

Short Note

2-((4-Phenyl-5-(2-(*p*-tolylamino)ethyl)-4*H*-1,2,4-triazol-3-yl)thio)-*N'*-(1-phenylethylidene)acetohydrazide

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Abstract: A synthesis of 2-((4-phenyl-5-(2-(*p*-tolylamino)ethyl)-4*H*-1,2,4-triazol-3-yl)thio)-*N'*-(1-phenylethylidene)acetohydrazide from 2-[(3-[2-[(4-methylphenyl)amino]ethyl]-4-phenyl-4,5-dihydro-1*H*-1,2,4-triazol-5-yl)sulfanyl]acetohydrazide and acetophenone is reported. The title compound has been tested to possess 1.5-fold higher antioxidant ability than the control, butylated hydroxytoluene, as determined by a Ferric reducing antioxidant power assay.

Keywords: 1,2,4-triazole; hydrazone; Schiff base; antioxidative; ferric reducing antioxidant power (FRAP) assay



Citation: Šermukšnytė, A.;

Jonuškienė, I.; Kantminienė, K.; Beresnevičius, Z.J.; Tumosiene, I. 2-((4-Phenyl-5-(2-(*p*-tolylamino)ethyl)-4*H*-1,2,4-triazol-3-yl)thio)-*N'*-(1-phenylethylidene)acetohydrazide. *Molbank* **2022**, *2022*, M1380. <https://doi.org/10.3390/M1380>

Academic Editor: Oleg A. Rakitin

Received: 25 May 2022

Accepted: 3 June 2022

Published: 5 June 2022

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1. Introduction

Oxidative stress is caused by an imbalance between the production and accumulation of reactive oxygen species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products. ROS are normally generated as by-products of oxygen metabolism; however, environmental stressors, such as pollutants, ionizing radiation, and heavy metals, contribute to a significant increase in ROS production, therefore causing the imbalance that leads to cell and tissue damage [1]. Oxidative stress occurs when there is an imbalance in redox homeostasis, with ROS producing processes overcoming antioxidant defensive processes [2]. Oxidative stress has been implicated in the pathogenesis of many diseases, such as diabetes, cancer, cardiovascular and neurodegenerative diseases, and different digestive disorders [3]. Antioxidants are involved in the defense mechanism of the organism against pathologies associated with the attack of free radicals by slowing or inhibiting completely the oxidation processes caused by reactive radicals. In recent years, several classes of organic compounds have attracted the attention of researchers as potential scaffolds for the synthesis of novel compounds possessing antioxidant activity.

1,2,4-Triazole derivatives exhibit a series of pharmacological activities such as antibacterial, antifungal, antitubercular, anticancer, antioxidant, antiviral, anti-inflammatory, antidepressant, etc. [4–7]. The triazole scaffold is present in molecules of several potent, biologically active compounds, such as trazodone (antidepressant drug), rizatriptan (anti-migraine drug), hexaconazole (antifungal drug) and alprazolam (hypnotic, sedative, and tranquilizer drug) [8]. The lipophilicity, polarity, and hydrogen bonding capacity of a molecule can be influenced by the presence of the 1,2,4-triazole moiety, thus improving the pharmacokinetic, pharmacological, toxicological, and physicochemical properties of the derivatives [9]. Presence of sulfur atom adjacent to the triazole moiety often enhances pharmacological activity compared to that of the parent derivatives [10,11]. 1,2,4-Triazole-3-thione derivatives are attractive scaffolds in medicinal chemistry for their broad biological

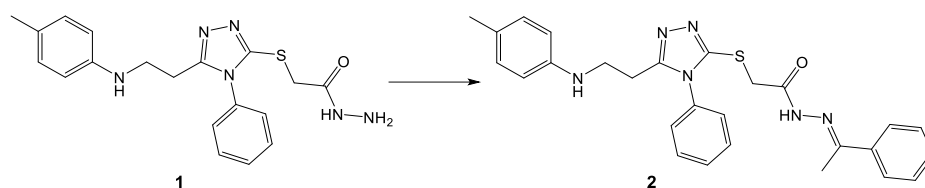
activity, e.g., antibacterial, antifungal, antioxidant, antiviral, anticonvulsant, antidepressant, anti-inflammatory, etc. [12–18].

Hydrazone, a separate type of Schiff bases, constitute another significant class of biologically active compounds in medicinal and pharmaceutical chemistry. Schiff bases have been shown to possess potent antioxidant activity to scavenge free radicals due to the presence of labile hydrogen in the NH group [19].

As a continuation of our interest in search for the potent antioxidant agents bearing heterocyclic moieties [20–23], herein we report the synthesis of 2-((4-phenyl-5-(2-(*p*-tolylamino)ethyl)-4*H*-1,2,4-triazol-3-yl)thio)-*N'*-(1-phenylethylidene)acetohydrazide (**2**) and evaluation of its antioxidant activity.

2. Results and Discussion

2-((4-Phenyl-5-(2-(*p*-tolylamino)ethyl)-4*H*-1,2,4-triazol-3-yl)thio)-*N'*-(1-phenylethylidene)acetohydrazide (**2**) was synthesized by the reaction of 2-[(3-[2-[(4-methylphenyl)amino]ethyl]-4-phenyl-4,5-dihydro-1*H*-1,2,4-triazol-5-yl)sulfanyl]acetohydrazide (**1**) [20] and acetophenone in methanol at reflux temperature of the reaction mixture in 89% yield (Scheme 1). In the ¹H-NMR (nuclear magnetic resonance) spectrum for **2**, the protons of the methyl group in the *p*-tolylamino moiety gave a singlet at 2.11 ppm [20], while the methyl group protons in the 1-phenylethylidene moiety gave a double set of singlets at 2.25 ppm and 2.30 ppm in the intensity ratio 1.8:1.2 (Supplementary Materials).



Scheme 1. Synthesis of the target compound **2**.

The protons of the methylene group adjacent to the S atom also resonated in a double set of resonances at 4.12 ppm and 4.45 ppm with the intensity ratio of 0.7:1.3. This splitting of the proton resonances indicates that the title hydrazone exists as a mixture of *Z/E* isomers in DMSO-*d*₆ solution due to the hindered rotation around the amide bond. In most cases, the *Z* isomer predominates [22,24,25]. A multiplet in the range of 7.55–7.64 ppm attributed to phenyl protons additionally proves the presence of the phenylethylidene moiety in the target molecule. In the ¹³C NMR spectrum for **2**, the resonance at 169.48 ppm has been attributed to the triazole ring carbon to which S atom is attached.

The antioxidant activity of the title compound **2** was evaluated using the Ferric reducing antioxidant power assay (FRAP) [26]. The FRAP assay is based on reduction of colourless Fe³⁺-2,4,6-tripyridyl-*s*-triazine complex to the intensively blue Fe²⁺-2,4,6-tripyridyl-*s*-triazine complex in acidic medium once it interacts with a potential antioxidant. At low cost, this method has been proven to be useful for screening of antioxidant capacities and comparing efficiencies of different compounds [27]. Reducing power as a measure of an antioxidant activity of 2-((4-phenyl-5-(2-(*p*-tolylamino)ethyl)-4*H*-1,2,4-triazol-3-yl)thio)-*N'*-(1-phenylethylidene)acetohydrazide (**2**) was 106.25 μM Fe²⁺, whereas those of a commonly used antioxidant butylated hydroxytoluene (BHT) and a well-known antioxidant ascorbic acid were 70.61 μM Fe²⁺ and 103.41 μM Fe²⁺, respectively, thus proving this type of hybrid compounds to be potent candidates for the development of antioxidant agents.

3. Materials and Methods

3.1. Synthesis

The reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). The reaction course and purity of the synthesized compounds were monitored by TLC using aluminium plates precoated with silica gel 60 F254 (MerckKGaA, Darmstadt, Germany). Melting point was determined on a MEL-TEMP melting point apparatus (Electrothermal, A Bibby

Scientific Company, Burlington, NJ, USA). The ^1H and ^{13}C -NMR spectra were recorded in $\text{DMSO-}d_6$ on Varian Unity Inova 300 spectrometer (300 and 75 MHz, respectively) operating in the Fourier transform mode. Chemical shifts (δ) are reported in parts per million (ppm) calibrated from TMS (0 ppm) as an internal standard for ^1H NMR, and $\text{DMSO-}d_6$ (39.43 ppm) for ^{13}C -NMR. FT-IR spectra (ν , cm^{-1}) were recorded on a Perkin-Elmer Spectrum BX FT-IR spectrometer using KBr pellets. Elemental analyses (C, H, N) were performed using the CE-440 Elemental Analyzer (Exeter Analytical, Inc., North Chelmsford, MA, USA).

2-((4-Phenyl-5-(2-(p-tolylamino)ethyl)-4H-1,2,4-triazol-3-yl)thio)-N'-(1-phenylethylidene)acetohydrazide (2)

To **1** [20] (0.58 g, 1.5 mmol) dissolved in methanol (20 cm^3), acetophenone (0.23 cm^3 , 2 mmol) was added dropwise. The reaction mixture was heated at reflux for 2 h; the precipitate started to form from the reaction mixture while still hot. The reaction mixture was cooled and water (40 cm^3) was added. The precipitate was isolated by filtration, washed with diethyl ether, and recrystallized from ethanol to give 0.71 g (89%); m.p.: 160–161 $^\circ\text{C}$; ^1H NMR (300 MHz, $\text{DMSO-}d_6$): δ = 2.11 (s, 3H, CH_3), 2.25 (s, 1.8H, CH_3), 2.30 (s, 1.2H, CH_3), 2.74 (q, J = 7.5 Hz, J = 15.5 Hz, 2H, $\text{CH}_2\text{-C}$), 3.22 (q, J = 7.5 Hz, J = 15.5 Hz, 2H, NHCH_2), 4.12 (s, 0.7H, CH_2), 4.45 (s, 1.3H, CH_2), 5.44 (t, 1H, J = 8 Hz, NHAr), 6.25 (d, J = 8.4 Hz, 2H, $\text{H}(2,6)\text{Ar}$), 6.80 (d, J = 8.4 Hz, 2H, $\text{H}(3,5)\text{Ar}$), 7.38–7.44 (m, 3H, $\text{H}(3,4,5)\text{Ar}'$), 7.46–7.52 (m, 2H, $\text{H}(2,6)\text{Ar}'$), 7.55–7.64 (m, 3H, $\text{H}(3,4,5)\text{Ar}''$), 7.70–7.83 (m, 2H, $\text{H}(2,6)\text{Ar}'$), 10.90 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $\text{DMSO-}d_6$): δ = 13.78, 14.25 (CH_3), 20.04 (Ar-CH_3), 24.71 (CH_2), 34.99, 35.58 (CH_2), 40.73 (C-7), 112.08 (C-2, 6), 124.24 (C-4), 126.09, 126.35, 127.45 (C-3', 5'), 128.32, 128.38, 129.20 (C-4'), 129.35, 129.94 (C-2', 6'), 130.00 (C-3, 5), 130.13, 132.82 (C-1'), 133.01, 137.89, 137.98, 145.72 (C-1), 148.42, 149.78 (C-9), 152.13, 154.02, 154.18 (C=O), 164.00, 169.48 (C-10) ppm; IR (KBr) ν_{max} (cm^{-1}): 3323–2858 (NH), 1677 (C=O) cm^{-1} ; Anal. Calcd. (%) for $\text{C}_{27}\text{H}_{28}\text{N}_6\text{O}_2$: C, 66.92; H, 5.82; N, 17.34., found: C, 66.99; H, 5.92; N, 17.53.

3.2. Evaluation of Antioxidant Activity

The FRAP reagent was freshly prepared to contain: 2.5 mL of a 10 mM TPTZ (2,4,6-tripyridyl-*s*-triazine) solution in 40 mM HCl, 2.5 mL of FeCl_3 (20 mM) and 25 mL of acetate buffer (0.3 M, pH = 3.6). 100 μL of the tested compound (20 mM) were mixed with 3 mL of the FRAP reagent. The absorbance of the reaction mixture at 593 nm was measured spectrophotometrically on a UV-200-RS spectrophotometer (MRC Ltd., Israel). For comprising of the calibration curve, five concentrations of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (5, 10, 15, 20, 25 μM) were used and the absorbances were measured as a sample solution [28]. Each experiment was conducted in triplicate.

Supplementary Materials: The following can be download online. Figure S1: ^1H -NMR Spectrum of **2**; Figure S2: ^{13}C -NMR Spectrum of **2**.

Author Contributions: Conceptualization, Z.J.B. and I.T.; methodology, I.J. and I.T.; formal analysis, I.J., K.K. and I.T.; investigation, A.Š.; resources, I.J. and I.T.; writing—original draft preparation, I.J., K.K. and I.T.; writing—review and editing, K.K.; visualization, A.Š. and I.T.; supervision, I.T. All authors have read and agreed to the published version of the manuscript.

Funding: Part of the research leading to these results has received a funding from European Social Fund (project No 09.3.3-LMT-K-712-22-0083) under grant agreement with the Research Council of Lithuania (LMTLT).

Data Availability Statement: The data presented in this study are available in this article.

Conflicts of Interest: The authors declare no conflict of interest.

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