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Short Note

4-[Bis(thiazol-2-ylamino)methyl]phenol

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Abstract: We have designed and synthesized novel bis-thiazole derivative. A 4-[bis(thiazol-2-ylamino)methyl]phenol was efficiently prepared in 71% yield by the reaction of 2-aminothiazole with 4-hydroxybenzaldehyde in ethanol for 24 h. The structure of newly obtained compound was characterized by ^{1}H, ^{13}C NMR and mass spectrometry. Bis-thiazole derivative exhibits high tyrosinase inhibitory activity with an IC_{50} value of 29.71 μM. This inhibitory activity is 2.4 times higher than that of activity of kojic acid (IC_{50} 72.27 μM) and almost 13 times higher than that of ascorbic acid (IC_{50} 385.6 μM). Obtained data suggest that the presented compound may be a leading candidate for a tyrosinase inhibitor.

Keywords: synthesis; thiazole; tyrosinase inhibitor

1. Introduction

Many thiazole derivatives have been synthesized [1] and studied for their biological properties, such as antimicrobial [2,3], anticancer [4–7], anticonvulsant [8,9], anti-*Toxoplasma gondii* [10,11] or antioxidant activities [12].

Recently, the thiazole group was examined as tyrosinase inhibitor [13]. Tyrosinase is an enzyme that plays a key role in melanin biosynthesis, responsible for melanogenesis. Overproduction of melanin can lead to various skin diseases, such as melasma, age spots and cancer. Well-known tyrosinase inhibitors such as kojic acid or hydroquinone are used to treat these diseases. However, these inhibitors have numerous side effects, such as dermatitis, skin irritation, DNA damage and cancer induction. Therefore, the search for tyrosinase inhibitors with higher activity and lower toxicity is currently the subject of numerous studies. Tyrosinase has been found to be overexpressed in cancer-affected melanocytes and not in other cells. The use of tyrosinase to activate the prodrug allows the active drug to be released selectively only in melanoma cells.

Due to the wide range of activities of 2-aminothiazole derivatives, much attention is currently focused on the search for new methods of their synthesis [14–19]. Currently, several methods of bis(heterocyclic)methanes synthesis are known [20,21], but there are no efficient methods of bis(aminothiazole)methanes synthesis.

Considering the above-mentioned findings, we decided to design and synthesize the bis-thiazole derivative containing a 4-hydroxyphenyl moiety and evaluate its tyrosinase inhibitory potential along with mechanism of inhibition.

2. Results and Discussion

2.1. Synthesis of Compound **3**

The synthesis of the title compound **3** was prepared as shown in Scheme 1. An equimolar amount of 2-aminothiazole was added to the ethanolic solution of 4-hydroxybenzaldehyde. The mixture was stirred at room temperature for 24 h without any catalyst. The resulting solid was filtered, washed with ethanol and recrystallized to give 4-[bis(thiazol-2-ylamino)methyl]phenol (**3**) in 71% yield. Compound **3** was fully characterized by NMR

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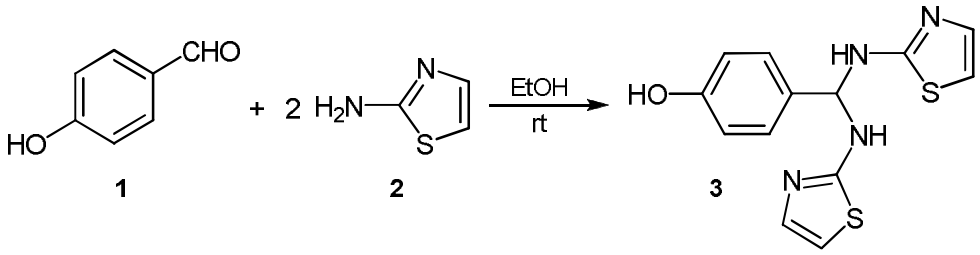
spectroscopy (^{1}H, ^{13}C and COSY NMR) and high resolution mass spectra, which showed a peak corresponding to its molecular ion $[M + H]^+$ (see Supplementary Materials).



Scheme 1. Synthesis of **3**.

Our method of synthesis based on N–C bond formation by the condensation reaction of 2-aminothiazole with aldehyde and nucleophilic addition reaction. The condensation takes place on position of primary amine of 2-aminothiazole.

In contrast to the methods described in the literature, our method does not require heating or transition metal catalysts or enzymes.

2.2. Tyrosinase Inhibitory Activity

2.2.1. Mushroom Tyrosinase Inhibition Assay

The synthesized compound **3** was tested for its inhibitory effects on mushroom tyrosinase using L-DOPA as substrate (Table 1). The results were compared with standard tyrosinase inhibitors such as kojic acid and ascorbic acid. Tested compound **3**, with IC_{50} value 29.71 μM, showed higher effect of tyrosinase inhibition than standard inhibitors. It is 2.4 times higher inhibitory effect than kojic acid (IC_{50} 72.27 μM) and almost 13 times higher inhibitory effect than ascorbic acid (IC_{50} 385.6 μM). Such high inhibitory activity may be due to the presence of two thiazole units in the molecule.

Table 1. Tyrosinase inhibitory activity of compound **3** and its mechanism of action.

Compound at 0.2 mM	Inhibitory Mechanism	V_{max}	K_m	$IC_{50} \pm$ SD [μM]
3	Competitive	0.6502	0.8048	29.71 ± 4.41
Ascorbic acid				385.6 ± 11.58
Kojic acid				72.27 ± 3.15

2.2.2. Kinetic Analysis of the Inhibition of Tyrosinase

Next, the mechanism of tyrosinase inhibition was determined using Lineweaver–Burk double reciprocal plots. The plot provides a useful graphical method for analysis of the Michaelis–Menten equations used to determine the inhibition constant K_m for possible competitive, uncompetitive, noncompetitive and mixed mechanisms of inhibitions. The obtained graph for the tested compounds is presented in Figure 1. As can be easily noticed, the tested compound **3** is a competitive inhibitor, which indicates that compound **3** binds to the enzyme at its active center, preventing binding of the substrate to the enzyme.

The low value of the inhibition constant, K_m 0.8048, indicates the strong inhibition of tyrosinase by this compound.

To observe the effect of the oxidation of L-DOPA by tyrosinase in the absence and in the presence of tested compound, the UV-Vis spectra were determined. UV-Vis spectra for tested compound **3** is presented in Figure 2. During L-DOPA oxidation by tyrosinase, a characteristic peak at 475 nm is observed on the UV-Vis spectrum, corresponding to the formation of dopachrome. After the addition of inhibitor **3** after 30 min, we observe a significant reduction in peak intensity at 475 nm in comparison with the absorbance of the mixture of L-DOPA with tyrosinase.

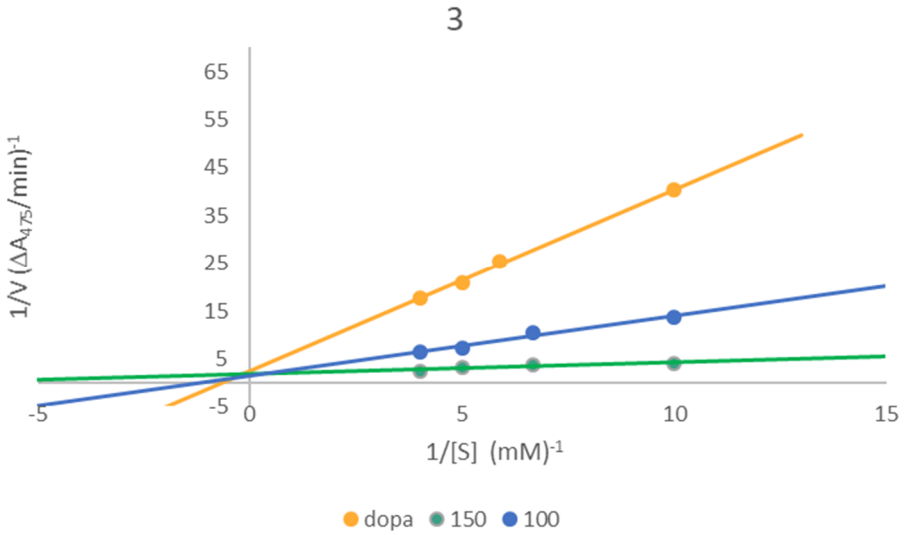


Figure 1. Lineweaver–Burk plots for tyrosinase inhibition for compounds **3** at concentration 0.2 mM using L-DOPA as substrate at concentrations 0.1, 0.15, 0.20 and 0.25 mM.

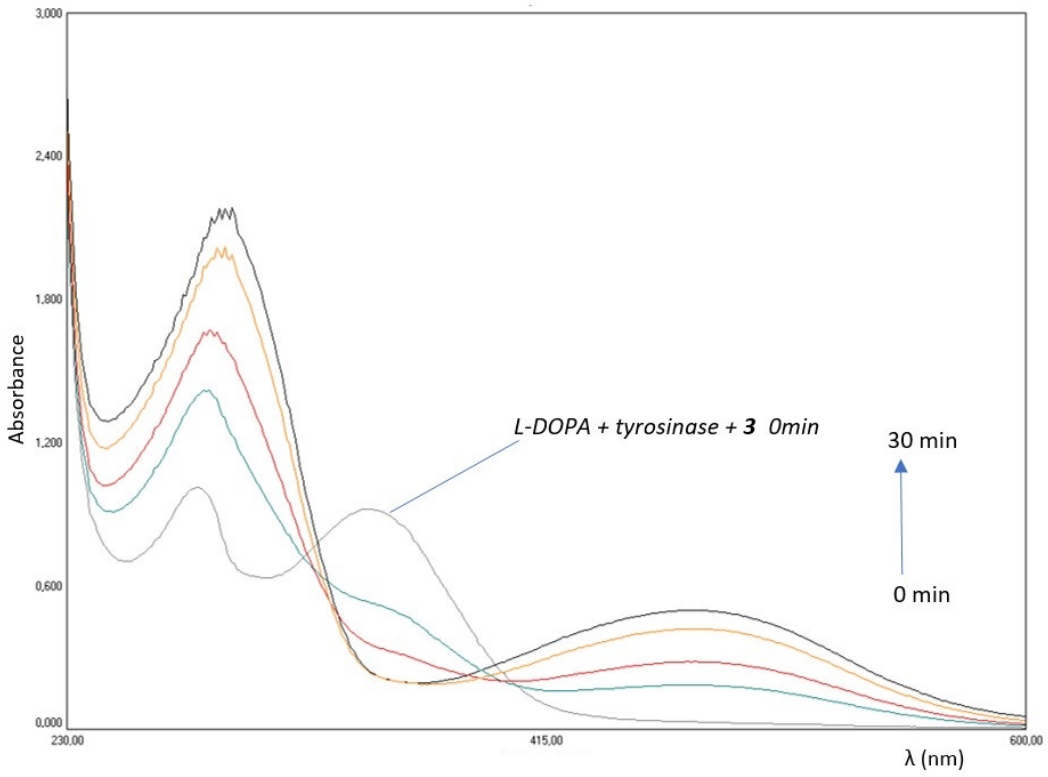


Figure 2. UV-Vis spectra obtained in the oxidation of L-DOPA by mushroom tyrosinase in the presence of inhibitors **3**.

In conclusion, we have developed an efficient one-step method for the synthesis of a new bis-thiazole derivative containing a 4-hydroxyphenyl moiety. Its structure was determined by spectroscopic methods. The obtained compound shows strong inhibition of tyrosinase, suggesting that this new derivative may reduce melanin production in melanoma cells by inhibiting tyrosinase. In addition, bis-thiazoles may be a so-far unexplored group of effective tyrosinase inhibitors.

3. Materials and Methods

All chemicals were purchased from commercially available sources and were used without any further purification. Synthesis was carried out in air atmosphere. The ^{1}H NMR (700 MHz) and ^{13}C NMR (176 MHz) spectra were recorded on a Bruker Avance III spectrometer (Bruker, Billerica, MA, USA) in dimethyl sulfoxide-d_6 (DMSO-d_6). The δ-values are reported in ppm. The HRMS analysis was carried out in the Laboratory for Analysis of Organic Compounds and Polymers of the Center for Molecular and Macromolecular Studies of the Polish Academy of Science in Łodź. The sample was ionized via electron spray ionization (ESI) in the positive mode. The chromatographic analysis was carried out on a Shimadzu HPLC Nexera XR combined with DAD SPD-M20A detector (Shimadzu, Kyoto, Japan). The mobile phase was acetonitrile and water in 20:80 volume ratios. The injection volume was 5 µL. The flow was set at 1 mL/min, the temperature of the chromatography oven at 25 °C. Compounds were detected in the range 190–800 nm. Open glass capillary was used to determine melting point value. Melting point value was determined in open glass capillary and is uncorrected. Macherey-Nagel Polygram Sil G/UV254 0.2 mm plates were used in TLC analysis. Visualization was performed under ultraviolet (UV) light. Fourier transform infrared (FTIR) spectra (4000–400 cm^{-1}) were recorded on a Shimadzu 8400S spectrometer as KBr pellets.

3.1. Synthesis of **3**

Compound **2** (0.250 g, 2.5 mmol) was added to a solution of 4-hydroxybenzaldehyde (0.610 g, 5 mmol) in ethanol (3 mL). The mixture was stirred at room temperature. After completion of the reaction (24 h, monitored by TLC, eluent—ethyl acetate:hexane 8:2), the obtained solid was filtered, washed by ethanol. The collected solid was recrystallized from mixture of chloroform with 5% methanol to give compound **3** with 71% yield (0.540 g), mp 138–141 °C.

^{1}H NMR (700 MHz, DMSO-d_6) δ (ppm): 6.32 (t, J = 7.0 Hz, 1H, CH-N), 6.35 (d, J = 3.5 Hz, 2H, CH_T), 6.73 (dm, J = 5.6 Hz, 2H, CH_{Ar}), 7.00 (d, J = 3.5 Hz, 2H, CH_T), 7.27 (dm, J = 5.6 Hz, 2H, CH_{Ar}), 8.21 (d, J = 7.0 Hz, 2H, NH), 9.41 (s, 1H, OH). ^{13}C NMR (176 MHz, DMSO-d_6) δ (ppm): 106.6, 116.1, 118.4, 126.2, 132.1, 138.7, 141.3, 162.2, 163.4, 168.9, 173.0. HPLC t_R (min): 1.826 (99.11%). UV-Vis (λ_{max}/nm): 346. FTIR (KBr, ν, cm^{-1}): 3340 (OH), 3178 (N-H), 3117 (N-H), 3075 (C-H), 2859 (NC-H), 2796 (NC-H), 1591 (N = C), 1506 (N-H), 1325 (S-C), 1245 (C-N), 824 (=C-H). HRMS (ESI) *m*/*z*: calculated for $C_{13}H_{13}N_4OS_2$ 305.0531 $[M + H]^+$. Found: 305.0532 $[M + H]^+$.

3.2. Mushroom Tyrosinase Inhibition Assay

The mushroom tyrosinase (Sigma-Aldrich) inhibition was performed following previously reported methods [10,22]. All the assays were carried out with solutions containing phosphate buffer (50 mM, pH 6.8), L-DOPA (0.17 mM), EDTA (0.022 mM), tyrosinase (50–100 units) and varying concentrations of tested compound **3** and were performed in triplicate at room temperature. The inhibitor solution was prepared in DMSO with an initial concentration of 1 mM. Different aliquots were added to the solution containing buffer, L-DOPA and EDTA, the enzyme being added in the end. Formation of dopachromone was determined by monitoring the absorbance at 475 nm with a T60U spectrophotometer (PG Instruments) equipped with quartz cells of 1 cm path length. Kojic acid and ascorbic acid were used as a reference inhibitor with an initial concentration of 1 mM. The IC_{50} values were calculated from the equation generated by exponential fit of the experimental data. The effectiveness of inhibition was expressed for the investigated compounds as the percentage of concentration necessary to achieve 50% inhibition (IC_{50}), calculated using the following equation:

$$\% \text{ of Inhibition} = \{[(B_{30} - B_0) - (A_{30} - A_0)] / (B_{30} - B_0)\} \times 100$$

where B_0 = absorbance of L-DOPA + tyrosinase at t = 0 min, B_{30} = absorbance of L-DOPA + tyrosinase at t = 30 min, A_0 = absorbance of L-DOPA + tyrosinase + inhibitor at t = 0 min and A_{30} = absorbance of L-DOPA + tyrosinase + inhibitor at t = 30 min.

3.3. Kinetic Analysis of the Inhibition of Tyrosinase

A series of experiments was performed to determine the inhibition kinetics of compound **3** by following the already reported method [23,24]. The inhibitor concentrations for tested compounds were 0.1 and 0.15 mM. Substrate L-DOPA concentration was between 0.1 and 0.25 mM in all kinetic studies. Maximal initial velocity was determined from the initial linear portion of absorbance up to 10 minutes after addition of enzyme. The inhibition type of the enzyme, Michaelis constant (K_m) and maximal velocity (V_{max}) were determined by Lineweaver–Burk plots of inverse of velocities (1/V) versus inverse of substrate concentration 1/[L-DOPA] mM^{-1}.

Supplementary Materials: The following supporting information can be downloaded online. ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY NMR, UV-Vis, HRMS, FTIR spectra and HPLC chromatogram of compound **3**.

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