3.59 (s, 3H, -N-CH_3), 4.01 (s, 2H, -CH_2-COO), 4.54 (s, 2H, -CH_2-), 7.05 (s, 2H, -NH_2), 7.20 (t, J = 3.7 Hz, 1H, Ar), 8.52 (d, J = 3.7 Hz, 2H, Ar). Mass spectrum, *m*/*z* (I_{rel}, %) 297 $[M + H]^+$ (100). Anal. calcd. for $C_{10}H_{12}N_6OS_2$: C: 40.53%; H: 4.08%; N: 28.36%; S: 21.64%. Found: C: 40.38%; H: 4.16%; N: 28.96%; S: 21.50%.

Antiradical activity. Free radical absorption was measured using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical test [9]. An exact weight of the substance (0.001 M) was placed in a 25.00 mL volumetric flask, dissolved in DMSO, and diluted to the mark before mixing. Then, 1.00 mL of the solution was transferred to a 10.00 mL volumetric flask (0.0001 M), filled to the mark with DMSO, and mixed. Next, 2.00 mL of the resulting solution was placed in a test tube, combined with 2.00 mL of a 0.1 mM DPPH solution in

methanol (Sigma-Aldrich, Steinheim, Germany), mixed, and tightly sealed. The tubes were vigorously shaken and left for 30 min at room temperature in the dark. Absorbance was measured at 516 nm. The control was a solution of 2.00 mL of 0.1 mM DPPH in the presence of 2.00 mL of methanol, and the standard was ascorbic acid. The free radical scavenging activity was expressed as a percentage of inhibition and calculated using formula (1):

$$\%\ \text{antiradical activity} = \frac{(A_0 - A_1)}{A_0} \cdot 100, \quad (1)$$

where A_0—is the absorption coefficient of the control sample, and A_1—is the absorption coefficient of the test sample. The absorption of the studied solutions was measured in aqueous-organic solutions, and the absorption maximum at 516 nm was recorded using a SPECORD 250 spectrophotometer.

3. Results

One of the known methods for the synthesis of 5-substituted-1,2,4-triazole-3(2*H*)-thiones involves the formation of intermediate carbothioamides followed by heterocyclization in an alkaline medium [9,10]. The starting pyrimidine-2-thione was synthesized via [3 + 3] cyclization of thiourea with 1,1,3,3-tetraethoxypropane, and ethyl 2-(pyrimidin-2-ylthio)acetate was then obtained through an alkylation reaction in an acetone medium in the presence of K_2CO_3 (Figure 1).

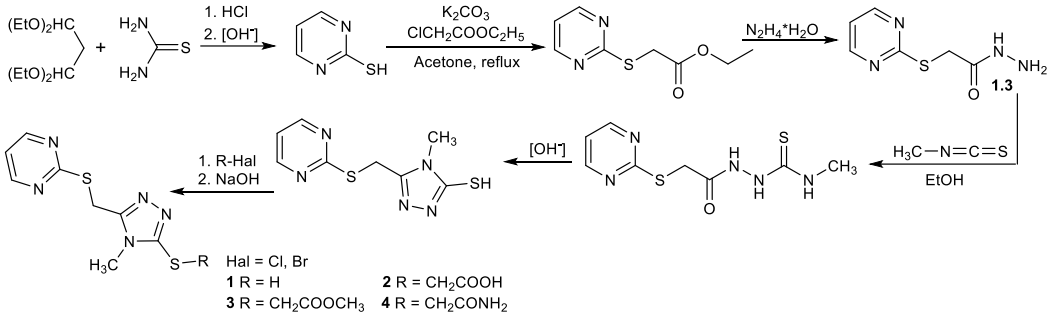

Figure 1. Synthesis of derivative hybrids of two pharmacophore fragments—pyrimidine-2-thione and 1,2.4-triazole.

Subsequently, the ester underwent hydrazinolysis, and the resulting hydrazide reacted with methyl isothiocyanate in an ethanol medium to form the intermediate product carbothioamide. Further cyclization was carried out by stirring with an aqueous sodium hydroxide solution for 2 h on a magnetic stirrer. The resulting solution was then acidified with glacial acetic acid to precipitate 4-methyl-5-((pyrimidin-2-ylthio)methyl)-4*H*-1,2,4-triazole-3-thiol (**1**). It is known that the presence of substituents at the sulfur atom in 1,2,4-triazol-3(2*H*)-thiones enhances their biological activity. For this reason, it was considered worthwhile to synthesize S-derivatives of (1,2,4-triazol-3(2*H*)-yl)methyl)thiopyrimidines. Alkyl derivatives and acyl derivatives (**2**–**4**) were obtained by reacting the original thione (**1**) with the corresponding halogen derivative in a polar solvent—ethanol—with the addition of an equimolar amount of sodium hydroxide.

Among the various groups of antioxidants with different mechanisms of action, the most important role is played by anti-radical antioxidants—substances that interact with free radicals to form products incapable of continuing oxidation chain reactions or that reduce the reaction rate. The antiradical activity of the synthesized compounds, assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical test, shows high activity levels (Table 1).

Table 1. Antiradical activity and absorption coefficients of 1,2,4-triazole derivatives.

Compounds	Absorption Coefficient, A	% Antiradical Activity
Control	0.4305	–
Ascorbic acid	0.2959	31.26
1	0.1864	56.7
2	0.0681	83.86
3	0.4304	−1.99
4	0.2572	39.05

The absorption spectra of 1,1-diphenyl-2-picrylhydrazyl (DPPH) with the compounds are shown in Figure 2, with absorption values at 516 nm. The negative value of antiradical activity (%) may be attributed to two factors: the similarity between the absorption spectra of the substance and DPPH, causing overlapping absorption bands, or an increase in the degradation of the compound, resulting in the release of free radicals. This is evident in the case of a compound with an ethanoic acid ester group at the 5-position of 1,2,4-triazol-3(2H)-thione and may be due to a non-ideal electronic configuration of the framework for reducing free radicals via dissociation.

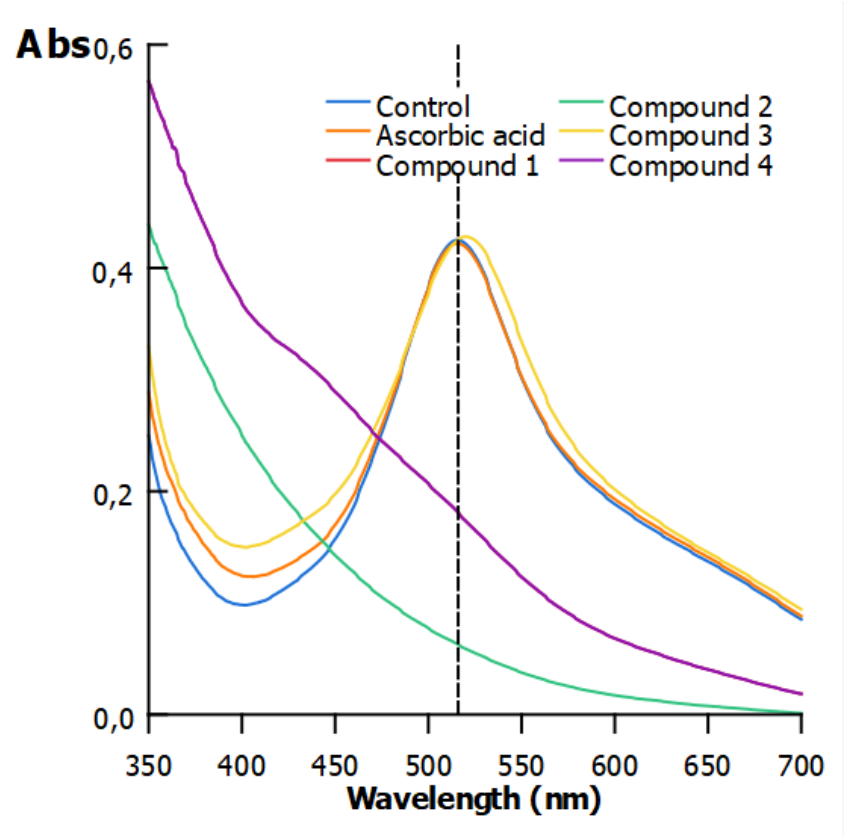

Figure 2. Absorption spectra of compound solutions with 1,1-diphenyl-2-picrylhydrazyl (DPPH).

It is worth noting that three compounds (**1**, **2**, **4**) demonstrate higher activity than the reference preparation, the natural antioxidant ascorbic acid. This high activity may be related to the presence of pharmacophore fragments, specifically the pyrimidine skeleton and the sulfur atom linked to 1,2,4-triazole. A more detailed analysis of the compounds suggests a relationship between "antiradical activity and structure", where the increase in activity may be attributed to the presence of free proton donors in the compounds, particularly in the protonated atoms of the pyrimidine ring and proton donors within the carboxylic acid and amide residues.

4. Conclusions

The negative value of antiradical activity (%) can be attributed to two factors: the similarity between the absorption spectra of the substance itself and DPPH, causing overlap of the absorption bands, or the increased degradation of the compound, leading to the release of free radicals. Notably, three compounds (1, 2, 4) exhibit higher activity than the reference drug, the natural antioxidant ascorbic acid. This high activity may be related to the presence of pharmacophore fragments, specifically the pyrimidine skeleton and the sulfur atom linked to 1,2,4-triazole.

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