JACOBS PUBLISHERS

OPEN ACCESS

Jacobs Journal of Medicinal Chemistry

Research Article

Synthesis of New Bis-Triazole Compounds Including Schiff Bases and Enzyme Inhibition and Antioxidant Activities

Yasemin Ünver[1*], Fatih Çelik[1], Esra Düğdü[1], Burak Barut[2], Kemal Sancak[1]

[1]*Department of Chemistry, Faculty of Sciences, Karadeniz Technical University, 61080 Trabzon, Turkey*

[2]*Karadeniz Technical University, Faculty of Pharmacy, Department of Biochemistry, Trabzon, Turkey*

**Corresponding author: Dr. Yasemin Ünver, Department of Chemistry, Faculty of Sciences, Karadeniz Technical University, 61080 Trabzon, Turkey, Tel: +90 462 377 2485; Fax: +90 462 325 3196; Email: unver.yasemincan@hotmail.com*

Received: 01-03-2016

Accepted: 27-04-2016

Published: 05-05-2016

Abstract

Compounds 1 and 2 possessing bis-triazole rings were reacted with four aromatic aldehydes and compounds 3-4(a-d) carrying Schiff bases were synthesized. All newly compounds well characterized by spectroscopic data such as FT-IR, ^{1}H NMR, ^{13}C NMR, LC-MS/MS, and elemental analyses. All the newly synthesized compounds were screened for their enzyme and antioxidant activities. This study was explained the acetylcholinesterase/butyrcholinesterase and tyrosinase inhibitory effects, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and phosphomolydenum reducing antioxidant power of the newly synthesized compounds for the first time. While compound 4a might be effective neurological agent, compounds 3c and 4c have antioxidant potential.

Keywords: Bis-Triazole; Schiff Base; Antioxidant; Enzyme Activities

Introduction

Antioxidants have capacity to protect organisms and cells from damage caused by oxidative stress during metabolism. For this reason, the synthetic compounds are extensively studied for their antioxidant activities using different methodologies. The search for active components that prevent or reduce the impact of oxidative stress on cells is a quite contemporary field. Exogenous chemicals involved in food systems and endogenous compounds involved in metabolic processes in human body produce highly reactive free radicals, particularly oxygen derived ones. They have the potential to oxidize biomolecules and cause cell death, consequently causing tissue damage. It is known that free radical oxidative processes also play a significant pathological role in causing many human diseases together with aging [1-2]. As oxidative stress plays an important role in Parkinson, Alzheimer, heart failure and cancer, the use of antioxidants is intensively studied in medicinal chemistry, particularly as a means for the treatment of these widespread diseases [3-7].

Heterocyclic compounds have an increasing importance in medicinal chemistry among the five membered heterocyclics. Especially various 1,2,4-triazole derivatives have been investigated as therapeutically interesting drug candidates because of their properties as esterase, antimicrobial and antimycotic agents, anti-inflammatory, anti-tubercular, antioxidant, anticancer, antimicrobial activity [8-14]. Some of the modern day drugs with triazole ring are Fluconazole, Itraconazole (antifungal agent), Ribavirin (antiviral agent), Rizatriptan (antimigrane agent) and Alprazolam (anxiolytic agent) Schiff bases obtained from various heterocycles have a wide range of biological activities including antifungal, antibacterial, antimalarial, antimycobacterial, antimicrobial, anti-inflammatory, antiviral, cytotoxic, anticonvulsant, antiproliferative, anticancer, antifungal and antipyretic activities [15-22].

On the basis of these observations, we thought of designing and synthesizing a new class of bis-heterocycles which possess triazole-schiff base. Additionally, we reported the investigation results of antioxidant with five different methods and enzyme

inhibition activities of all newly synthesized compounds in this paper.

Experimental

Chemistry

The ^{1}H-, and ^{13}C-Nuclear Magnetic Resonance spectra were recorded on an Agilent 400 MHz spectrometer, where tetramethylsilane (TMS) as an internal standard and Dimethyl sulfoxide-d_6 (DMSO-d_6) as solvent are used. IR spectra were recorded on a Perkin-Elmer Spectrum one FT-IR spectrometer (resolution 4) in KBr pellets. The MS spectra were measured with an Micromass Quattro LC-MS/MS spectrometer with methanol as solvent. Elemental analyses were carried out on a C,H, N-O rapid elemental analyzer Hewlett-Packard 185 for C, H and N and results are with in 0.4 % of the therotical values. Melting points were measured on an electro thermal apparatus and are uncorrected.

Synthesis of the Compounds 3-4

0.01 mol compounds 1-2 and 0.02 mol aromatic aldehydes was heated in oil bath dry to dry for 2-3 h. at 160-180 ^{0}C. After cooling it to room temperature, the solids 3-4 were obtained and recrystallized from a mixture of dimethyl formamide (DMF) and ethanol.

*2,2'-(4,4'-(butane-1,4-,diyl) bis (3-methy1-5-oxo-1H-1,2,4-triazole-4,1(4H,5H)-diyl))bis(N'-(thiophen-2-ylmethylene)acetohydrazide (**3a**)*. Yield: 86.40 %, m.p. 286-287 ^{o}C. IR (KBr, cm^{-1}): 3052 (aromatic CH), 1695 (C=O), 1624 (C=N), 1575 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.57 (s, 4H, 2N-CH_2-C$\underline{H}_2$), 2.17 (s, 6H, 2CH_3), 3.60 (s, 4H, 2N-C$\underline{H}_2$), 4.33 (s, 4H, N-C$\underline{H}_2$-C=O), 7.12-8.86 (m, 6H, Arom.H), 8.86 (s, 2H, 2N=CH), 10.17 (s, 2H, 2NH);^{13}C NMR (100 MHz, DMSO-d_6) δ :11.66 (CH_3), 25.92 (N-CH_2-$\underline{C}H_2$), 40.57 (N-CH_2), 46.60 (N-$\underline{C}H_2$-C=O), thiophene C [127.29 (CH), 128.37 (CH), 131.44 (CH), 136.63(C)], 143.06 (C=N), 154.18 (triazole C=O), 156.25 (N=CH), 169.85 (C=O); LC-MS (m/z): 721.41 (M^{+}+2+Na, 60%). Analysis (% Calculated/found) for $C_{26}H_{28}N_{14}S_4O_2$: C:44.81/44.56, H:4.05 /3.90, N:28.14/28.68.

*2,2'-(4,4'-(hexane-1,6-,diyl) bis (3-methy1-5-oxo-1H-1,2,4-triazole-4,1(4H,5H)-diyl))bis(N'-(thiophen-2-ylmethylene)acetohydrazide (**4a**)*. Yield: 90.45 %, m.p. 215-216 ^{o}C. IR (KBr, cm^{-1}): 3060 (aromatic CH), 1730 (C=O), 1644 (C=N), 1584 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.27 (s, 4H, 2N-CH_2-CH_2-C$\underline{H}_2$), 1.53 (s, 4H, 2N-CH_2-C$\underline{H}_2$), 2.15(s, 6H, 2CH_3), 3.50 (s, 4H, 2N-CH_2), 4.07 (s, 4H, 2N-$\underline{C}H_2$-C=O), 7.00-8.81 (m, 6H, Arom.H), 8.70 (s, 2H, 2N=CH), 10.01 (s, 2H, 2NH); ^{13}C NMR (100MHz, DMSO-d_6) δ :11.68 (CH_3), 25.86 (N-CH_2-CH_2-$\underline{C}H_2$), 28.70 (N-CH_2-$\underline{C}H_2$), 41.02 (N-CH_2), 46.55 (N-$\underline{C}H_2$-C=O), thiophene C [126.56 (CH), 128.68 (CH), 130.78 (CH), 136.00 (C)], 144.10 (C=N), 154.40 (triazole C=O), 155.85 (N=CH), 169.00 (C=O); LC-MS (m/z): 721.20 (M^{+}, 60%). Analysis (% Calculated/found) for $C_{28}H_{32}N_{14}S_4O_2$ C:46.39/46.16, H:4.45/4.07, N:27.05/27.80.

*2,2'-(4,4'-(butane-1,4-diyl) bis (3-methy1-5-oxo-1H-1,2,4-triazole-4,1(4H,5H)-diyl))bis(N'-benzylideneacetohydrazide) (**3b**)*. Yield: 80.10 %, m.p. 160-161 ^{o}C. IR (KBr, cm^{-1}): 3101 (aromatic CH), 1736 (C=O), 1657 (C=N), 1608 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ:1.56 (s, 4H, 2N-CH_2-C$\underline{H}_2$), 2.18 (s, 6H, 2CH_3), 3.60 (s, 4H, 2N-C$\underline{H}_2$), 4.37 (s, 4H, N-C$\underline{H}_2$-C=O), 7.12-8.7.65 (m, 10H, Arom.H), 8.85 (s, 2H, 2N=CH), 10.15 (s, 2H, 2NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ :11.67 (CH_3), 25.93 (N-CH_2-$\underline{C}H_2$), 40.56 (N-CH_2), 47.10 (N-$\underline{C}H_2$-C=O), Arom C [128.32 (CH), 128.39 (CH), 129.11 (CH), 131.08 (CH), 131.66 (CH), 134.26(C)], 143.90 (C=N), 154.40 (triazole C=O), 160.52 (N=CH), 169.86 (C=O); LC-MS (m/z): 685.50 (M^{+}+1, 60%). Analysis (% Calculated/found) for $C_{30}H_{32}N_{14}S_2O_2$ C:52.62/53.25, H:4.71/4.00, N:28.64/28.30.

*2,2'-(4,4'-(hexane-1,6-,diyl) bis (3-methy1-5-oxo-1H-1,2,4-triazole-4,1(4H,5H)-diyl))bis(N'-benzylideneacetohydrazide) (**4b**)*. Yield: 80.65 %, m.p. 220-221 ^{o}C. IR (KBr, cm^{-1}): 3076 (aromatic CH), 1732 (C=O), 1645 (C=N), 1625 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.27 (s, 4H, 2N-CH_2-CH_2-CH_2-C$\underline{H}_2$), 1.56 (s, 4H, 2N-CH_2-C$\underline{H}_2$), 2.18 (s, 6H, 2CH_3), 3.60 (s, 4H, 2N-CH_2), 4.38 (s, 4H, 2N-$\underline{C}H_2$-C=O), 7.52-7.89 (m, 10 H, Arom.H), 8.73 (s, 2H, 2N=CH), 10.06 (s, 2H, 2NH); ^{13}C NMR (100MHz, DMSO-d_6) δ :11.68 (CH_3), 25.86 (N-CH_2-CH_2-$\underline{C}H_2$), 28.71 (N-CH_2-$\underline{C}H_2$), 41.01 (N-CH_2), 46.56 (N-$\underline{C}H_2$-C=O), Arom C [128.83 (CH), 129.38 (CH), 131.56 (CH), 131.83 (CH), 134.26 (C)], 144.06 (C=N), 154.18 (triazole C=O), 161.94 (N=CH), 169.86 (C=O); LC-MS (m/z): 635.40 (M^{+}+Na, 60%). Analysis (%Calculated/found) for $C_{32}H_{36}N_{14}S_2O_2$ C:53.92/54.05, H:5.09/4.90, N:27.51/27.50.

*2,2'-(4,4'-(butane-1,4-,diyl) bis (3-methy1-5-oxo-1H-1,2,4-triazole-4,1(4H,5H)-diyl))bis(N'-(4-metoxybenzylidene)acetohydrazide) (**3c**)*. Yield: 81.50 %, m.p. 275-276 ^{o}C. IR (KBr, cm^{-1}): 3068 (aromatic CH), 1733 (C=O), 1655 (C=N), 1582 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ:1.56 (s, 4H, 2N-CH_2-C$\underline{H}_2$), 2.18 (s, 6H, 2CH_3), 3.60 (s, 4H, 2N-C$\underline{H}_2$), 3.83 (s, 6H, 2 OCH_3), 4.37 (s, 4H, N-C$\underline{H}_2$-C=O), 7.05-7.07 (m, 4H, Arom.H), 7.64-7.94 (m,4H, Arom.H) 8.64 (s, 2H, 2N=CH), 10.16 (s, 2H, 2NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ :11.67 (CH_3), 25.93 (N-CH_2-$\underline{C}H_2$), 40.61 (N-CH_2), 46.59 (N-$\underline{C}H_2$-C=O), 55.86 (OCH_3), Arom C [114.87 (CH), 127.04 (C), 130.45 (CH), 162.14 (C)], 144.06 (C=N), 154.18 (triazole C=O), 160.96 (N=CH), 169.85 (C=O); LC-MS (m/z): 744.25 (M^{+}, 60%). Analysis (% Calculated/found) for $C_{32}H_{36}N_{14}S_2O_4$ C:51.60/52.20, H:4.87/4.75, N:26.33/26.02.

*2,2'-(4,4'-(hexane-1,6-,diyl) bis (3-methy1-5-oxo-1H-1,2,4-triazole-4,1(4H,5H)-diyl))bis(N'-(4-metoxybenzylidene)acetohydrazide) (**4c**)*. Yield: 81.50 %, m.p.170-171 ^{o}C. IR (KBr, cm^{-1}): 3093 (aromatic CH), 1692 (C=O), 1619 (C=N), 1601 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.25 (s, 4H, 2N-CH_2-CH_2-C$\underline{H}_2$), 1.54 (s, 4H, 2N-CH_2-C$\underline{H}_2$), 2.19 (s, 6H, 2CH_3), 3.68 (s, 4H, 2N-CH_2), 3.83 (s, 6H, 2OCH_3), 4.38 (s, 4H, 2N-$\underline{C}H_2$-C=O), 6.86-7.07 (m, 4H, Arom.H), 7.81-7.83 (m, 4H, Arom.H), 8.84 (s, 2H, 2N=CH), 9.87 (s, 2H, 2NH); ^{13}C NMR (100MHz, DMSO-d_6) δ :11.69 (CH_3), 25.91 (N-CH_2-CH_2-$\underline{C}H_2$), 28.75 (N-CH_2-$\underline{C}H_2$), 40.98 (N-CH_2), 46.01 (N-$\underline{C}H_2$-C=O), 55.85 (OCH_3), Arom C [114.86

(CH), 127.05 (C), 130.44 (CH), 162.14(C)], 144.09 (C=N), 154.30 (triazole C=O), 160.95 (N=CH), 169.60 (C=O);LC-MS (m/z): 772.12 (M^+, 60%). Analysis (% Calculated/found) for $C_{32}H_{36}N_{14}S_2O_4$ C:51.60/52.20, H:4.87/4.75, N:26.33/26.02.

*2,2'-(4,4'-(butane-1,4-,diyl) bis (3-methy1-5-oxo-1H-1,2,4-triazole-4,1(4H,5H)-diyl))bis(N'-(2-hydroxybenzylidene)acetohydrazide) (**3d**).* Yield: 78.90 %, m.p. 246-247 °C. IR (KBr, cm^{-1}): 3085 (aromatic CH), 1734 (C=O), 1659 (C=N), 1582 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.56 (s, 4H, 2N-CH_2-C$\underline{H}_2$), 2.18 (s, 6H, 2CH_3), 3.60 (s, 4H, 2N-CH_2), 4.37 (s, 4H, 2N-CH_2-C=O), 4.98 (s, 2H, 2OH), 7.12-8.43 (m, 8H, Arom. H), 8.86 (s, 2H, N=CH), 10.15 (s, 2H, 2NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ :11.67 (CH_3), 25.92 (N-CH_2-$\underline{C}H_2$), 40.56 (N-CH_2), 46.58 (N-CH_2-C=O), Arom.C [127.30 (CH), 128.83 (CH), 131.07 (CH), 131.47 (CH), 132.34 (C), 138.67 (C)], 144.06 (C=N), 150.78 (triazole C=O), 159.28 (N=CH), 169.84 (C=O); LC-MS (m/z): 739.51 (M^++Na, 60%). Analysis (% Calculated/found) for $C_{30}H_{32}N_{14}S_2O_4$ C:50.27/50.98, H:4.50/4.20, N:27.36/26.97.

*2,2'-(4,4'-(hexane-1,6-,diyl) bis (3-methy1-5-oxo-1H-1,2,4-triazole-4,1(4H,5H)-diyl))bis(N'-(2-hydroxybenzylidene)acetohydrazide) (**4d**).* Yield: 75.48 %, m.p. 221-222 °C. IR (KBr, cm^{-1}): 3097 (aromatic CH), 1687 (C=O), 1621 (C=N), 1584 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.26 (s, 4H, 2N-CH_2-CH_2-C$\underline{H}_2$), 1.55 (s, 4H, 2N-CH_2-C$\underline{H}_2$), 2.17(s, 6H, 2CH_3), 3.38 (s, 4H, 2N-C$\underline{H}_2$), 4.30 (s, 4H, 2N-CH_2-C=O), 4.29 (s, 2H, 2OH), 7.14-8.78 (m, 6H, Arom.H), 8.88 (s, 2H, 2N=CH), 9.97 (s, 2H, 2NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ : 11.86 (CH_3), 25.80 (N-CH_2-CH_2-$\underline{C}H_2$), 28.88 (N-CH_2-$\underline{C}H_2$), 40.30 (N-CH_2), 47.11 (N-CH_2-C=O), Arom.C [127.35 (CH), 128.76 (CH), 131.12 (CH), 131.48 (CH), 132.67 (C), 144.01 (C)], 144.39 (C=N), 150.81 (triazole C=O), 158.59 (N=CH), 169.70 (C=O); LC-MS (m/z): 767.61 (M^++Na,60%). Analysis (%Calculated/found) for $C_{32}H_{36}N_{14}S_2O_4$ C:51.60/51.40, H:4.87/4.33, N:26.33/26.30.

Materials and Methods for Biological Activities

Chemicals and Reagents

Acetylcholinesterase enzyme (AChE) from electric eel, acetylthiocholine iodide, 5,5-dithio-bis(2-nitrobenzoic) acid (DTNB), galantamine, Trisma-base, tyrosinase from mushroom, *L*-DOPA, kojic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid (GA), methanol, ethanol, phoshomolybdic acid, and quercetin (QE) were purchased from Sigma-Aldrich.

Enzyme Inhibitions

Acetylcholinesterase/Butyrylcholinesterase(AChE/BChE) Inhibition

Acetylcholinesterase/Butyrylcholinesterase (AChE/BChE) inhibiton was determined by Ingkaninan et al. [23] colorimetric method. Galantamine was used as the reference drug. First of all, the stock solutions (2.5 mg/mL) were prepared from the all of the compounds in 25% DMSO and then five different concentrations made from stock solutions in the buffer (Tris-HCI pH 8.00) for the experiments. 50 mM Tris-HCl buffer (pH 8.00), 3 mM DTNB (in buffer), 0.2 U/mL AChE/ BChE and the compounds at different concentrations were added in a 96-well microplate. The mixtures were incubated for 15 min at 25°C. After incubation, 15 mM acetylthiocholine iodide/butyrylcholine chloride were added in the microplate and incubated 5 min at room temperature. The absorbance was measured at 412 nm using a 96-well microplate reader.

Inhibition of AChE/BChE was calculated by using the formula 1 and IC_{50} values obtained plotting the inhibition percentage against the compounds or reference drug. $A_{control}$ is the activity of enzyme without the compounds (solvent in buffer pH= 8) and A_{sample} is the activity of enzyme with the compounds at different concentrations. The experiments were carried out in triplicate and results were expressed as the mean ± standard deviation (SD).

Formula 1. $$Enzyme\ Inhibition\ (\%) = \left[\frac{(A_{control} - A_{sample})}{A_{control}}\right] \times 100$$

Tyrosinase Inhibition

Tyrosinase inhibiton was determined using the method described by Masuda et al. [24]. Kojic acid was used as the reference drug. Firstly, the stock solutions (2.5 mg/mL) were prepared from the all of the compounds in 25% DMSO. Then five different concentrations were prepared from the stock solution in the phosphate buffer (pH 6.8). 20 µL of the compounds at different concentrations 20 µL of 250 U/mL tyrosinase and 100 µL of 100 mM pH 6.8 phosphate buffer solutions were added in a 96-well microplate. The reaction was initiated with addition of 20 µL of 3 mM *L*-DOPA and the absorbance was measured at 475 nm using a 96-well microplate reader. Inhibition of tyrosinase was calculated by using the formula 1 and IC_{50} values obtained plotting the inhibition percentage against the compounds or reference compound. The experiments were carried out in triplicate and results were expressed as the mean ± standard deviation (SD).

Antioxidant Activities

DPPH Radical Scavenging Assay

The DPPH radical scavenging activities were examined using the method described by Blois et al [25] compared to gallic acid and as the reference compound. Total volume of assay mixture which was 1 mL, contained methanolic DPPH solution (0.4 mM) and different concentrations of the compounds. The mixtures were incubated for 30 min at room temperature in the dark. After incubation, the absorbance of the compound ($A_{compound}$) was measured at 517 nm. Assay mixture without

samples was used as a control ($A_{control}$). DPPH scavenging was calculated by using the formula 2 and IC_{50} values obtained plotting the inhibition percentage against the compounds or reference drug. The experiments were carried out in triplicate and results were expressed as the mean ± standard deviation (SD).

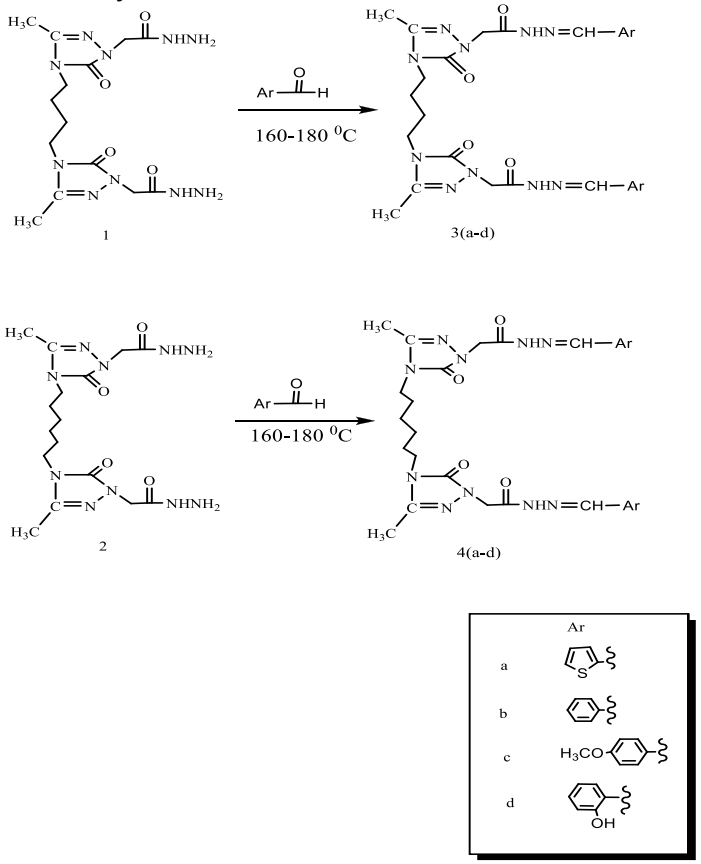
Formula 2. $$Scavenging\ effects\ (\%) = \left[\frac{(A_{compound} - A_{sample})}{A_{control}}\right] \times 100$$

Phosphomolibdenum-Reducing Antioxidant Power (PRAP) Assay

Phosphomolibdenum-reducing antioxidant power assay of the compounds were determined using phosphomolybdic acid compared to QE as the reference compound [26]. Total volume of assay mixture which was 1 mL, contained 10% phoshomolybdic acid solution in ethanol (w/v) and different concentrations (250 µg/mL, 500 µg/mL, and 1000 µg/mL) of the compounds. The mixtures were incubated for 30 min at 80 °C. After incubation, the absorbance was measured at 600 nm and compared to reference compound. The experiments were carried out in triplicate and results were expressed as the mean ± standard deviation (SD).

Results and Discussion

Chemistry



Scheme1. Synthetic pathway for the preparation of compounds 3-4(a-d)

The synthesis of bis-triazole compounds was performed following the steps shown in reaction the Scheme 1. The synthesis of known compounds 1 and 2 was performed according to previously reported procedure [8]. The reaction of compounds 1 and 2 with several aromatic aldehydes gave the bis triazoles 3-4(a-d) including Schiff bases respectively. NH2 peaks belonging to compounds 1 and 2 disappeared and the imine N=CH protons resonated as singlet at 8.64-8.88 ppm integrating for 2 protons (there are two N=CH groups in the structure) in the 1H-NMR spectra of compounds 3-4(a-d). In addition, imine N=CH appeared 155.85-161.94 ppm in the 13C NMR spectra of compounds 3-4(a-d). 13C and 1H NMR spectra of compounds 3-4 exhibited additional signals due to aromatic group moiety at the related chemical shift values. Morever, compounds 3-4 gave relatively stable molecular ion peaks in the Mass spectra.

Enzyme Inhibition

Alzheimer Disease (AD), described to dementia, has become a major health issue in countries because of expected number of patients increased to 25 million by 2025 [27]. This disease is related to shortage of acetycholine and butyrycholine which are hydrolyzed by acetylcholinesterase and butyrycholinesterase [28]. Therefore, acetylcholinesterase and butyrycholinesterase inhibitors are currently used for this disease. In this work, the AChE/BChE inhibiton of the compounds were examined by Ingkaninan's [23] assay with galantamine as a standard drug. AChE/BChE inhibition rates of the compounds were measured at different concentrations and the results of AChE/BChE of the compounds in this study were summarized in Table 1, Figure 1 and Figure 2 as IC_{50} values. In this work, the lowest IC_{50} values of the compounds indicate a higher inhibition effectiveness. IC_{50} values of the compounds were determined lower than 0.25 mg/mL. Compounds **4c** and **4a** were exhibited the lowest IC50 values of AChE with 0.113 ± 0.03 mg/mL and **4a** (0.113 ± 0.01 mg/mL) respectively, followed by compound **4d** (0.116 ±0.03 mg/mL). At the same time, compound **4d** was

Compounds	AChE	BChE	Tyrosinase	DPPH
3a	0.228 ± 0.05	0.180 ± 0.007	160.65 ± 0.01	0.941 ± 0.05
4a	0.113 ± 0.01	0.168 ± 0.01	144.51 ± 0.008	0.988 ± 0.08
3b	0.166 ± 0.009	0.114 ± 0.006	ND	0.845 ± 0.03
4b	0.155 ± 0.04	0.110 ± 0.009	ND	0.905 ± 0.01
3c	0.185 ± 0.04	0.207 ± 0.04	ND	0.781 ± 0.01
4c	0.113 ± 0.03	0.175 ± 0.01	ND	0.886 ± 0.009
3d	0.217 ± 0.006	0.100 ± 0.08	ND	0.918 ± 0.02
4d	0.116 ± 0.03	0.094 ± 0.08	ND	0.933 ± 0.07
Galantamine	0.019 ± 0.01	0.021 ± 0.03		
Kojic Acid			0.024 ± 0.01	
Gallic Acid				0.068 ± 0.005

Table1. Acetylcholinesterase, butyrylcholinesterase, tyrosinase and DPPH radical scavenging IC50 values (mg/mL) of the compounds(3-4)

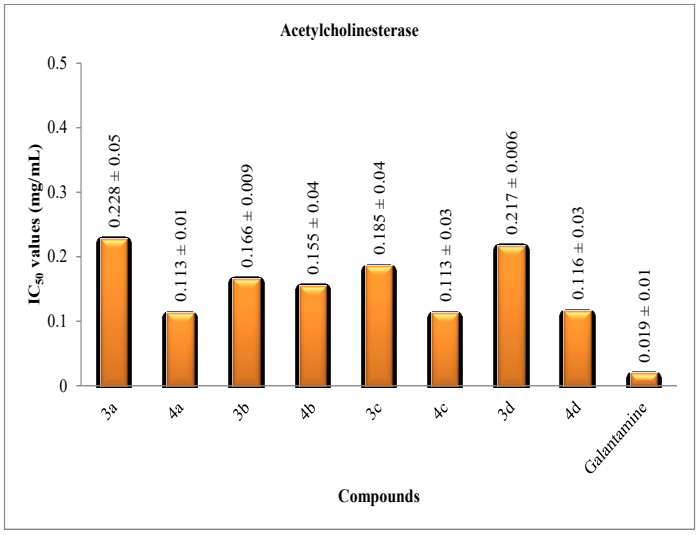


Figure1. Acetylcholinesterase IC_{50} values (mg/mL) of the compounds (3-4).

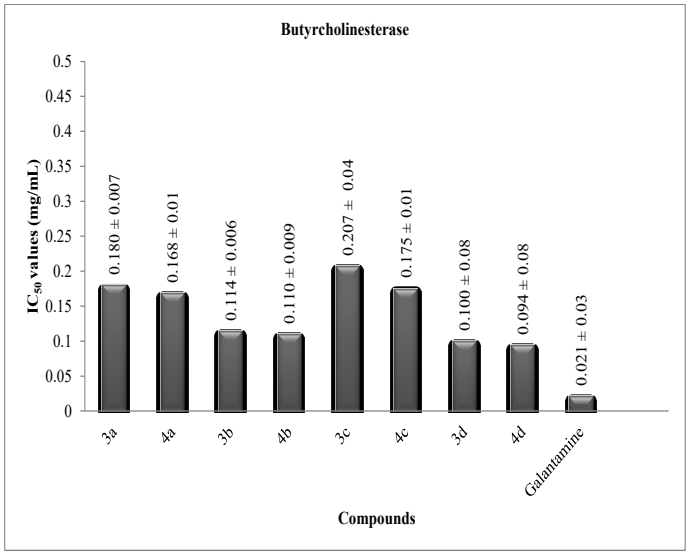


Figure2. Butyrylcholinesterase IC_{50} values (mg/mL) of the compounds (3-4).

showed lowest IC_{50} values of BChE with 0.094 ± 0.08 mg/mL followed by compound **3d** (0.100 ± 0.08 mg/mL) and compound **4b** (0.110 ± 0.03 mg/mL) respectively.

Tyrosinase enzyme plays a role formation of neuromelanin and damaged neurons related to Parkinson disease [28]. Therefore, researcher have aimed to find new tyrosinase enzyme inhibitor for against this disease. In this study, IC_{50} values of the compounds and reference drug on tyrosinase enzyme were summarized in Table 1. Compounds 3a and 4a inhibited tyrosinase enzyme and determined IC_{50} values among the all of the compounds. Compound 4a gave the lowest IC_{50} value with 0.144 ± 0.008 mg/mL, at the same time IC_{50} value of compound 3a was determined 0.160 ± 0.01 mg/mL. The other compounds had no activity against tyrosinase enzyme. All of the data of enzyme inhibition indicated that, compound 4a might be effective neurological agent.

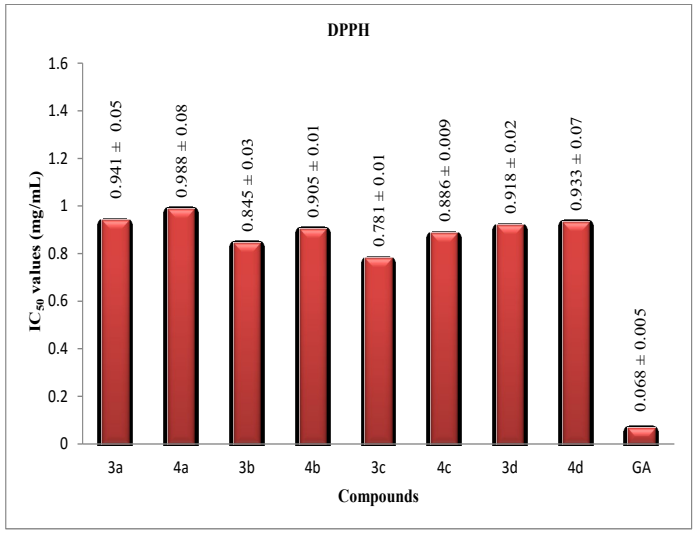


Figure3. DPPH radical scavenging activities IC_{50} values (mg/mL) of the compounds(3-4).

Antioxidant Activities

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule [29]. DPPH radical scavenging assay is a fast and simple method. DPPH radical scavenging activities of the compounds were examined based on reduction of absorbance at 517 nm compared to GA as reference compound [30]. The IC_{50} values for the DPPH radical scavenging activities of the compounds were presented in Table 1 and Figure 3. In this study, the lowest IC_{50} values of the compounds indicate a higher scavenging activities. All of the compounds exhibited radical scavenging activitites ranging from 0.781 ± 0.01 mg/mL to 0.988 ± 0.08 mg/mL. Compound **3c** showed the lowest IC_{50} value with 0.781 ± 0.01 mg/mL followed by compound **3b** (0.845 ± 0.03 mg/mL) and compound **4c** (0.886 ± 0.009 mg/mL), respectively. This results showed that, all of the newly synthesized compounds gave moderate scavenging activities to compare reference compound.

The reducing power activities of newly synthesized compounds were investigated by Phosphomolydenum reducing antioxidant power (PRAP). PRAP assay was based on the reduction of Mo(IV) to Mo(V) by the tested compounds [31]. PRAP was examined using phosphomolybdic acid compared to QE as the standard compound at 600 nm in this work. Absorbance of PRAP assay of the compounds and standard compound were summarized in Table 2. The highest absorbance values of the compounds showed a higher reducing antioxidant power. At 1 mg/mL, compound 3c gave the highest value with 2.0279 ± 0.049 followed by compound 4c as the DPPH scavenging assay. On the other hand, compound 4a showed the lowest value with 1.3191 ± 0.035. In this study, results of the antioxidant methods showed that compounds 3c and 4c have antioxidant potential.

mg/mL	0.25	0.5	1
3a	0.4754 ± 0.048	0.8474 ± 0.090	1.4632 ± 0.102
4a	0.4141 ± 0.050	0.704 ± 0.029	1.3191 ± 0.035
3b	0.4457 ± 0.059	0.8074 ± 0.024	1.6204 ± 0.034
4b	0.3681 ±0.092	0.7466 ± 0.031	1.3858 ± 0.047
3c	0.4842 ± 0.035	0.8244 ± 0.013	2.0279 ± 0.049
4c	0.4331 ± 0.078	0.6247 ± 0.037	1.9215 ± 0.126
3d	0.3531 ± 0.018	0.8064 ± 0.052	1.4431 ± 0.134
4d	0.3287 ± 0.040	0.8044 ± 0.033	1.4055 ± 0.102
QE	3.5339 ± 0.013	ND	ND

Table2. Absorbance of PRAP assay of the compounds at 600 nm.

Conclusion

In this study, the synthesis of new bis-1,2,4-triazole compounds possessing Schiff bases were reported. The structures of all the compounds were confirmed by recording their elemental analyses, IR, ^{1}H NMR, ^{13}C NMR and mass spectra. All the newly synthesized compounds were screened for their enzym and antioxidant activities. This study was explained the acetylcholinesterase/butyrcholinesterase and tyrosinase inhibitory effects, and DPPH radical scavenging and phosphomolydenum reducing antioxidant power of the newly synthesized compounds for the first time. While compound **4a** might be effective neurological agent, compounds **3c** and **4c** have antioxidant potential.

Acknowledgement

This study was supported by grants from Karadeniz Technical University.

References

1. Liu Z, Wang B, Yang Z, Li Y, Qin D et al. Synthesis, crystal structure, DNA interaction and antioxidant activities of two novel water-soluble Cu(2þ) complexes derivated from 2-oxo-quinoline-3- carbaldehyde Schiff-bases. Eur J Med Chem. 2009, 44(11): 4477-4484.

2. Hussain HH, Babic G, Durst T, Wright JS, Flueraru M et al. Development of Novel Antioxidants: Design, Synthesis, and Reactivity Org. Chem. 2003, 68(18): 7023-7032.

3. Sies H. Oxidative stress: oxidants and antioxidants. Exp Physiol. 1997, 82(2): 291-295.

4. Bekircan O, Menteşe E, Ülker S, Küçük C. Synthesis of some new 1,2,4-triazole derivatives starting from 3-(4-chlorophenyl)-5-(4-methoxybenzyl)-4H-1,2,4-triazol with anti-lipase and anti-urease activities. Arch Pharm Chem Life Sci. 2014, 347(6): 387-397.

5. Gangadhar BB, Prakash GA, Sangamesh AP, Prema SB. Synthesis, spectral characterization, in vitro antibacterial, antifungal and cytotoxic activities of Co(II), Ni(II) and Cu(II) complexes with 1,2,4-triazole Schiff bases. Eur J Med Chem. 2008, 43(12): 2639-2649.

6. Liu LJ, Leung KH, Chan DS, Wang YT, Ma DL et al. Identification of a natural product-like STAT3 dimerization inhibitor by structure-based virtual screening. Cell Death Dis. 2014, 5: e1293.

7. Ma DL, Lai TS, Chan FY, Chung WH, Abagyan R et al. Discovery of a Drug-Like G-Quadruplex Binding Ligand by HighThroughput Docking. ChemMedChem. 2008, 3(6): 881-884.

8. Düğdü E, Ünver Y, Ünlüer D, Sancak K. Synthesis and Biological Properties of Novel Triazole-Thiol and Thiadiazole Derivatives of the 1,2,4-Triazole-3(5)-one Class. Molecules. 2014, 19(2): 2199-2212.

9. Shiu-Hin C, Hui Y, Maria Hiu-Tung K, Zhen C, Paul L et al. Structure-based optimization of FDA-approved drug methylene blue as a c-myc G-quadruplex DNA stabilizer. Daniel Biochimie. 2011, 93(6): 1055-1064.

10. Gursoy A, Demirayak S, Cesur Z, Reisch J, Otuk G. Synthesis of some new hydrazide-hydrazones, thiosemicarbazides, thiadiazoles, triazoles and their derivatives as possible antimicrobials. Pharmazide. 1990, 45(4): 246-250.

11. Kritsanida M, Mouroutsou A, Marakos P, Pouli N, Pannecouque C et al. Synthesis and antiviral activity evaluation of some new 6-substituted 3-(1-adamantyl)-1,2,4-triazolo[3,4-b][1,3,4]thiadiazoles. IL Farmaco 2002, 57(3): 253-257.

12. Bayrak H, Demirbas A, Karaoglu ŞA, Demirbas N. Synthesis of some new 1,2,4-triazoles, their Mannich and Schiff bases and evaluation of their antimicrobial activities. Eur J Med Chem. 2009, 44(3): 1057-1066.

13. Khan KM, Siddiqui S, Saleem M, Taha M, Saad SM et al. Synthesis of triazole Schiff bases: Novel inhibitors of nucleotide pyrophosphatase/phosphodiesterase-1. Bioorg Med Chem Lett. 2014, 22(22): 6509-6514.

14. Tozkoparan B, Kupeli E, Yesilada E, Ertan M. Preparation of 5-aryl-3-alkylthio-l,2,4-triazoles and corresponding sulfones with antiinflammatory-analgesic activity. Bioorg Med Chem. 2007, 15(4): 1808-1814.

15. Kucukguzel I, Kucukguzel SG, Rollas S, Kiraz M. Some 3-Thioxo/Alkylthio-1,2,4-triazoles with a Substituted Thiourea Moiety as Possible Antimycobacterials. Bioorg Med Chem Lett. 2001, 11(13): 1703-1707.

16. Howell A, Cuzick J, Baum M, Buzdar A, Dowsett M et al. Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. Lancet. 2005, 365(9453): 60-62.

17. Ünver Y, Sancak K, Çelik F, Birinci E, Küçük M et al. New

thiophene-1,2,4-triazole-5(3)-ones: highly bioactive thiosemicarbazides, structures of schiff bases and triazole-thiols. Eur J Med Chem. 2014, 84: 639-650.

18. Bedi KK, Elcin O, Seda U, Fatma K, Nathaly S et al. Synthesis and characterization of novel hydrazide-hydrazones and the study of their structure-antituberculosis activity. Eur J Med Chem. 2006, 41(11): 1253-1261.

19. Kucukguzel SG, Rollas S, Habibe E, Kiraz M. Synthesis, characterization and antimicrobial evaluation of ethyl-2-arylhydrazono-3-oxobutyrates. Eur J Med Chem. 1999, 34(2): 153-160.

20. Maccari R, Ottana R, Vigorita MG. In vitro advanced antimycobacterial screening of isoniazid-related hydrazones, hydrazides and cyanoboranes: Part 14. Bioorg Med Chem Lett. 2005, 15(10): 2509-2513.

21. Rollas S, Gulerman N. Erdeniz H. Synthesis and antimicrobial activity of some new hydrazones of 4-fluorobenzoic acid hydrazide and 3-acetyl-2,5-disubstituted-1,3,4-oxadiazolines. IL Farmaco. 2002, 57(2): 171-174.

22. Panneererselvam P, Nair RR, Vijayalakshmi G, Subramanian EH, Sridhar SK. Synthesis of Schiff bases of 4-(4-aminophenyl)-morpholine as potential antimicrobial agents. Eur J Med Chem. 2005, 40(2): 225-229.

23. Ingkaninan K, de Best CM, van der Heijden R, Hofte AJ, Karabatak B et al. High-performance liquid chromatography with on-line coupled UV, mass spectrometric and biochemical detection for identification of acetylcholinesterase inhibitors from natural products. J. Chromatogr. A. 2000, 872(1-2): 61-73.

24. Masuda T, Yamashita D, Takeda Y, Yonemori S. Screening for tyrosinase inhibitors among extracts of seashore plants and identification of potent inhibitors from Garcinia subelliptica. Bioschi. Biotechnol. Biochem. 2005, 69(1): 197-201.

25. Blois M.S. Antioxidant Determinations by the Use of a Stable Free Radical. Nature. 1958, 181: 1199-1200.

26. Falcioni G, Fedeli D, Tiano L, Calzuola I, Mancinelli L et al. Antioxidant activity of wheat sprouts extract in vitro: inhibition of DNA oxidative damage. J. Food Sci. 2002, 67(8): 2918-2922.

27. Abbott A. Dementia: a problem for our age. Nature. 2011, 475: S2-S4.

28. Levent Altun M, Sever Yılmaz B, Ilkay Erdogan O, Saltan Citoglu G. Assessment of cholinesterase and tyrosinase inhibitory and antioxidant effects of Hypericum perforatum L. (St. John's wort) Industrial Crops and Products. 2013, 43: 87-92.

29. Soares JR, Dinis TC, Cunha AP. Almeida, LM. Antioxidant activities of some extracts of Thymus zygis. Free Radic. Res. 1997, 26(5): 469-478.

30. Smail A, Badiâ L, Maria G. Miguel. Antioxidant and Anti acetylcholinesterase Activities of Some Commercial Essential Oils and Their Major Compounds Molecules. 2011, 16, 7672-7690.

31. Apotrosoaei M, Vasincu IM, Dragan M, Buron F, Routier S et al. Design, Synthesis and the Biological Evaluation of New 1,3-Thiazolidine-4-ones Based on the 4-Amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one Scaffold. Molecules. 2014, 19(9): 13824-13847.