DOI: 10.17344/acsi.2024.8703 *Acta Chim. Slov.* **2024,** *71,* 566–579

Scientific paper

Synthesis of Schiff Bases of Usnic Acid and Investigation of Their Antidiabetic, Antidepressant, Anti-Parkinson's Disease, Neuroprotective and Antioxidant Potentials

Lawali Yabo-Dambagi,[1,*] Ahmet Cakir,[2,3] Mehmet Akyuz,[2,3] Aysegul Karakucuk-Iyidogan[1] and Ali Aslan[4,5]

[1] *Department of Chemistry, Faculty of Science and Letters, Gaziantep University, Gaziantep, Türkiye*

[2] *Department of Chemistry, Faculty of Science, Kilis 7 Aralık University, 79000-Kilis, Türkiye*

[3] *Advanced Technology Application and Research Center (ATARC), Kilis 7 Aralık University, 79000-Kilis, Türkiye*

[4] *Department of Pharmacology, Faculty of Pharmacy, Yüzüncü Yıl University, 5080-Van, Türkiye*

[5] *Department of Biology, Faculty of Arts and Science, Kyrgyz-Turkish Manas University, Bishkek, Kyrgyzstan*

** Corresponding author: E-mail: dambagi91@gmail.com, ld11012@mail2.gantep.edu.tr*

Received: 02-16-2024

Abstract

Schiff bases have various pharmacological activities due to the azomethine (–C=N–) group. Usnic acid is the most famous lichen metabolite and it contains two carbonyl groups to synthesize the Schiff base derivatives with primary amines. Therefore, in the current study, the known Schiff base derivatives **2**–**5** of usnic acid (**1**) were synthesized to explore their antidiabetic, neuroprotective, antioxidant, antidepressant and anti-Parkinson's disease properties. Among the tested compounds, compound **4** exhibited the strongest antidiabetic and antidepressant activities, inhibiting α-glycosidase, α-amylase and MAO-A enzyme activities, respectively. Moreover, all of the tested compounds strongly scavenged the ABTS and DPPH radicals and the ABTS radical scavenging activities of **3** and **4** were found to be higher than the commercial antioxidants BHA and trolox. None of the tested compounds showed any significant anti-Parkinson's disease activity or neuroprotective action. In conclusion, compound **4** can be suggested as a drug candidate molecule for further studies due to its strong antioxidant, antidiabetic and antidepressant properties.

Keywords: Usnic acid; Schiff base; Biological activity; Antidiabetic; Antidepressant; Antioxidant

1. Introduction

Schiff bases or imine bases have been frequently used in various fields of industry, such as the paint industry, polymer technology, pharmaceutical industry, medicine, agriculture, preparation of rocket fuel, and explanation of biological events, as well as in many other areas due to the groups in their structures.[1] Schiff bases can be synthesized from an aliphatic or aromatic amine and a carbonyl compound by nucleophilic addition, forming a hemiaminal, followed by dehydration to generate an imine. They contain the imine or azomethine (–C=N–) group in their chemical structures. Schiff bases have been used in medicine for various pharmaceutical purposes, such as anti-inflammatory, analgesic, antimicrobial, anticonvulsant, antituberculosis, anticancer, antioxidant and antihelminthic.[1–8] Imine bases are also known as good nitrogen ligands due to their ability to form complexes with metal ions. These ligands provide one or more electron pairs to the metal ion during the coordination compound's formation. Schiff bases can form highly stable 4-, 5-, and 6-membered ring complexes if they donate more than one electron pair.[2,9,10]

Medicinal plants or herbs have been used in the treatment of various diseases in traditional medicine practices since prehistoric times. The therapeutic properties of medicinal plants are frequently due to their secondary

metabolites.[11,12] Although lichens and mosses are both called non-vascular plants, lichens are not plants. Lichens are a complex life form that is a symbiotic partnership of two separate organisms, a fungus and an alga. Lichens are widespread symbionts and play important roles in many terrestrial ecosystems due to their quick adaptation to all climatic and geographical conditions.[13] Lichens, like plants, have been used in traditional medicine to treat various diseases since ancient times.[14–16] Unlike plants, lichens synthesize unique and characteristic secondary metabolites such as dibenzofurans, diphenyl ethers, depsides, depsidones and the degradation products of depsides and depsidones etc.[14,17–20] Usnic acid is a well-known metabolite synthesized by various lichen species and is notable for its diverse pharmacological properties, including analgesic, antibacterial, antiprotozoal, anti-inflammatory, antiulcer, anticholinergic, antiproliferative, and apoptotic effects against different cancer cell lines.[21–32] Usnic acid has two carbonyl groups in its chemical structure and thus, its Schiff base derivatives can be synthesized via condensation reactions with primary amines.[2–10,33–37] Therefore, in the current study, we aimed to evaluate the antidiabetic, antidepressant, anti-Parkinson's disease, anticholinesterases and antioxidant potentials of previously synthesized Schiff base derivatives of usnic acid with primary amines, 4-aminophenol, 3-aminophenol, 2-aminophenol and 4-aminomorpholine.

2. Experimental

2. 1. Reagents and Instrumentation

Solvents and all of the required reagents used in the synthesis and isolation process were provided by Merck (Darmstadt, Germany), Riedel de Haen, Fluka and Sigma-Aldrich (St. Louis, MO, USA). The ^{1}H NMR spectra of the synthesized compounds were measured in DMSO-d_6 using a Bruker 400 MHz instrument. An Agilent (Cary 600 Series) (4000–400 cm^{-1}) instrument with an ATR attachment was used to obtain the FTIR spectra. Melting points were measured with a EZ-Melt apparatus. Bioassay experiments were recorded on a UV-Visible Spectrophotometer (T80 + UV PG Instrument Ltd.). Thin layer chromatography (TLC) was performed on the silica gel 60F-254 (Merck) plate. The spots on TLC were visualized with UV light (wavelengths of 365 and 254 nm), and spraying (1% vanillin-H_2SO_4) and then heating at 105 °C. Column chromatography (CC) was carried out using silica gel (Merck, 70–230 and 200–400 mesh).

2. 2. Extraction of Lichen Sample and Isolation of Usnic Acid (1)

In this study, *Usnea longissima* used to isolate usnic acid was collected in the August–September period of 2021 from Northern Anatolian forests, then cleaned from other specimens and dried in a cool and shaded place by Dr. Ali Aslan.[25] Dried thalluses were powdered using a laboratory blender. In order to isolate a sufficient amount of usnic acid to be used in the synthesis, the lichen sample (1.13 kg) was extracted with hexane (5 × 1.5 L) by maceration during 24 h at room temperature to remove chlorophyll and other lipophilic constituents (4.33 g, 0.38%). After extraction with the hexane, the lichen sample was macerated with $CHCl_3$ (5 × 5 L) at room temperature and the solvent was evaporated from the extract via a rotary evaporator at a low temperature (60 °C). Afterward, it yielded 35.35 g (3.04%) of the extract that consisted of a high amount of acicular yellow crystals of usnic acid.[2,25] The crystals were quickly washed several times with hexane and then chloroform, and the purities of the chloroform phase and the crystals were controlled by TLC.

The chloroform phase containing usnic acid (**1**) with impurities was subjected to silica gel (70–230 mesh) column chromatography with $CHCl_3$ and $CHCl_3$-EtOAc (9:1) in order to isolate the remaining usnic acid (**1**) in the chloroform phase, and the fractions (25 mL) were checked by TLC. The fractions containing pure usnic acid (**1**) were combined, and the solvents were evaporated and weighed. At the end of the crystallization and chromatography processes, 25.35 g of usnic acid with a yield of 2.24% was purified as yellow acicular crystals.

2. 3. Synthesis of the Schiff Bases 2–5

(*S*,*E*)-6-Acetyl-3,7,9-trihydroxy-2-(1-(4-hydroxyphenylimino)ethyl)-8,9b-dimethyldibenzo[*b*,*d*]furan-1(9b*H*)-one (2). In order to synthesize the Schiff base derivative of usnic acid and 4-aminophenol, usnic acid (2 g, 5.80 mmol) was dissolved in 25 mL of $CHCl_3$ in a two-neck glass balloon (70 °C) and added dropwise 4-aminophenol (0.634 g, 5.80 mmol) dissolved in methanol (25 mL) to the reaction medium every 10 minutes. The reaction was continued for 2 days at the same temperature (70 °C) by refluxing, and the medium was cooled to room temperature. At the end of the process, a yellowish product **2** precipitated, and then it was carefully separated from the liquid phase using a dropper. Yield: 2.28 g (86.56%) of yellowish solid; mp: 255 °C (decomp.). ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.57 (s, 1H, O<u>H</u>,), 13.36 (s, 1H, O<u>H</u>,), 12,04 (s, 1H, O<u>H</u>, 8), 8.88 (s, 1H, O<u>H</u>, 4'), 7.17 (d, 2H, *J* = 8.68 Hz, 2' and 6'), 6.85 (d, 2H, *J* = 8.68 Hz, 3' and 5'), 5.89 (s, 1H, 4), 2.60 (s, 3H, 18), 2.49 (s, 3H, 15), 1.93 (s, 3H, 16), 1.65 (s, 3H, 13). ^{13}C NMR (100 MHz, DMSO-d_6): 198.3 (1), 102.6 (2), 189.9 (3), 102,6 (4), 173.9 (5), 157,7 (6), 101.3 (7), 163.1 (8), 106.9 (9), 158.0 (10), 105.4 (11), 57.1 (12), 32.2 (13), 174.3 (14), 20.7 (15), 7.9 (16), 201.3 (17), 31.4 (18), 127.2 (1'), 127.3 (2'), 116.4 (3'), 156.1 (4'), 116.4 (5'), 127.3 (6'). FTIR (ATR, ν cm^{-1}): 3275 (strong Ar-OH bands), 3000–2860 (week aliphatic C–H bands), 1689 and 1627 (C=O and –C=N– bands), 1600–1200 (aromatic C=C bands), 1200–1000 (C–O and C–N bands).[2] $[\alpha]_D^{23}$ +141.2° (*c* = 0.08, CH_2Cl_2).

(*S,E*)-6-Acetyl-3,7,9-trihydroxy-2-(1-(3-hydroxyphenylimino)ethyl)-8,9b-dimethyldibenzo[*b,d*]furan-1(9b*H*)-one (3). Usnic acid (2 g, 5.80 mmol) was dissolved in 25 mL of $CHCl_3$ in two-neck glass balloon via refluxing (70 °C), and 3-aminophenol (0.634 g, 5.80 mmol) dissolved in 25 mL of methanol was added dropwise to the reaction medium every 10 minutes. The reaction mixture was refluxed at 70 °C for 2 days, and the reaction was visualized intervally by TLC with CH_2Cl_2 : ethyl acetate (9 : 1) mobile phase. At the end of the 2nd day, TLC showed that the spot belonging to usnic acid decreased and that of the product increased. In order to isolate the product, the reaction mixture (2.5 g) was fractioned over silica gel (30 g, 70–230 mesh) CC using CH_2Cl_2 : EtOAc (9 : 1), and the fractions (10 mL) were controlled on TLC using the same mobile phase. The fractions containing the product were collected, and the solvent was evaporated. Yield: 1.88 g (71.30%) of yellowish solid; mp: 220 °C (decomp.). ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.72 (s, 1H, OH$_1$), 13.33 (s, 1H, OH$_1$), 11.92 (s, 1H, OH, 8), 9.97 (s, 1H, OH), 7.28 (t, 1H, J = 8.02 Hz, 5'), 6.81 (dd, 1H, J_1 = 8.19 Hz, J_2 = 1.88 and 1.92 Hz, 4'), 6.76 (d, 1H, J_1 = 7.92 Hz, 6'), 6,73 (d, 1H, J_1 = 1.84 Hz, 2'), 5.85 (s, 1H, 4), 2.56 (s, 3H, 18), 2.51 (s, 3H, 15), 1.91 (s, 3H, 16), 1.62 (s, 3H, 13). ^{13}C NMR (100 MHz, DMSO-d_6): 198.5 (1), 102.7 (2), 190.1 (3), 102,5 (4), 173.9 (5), 157.9 (6), 101.2 (7), 163.1 (8), 107.0 (9), 158.8 (10), 105.3 (11), 57.1 (12), 32.1 (13), 174.2 (14), 20.8 (15), 7.9 (16), 201.1 (17), 31.4 (18), 137.1 (1'), 112.8 (2'), 156.0 (3'), 115.7 (4'), 130.9 (5'), 116.5 (6'). FTIR (ATR, ν cm^{-1}): 3400–3200 (Ar–OH bands), 3000–2850 (week aliphatic C–H bands), 1694 and 1625 (C=O and –C=N– bands), 1600–1200 (aromatic C=C bands), 1100–1000 (C–O and C–N bands).[2] $[\alpha]_D^{23}$ +178.8° (c = 0.08, CH_2Cl_2).

***(S,E)*-6-Acetyl-3,7,9-trihydroxy-2-(1-(2-hydroxyphenylimino)ethyl)-8,9b-dimethyldibenzo[*b,d*]furan-1(9b*H*)-one (4).** In order to synthesize compound **4**, 2 g of usnic acid (5.80 mmol) was dissolved in $CHCl_3$ (25 mL) in a two-neck glass balloon via refluxing at 70 °C, and 2-aminophenol (0.634 g, 5.80 mmol) dissolved in methanol (25 mL) was added dropwise to the reaction medium every 10 minutes. The reaction was refluxed at 70 °C for 3 days and was controlled intermittently with TLC using CH_2Cl_2 : hexane (8.5 : 1.5) and CH_2Cl_2 : EtOAc (9 : 1). At the end of the third day, the reaction mixture was cooled to room temperature, and the product **4** precipitated as a light brownish solid. The precipitate was carefully separated from the liquid part using a dropper, and then its purity was checked on TLC with CH_2Cl_2 : EtOAc (9 : 1). Yield: 2.18 g (82.76%); mp: 259 °C (decomp.). ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.54 (s, 1H, OH$_1$), 13.37 (s, 1H, OH$_1$), 12.06 (s, 1H, OH, 8), 10.37 (s, 1H, OH), 7.28 (d, 1H, J = 7.88 Hz, 3'), 7.24 (td, 1H, J_1 = 7.82 and 7.76 Hz, J_2 = 1.15 and 1.40 Hz, 5'), 7.02 (d, 1H, J = 8.12 Hz, 6'), 6.90 (td, 1H, J_1 = 7.56 and 7.22 Hz, J_2 = 0.84 Hz, 4'), 5.93 (s, 1H, 4), 2.62 (s, 3H, 18), 2.50 (s, 3H, 15), 1.94 (s, 3H, 16), 1.67 (s, 3H, 13). ^{13}C NMR (100 MHz, DMSO-d_6): 198.4 (1), 102.8 (2), 190.0 (3), 102.7 (4), 174.0 (5), 156.1 (6), 101.3 (7), 163.0 (8), 106.9 (9), 158.0 (10), 105.5 (11), 57.1 (12), 32.2 (13), 174.6 (14), 20.8 (15), 8.0 (16), 201.3 (17), 31.5 (18), 123.4 (1'), 152.0 (2'), 127.3 (3'), 119.8 (4'), 130.0 (5'), 116.9 (6'). FTIR (ATR, ν cm^{-1}): 3371 (Ar–OH bands), 3000–2850 (week aliphatic C–H bands), 1687, 1624 and 1601 (strong C=O and –C=N– bands), 1600–1200 (strong aromatic C=C bands), 1100–1000 (strong C–O and C–N bands).[2] $[\alpha]_D^{23}$ +193.8° (c = 0.08, CH_2Cl_2).

***(S,E)*-6-Acetyl-3,7,9-trihydroxy-8,9b-dimethyl-2-(1-(morpholinoimino)ethyl)dibenzo[*b,d*]furan-1(9b*H*)-one (5)**. For the synthesis of the Schiff base **5**, usnic acid (3 g, 8.71 mmol) was disolved in $CHCl_3$ (25 mL) at 70 °C using a two-neck glass balloon, and then 1.80 g (17.42 mmol) of 4-aminomorpholine dissolved in methanol (25 mL) was added dropwise to the reaction medium every 10 minutes. The reaction was refluxed (70 °C) for 3 days, and it was checked intermittently with TLC using CH_2Cl_2 : EtOAc (9 : 1). It was observed that the product **5** was synthesized at a high rate at the end of the 3rd day. The solvent was evaporated and the solid residue was subjected to silica gel (30 g, 70–230 mesh) CC with CH_2Cl_2 : EtOAc (9 : 1) and the collected fractions (15 mL) were controlled over TLC. Yield: 3.61 g (75.20%) of yellowish solid; mp 189–191 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.62 (s, 1H, OH), 14.18 (s, 1H, OH), 11.43 (s, 1H, OH, 8), 5.71 (s, 1H, 4), 3.84 (m, 2H, 3' and 5'), 2.88 (m, 2H, 2' and 6'), 2.57 (s, 3H, 18), 2.15 (s, 3H, 18), 2.11 (s, 3H, 16), 1.67 (s, 3H, 13). ^{13}C NMR (100 MHz, DMSO-d_6): 198.8 (1), 99.2 (2), 190.9 (3), 101.3 (4), 173.9 (5), 167.8 (6), 99.2 (7), 161.0 (8), 104.2 (9), 154.1 (10), 108.0 (11), 57.5 (12), 31.9 (13), 175.3 (14), 17.8 (15), 8.0 (16), 206.9 (17), 17.9 (18), 55.8 (2'), 65.9 (3'), 66.0 (5'), 55.4 (6'). FTIR (ATR, ν cm^{-1}): 3375 (Ar–OH bands), 3000–2800 (aliphatic C–H bands), 1639, 1624 and 1609 (strong C=O and –C=N– bands), 1600–1200 (strong aromatic C=C bands), 1100–1000 (strong C–O and C–N bands). $[\alpha]_D^{23}$ +233.8° (c = 0.08, CH_2Cl_2).

2. 4. Enzyme Inhibition Assays

α-Glucosidase and α-amylase inhibition assays**.** α-Glucosidase and α-amylase enzyme measurements were made according to the previous methods,[38,39] and the experimental details were published in our previous studies.[6,40]

MAO sample preperation and MAO-A and MAO-B enzyme inhibition assays. A mitochondrial MAO sample was isolated by means of the previously described method from sheep liver.[41,42] MAO-A and MAO-B enzymes inhibition tests were performed according to the previous method with minor modifications.[42]

AChE and BChE enzyme inhibition assays. AChE and BChE enzyme measurements were made according to the previous method,[43] and the experimental details were published in our previous study.[6]

2. 5. Antioxidant Potentials

Radical scavenging activity. ABTS and DPPH radical scavenging activity assays of usnic acid and the synthesized Schiff bases were carried out according to the previous methods,[38,39,43–45] and the experimental details were published in our previous studies.[6,40,44]

Reducing powers. The experimental details for the total reducing powers of the Schiff bases and usnic acid were reported in our previous study.[44]

3. Results and Discussion

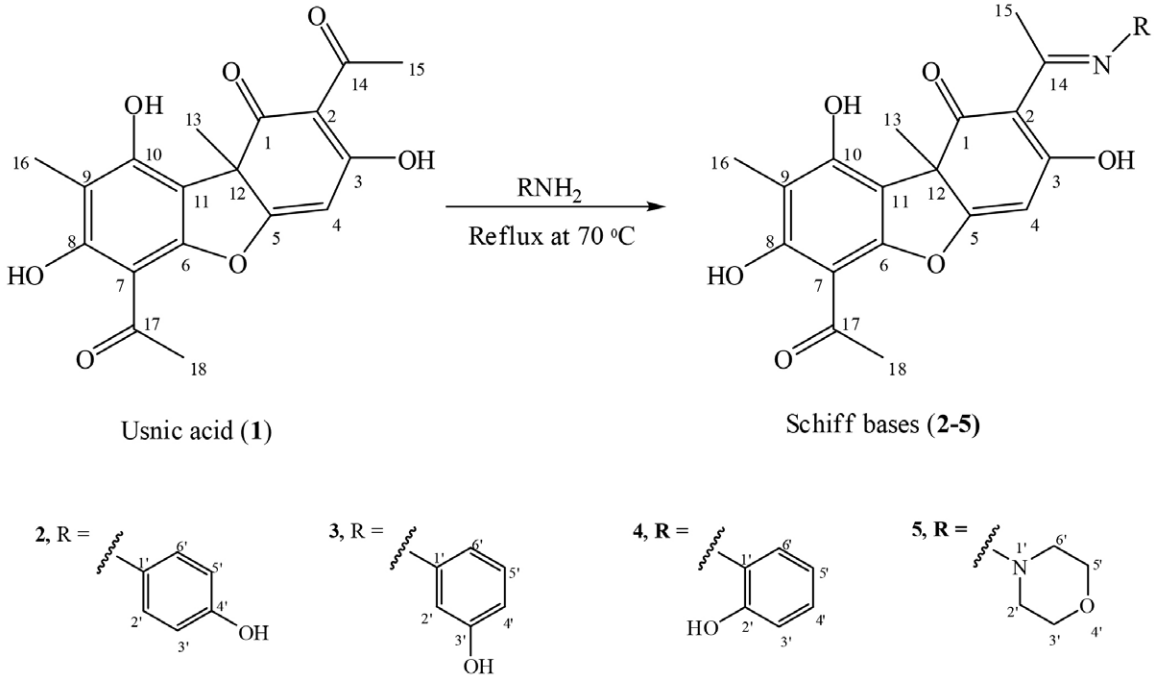
3. 1. Synthesis of the Schiff Base Derivatives of Usnic Acid (1)

Schiff bases have different pharmacological activities such as anti-inflammatory, analgesic, antimicrobial, anticonvulsant, antituberculosis, anticancer, antioxidant, antihelminthic, etc. due to the azomethine (–C=N–) group.[1–8] Usnic acid is the most famous lichen metabolite, and it contains two carbonyl groups that enable the synthesis of the Schiff base derivatives with primary amines. Hence, in the literature, there are numerous reports on the Schiff bases derivatives of usnic acid synthesized with different primary amines and their various pharmacological activities.[2,4,5,7,33–37] Therefore, in the current study, the known Schiff bases derivatives **2–5** of usnic acid (**1**) were synthesized via condensation reaction with 4-aminophenol, 3-aminophenol, 2-aminophenol and 4-aminomorpholine[2] (Scheme 1) to explore their new pharmacological activities. The chemical structures of the synthesized compounds were characterized by means of FTIR, ^{1}H and ^{13}C NMR, 1D- and 2D-NMR techniques (DEPT, APT ^{1}H,^{1}H-COSY, HMQC and HMBC) and confirmed comparing with previously published spetroscopic data.[2,4,5,7,35–37]

3. 2. Biological Activities of Usnic acid (1) and its Schiff Base Derivatives 2–5

3. 2. 1. Antidiabetic Activities

Diabetes is a chronic disease that occurs as a result of insulin production deficiency and/or insulin resistance and is characterized by hyperglycemia.[46,47] Nowadays, uncontrolled hyperglycemia is considered one of the most important health problems, leading to blindness, amputation, kidney failure, heart attacks, retinopathy, neuropathy, nephropathy, stroke and lower limb amputation.[46–50] There are two types of diabetes: type 1 (insulin-dependent) and type 2 (non-insulin-dependent).[46,47] Type 2 diabetes corresponding to 80–90% of the patients–and is becoming more common gradually due to the increase in the world population, the aging of people in society, the increase in obesity and the sedentary lifestyle.[49–52] Digestive enzymes, α-amylase and α-glucosidase, are primarily responsible enzymes for hyperglycemia in type 2 patients, and nowadays, the most preferred approach for the treatment of type 2 diabetes is to reduce hyperglycemia by inhibiting these enzymes after feeding.[46,53–55] Herewith, in the current study, the inhibitory effects of the Schiff bases **2–5** and usnic acid (**1**) on α-glucosidase and α-amylase enzyme activities were investigated to explore potential new antidiabetic agent(s) (Table 1). The tested compounds exhibited



Scheme 1. The chemical structures of the Schiff bases derivatives **2–5** of usnic acid (**1**).

different inhibitory effects in a concentration-dependent manner on the digestive enzymes. Furthermore, IC_{50} and IC_{90}, whose low values point out a potent inhibitor, were computed to compare the inhibitory properties of the tested compounds and the antidiabetic agent acarbose on the digestive enzymes (Table 2). As shown in these tables, usnic acid (**1**) and the synthesized Schiff bases **2**–**5** exhibited a stronger inhibitory effect against the α-glycosidase enzyme compared to α-amylase. For instance, all of the tested compounds inhibited α-glycosidase at low concentrations with IC_{50} values of 0.21–1.32 mg/mL (0.48–3.08 mM). However, they showed significant inhibitory effects against α-amylase at much higher concentrations, with IC_{50} values ranging from 1.52 to 79.41 mg/mL (3.49–230.84 mM) (Table 2). Among the tested compounds, compound **2** (IC_{50} = 0.21 mg/mL, 0.48 mM) was found to be the strongest inhibitor for α-glycosidase, whereas compound **4** acted as the strongest inhibitor of both α-glycosidase (IC_{50} = 0.55 mg/mL, 1.26 mM) and α-amylase (IC_{50} = 1.52 mg/mL, 3.49 mM). These results suggest that compound **4** has antidiabetic potential by inhibiting both digestive enzymes. Furthermore, our results indicate that usnic acid (**1**) and the Schiff bases **2**–**5** showed very strong inhibitory activity against α-glycosidase with lower IC_{50} values (0.21–1.32 mg/mL and 0.48–3.08 mM) compared to acarbose (IC_{50} = 22.41 mM and 14.47 mg/mL).On the other hand, acarbose was found to be more effective against α-amylase with an IC_{50} of 0.11 mg/mL compared to the inhibitory effects of usnic acid (**1**) and the Schiff bases **2**–**5**. Our findings are consistent with previous reports.[6,40,44,56–58] Our results also reveal that the new functional groups introduced via azomethine bonds in usnic acid affect the antidiabetic activity of the synthesized compounds (Table 2). For instance, Schiff bases **2** (IC_{50} = 0.21 mg/mL), **3** (IC_{50} = 0.54 mg/mL), and **4** (IC_{50} = 0.55 mg/mL) were more effective against α-glycosidase than usnic acid (**1**) (IC_{50} = 0.67 mg/mL), whereas compound **5** (IC_{50} = 1.32 mg/mL) showed a weaker inhibitory effect compared to usnic acid (**1**). Similar results were also found against α-amylase; all synthesized Schiff bases **2**–**5**, with IC_{50} values ranging from 1.52

Table 1. Antidiabetic effects of usnic acid and the synthesized Schiff bases

Treatments	Conc. (mg/mL)	α-Glycosidase Abs±SS	Inh (%)	Conc. (mg/mL)	α-Amylase Abs±SS	Inh (%)
Enzyme + substrate	-	**0.594±0.006f**	-	-	**0.433±0.008f**	-
	0.156	0.522±0.004e	12.12*	10	0.397±0.08e	8.31
	0.312	0.422±0.006d	28.96*	20	0.370±0.005d	14.55*
Enzyme + substrate + **1**	0.625	0.332±0.009c	44.10*	40	0.299±0.005c	30.95*
	1.25	0.052±0.009b	91.24*	80	0.227±0.007b	47.57*
	2.5	0.000±0.000a	100.00*	160	0.010±0.005a	97.69*
Enzyme + substrate	-	**0.626±0.006e**	-	-	**0.446±0.011d**	-
	0.062	0.488±0.006d	22.04*	10	0.240±0.011c	46.19*
	0.125	0.382±0.005c	38.97*	20	0.222±0.004c	50.22*
Enzyme + substrate + **2**	0.25	0.228±0.005b	63.58*	40	0.146±0.003b	67.26*
	0.5	0.026±0.003a	95.84*	80	0.000±0.000a	100*
Enzyme + substrate	-	**0.608±0.015e**	-	-	**0.356±0.003e**	-
	0.125	0.559±0.010d	8.05	20	0.301±0.003d	15.45*
Enzyme + substrate + **3**	0.25	0.504±0.006c	17.26*	40	0.205±0.002c	23.00*
	0.5	0.347±0.010b	42.93*	80	0.155±0.003b	56.00*
	1	0.006±0.000a	99.01*	160	0.000±0.000a	100*
Enzyme + substrate	-	**0.363±0.011e**	-	-	**0.510±0.011e**	-
	0.125	0.285±0.005d	21.49*	0.625	0.330±0.006d	35.30*
Enzyme + substrate + **4**	0.25	0.247±0.003c	31.96*	1.25	0.258±0.017c	49.41*
	0.5	0.209±0.010b	42.42*	2.5	0.190±0.006b	62.74*
	1	0.061±0.004a	83.20*	5	0.000±0.000a	100*
Enzyme + substrate	-	**0.369±0.037d**	-	-	**0.471±0.030e**	-
	0.125	0.354±0.001d	4.06	20	0.337±0.003d	28.30*
	0.25	0.337±0.007d	8.67	40	0.256±0.003c	45.53*
Enzyme + substrate + **5**	0.5	0.307±0.008c	16.80*	80	0.159±0.001b	66.17*
	1	0.231±0.006b	37.40*	160	0.000±0.001a	100*
	2	0.085±0.008a	76.80*			

Abs: Absorbance. Conc.: Concentration. Inh.: Inhibition. SD: Standard deviation.
*: Statistically different from enzyme + substrate applications ($p < 0.05$). The different letters in the lines are statistically different according to the Duncan test

to 77.33 mg/mL, acted as stronger inhibitors compared to usnic acid (**1**) (IC_{50} = 79.41 mg/mL) (Tables 1 and 2). In particular, compound **4** was noted to be a much stronger inhibitor of α-amylase, with IC_{50} values of 1.52 mg/mL and 2.58 mM. These results provide evidence that the new functional groups bonded to usnic acid (**1**) alter the interactions with the enzymes.

The previous reports indicated that the antidiabetic agents including acarbose, voglibose and miglitol strongly inhibit α-amylase but weakly inhibit α-glucosidase.[40,56–58] These agents also have side effects including diarrhea and abdominal bloating and abdominal pain due to the strong inhibition of the α-amylase enzyme. Therefore, it is an important advantage in the treatment of type 2 diabetes that the α-glucosidase enzyme is strongly inhibited and the α-amylase enzyme is weakly inhibited.[40,56–58] Tables 1 and 2 demonstrate that compounds **2**, **3**, and usnic acid (**1**) exhibited stronger inhibitory effects against α-glucosidase, while their inhibitory effects against α-amylase were comparatively weaker. Hereof, these compounds (**1**, **2**, and **3**) can also be recommended as potential antidiabetic agents besides compound **4**. Furthermore, it has been documented that potent α-glucosidase inhibitors can be used in the treatment of obesity as a result of slowing down glucose absorption from the blood.[59,60] Hence, usnic acid (**1**) and the synthesized Schiff bases **2**–**5** are the potential molecules to be used in the treatment of obesity.

Table 2. IC_{50} and IC_{90} values for antidiabetic effects of the treatments

Treatments	α-Glycosidase				α-Amylase			
	IC_{50}		IC_{90}		IC_{50}		IC_{90}	
	(mg/mL)	(mM)	(mg/mL)	(mM)	(mg/mL)	(mM)	(mg/mL)	(mM)
Acarbose*	14.47	22.41	29.66	45.94	**0.11**		**0.23**	
Usnic Asid (**1**)	**0.67**	**1.95**	**1.24**	**3.61**	79.41	230.84	147.77	429.56
synthesized Schiff bases								
2	**0.21**	**0.48**	**0.45**	**1.03**	17.33	39.84	68.02	156.37
3	**0.54**	**1.24**	**0.93**	**2.14**	77.33	177.56	141.80	325.98
4	**0.55**	**1.26**	**1.12**	**2.58**	**1.52**	**3.49**	**4.31**	**9.91**
5	**1.32**	**3.08**	**2.34**	**5.46**	54.76	127.94	135.73	317.13

*The data was acquired from our previous reports.[6]

3. 2. 2. Antioxidant Potentials

Reactive oxygen species (ROS) as unstable and highly reactive molecules are produced in normal or pathological cell metabolism as a result of cellular oxidation and play an important role in the pathogenesis of various diseases by causing tissue and organ damage.[61–63] The most common types of ROS are the superoxide ($O_2^{\bullet-}$), peroxyl ($ROO^{\bullet}$), hydroxyl ($OH^{\bullet}$), hydroperoxyl ($HO_2^{\bullet}$) and alkoxy ($RO^{\bullet}$), hydrogen peroxide (H_2O_2), ozone (O_3), singlet oxygen ($^1O_2$), hypochlorous acid (HOCl), peroxynitrite ($ONOO^-$), nitric acid (HNO_2) and nitrogen trioxide (N_2O_3).[64,65] It is well known that ROS damage functional molecules such as DNA, proteins and lipids, which have important functions in tissues by leading to the oxidative stress.[61–65] Cardiovascular diseases, cancer, diabetes, ischemia, asthma, arthritis, inflammation, rapid aging, Parkinson's, and Alzheimer's diseases are the diseases associated with the oxidative stress caused by ROS.[61–66] Living organisms have an oxidant/antioxidant balance against the damage of ROS, whereas some external factors like depression, environmental pollution, radiation, an unbalanced diet, pesticides, drugs and smoking may disrupt this balance in favor of oxidants.[64,65,67] Hence, it could be mandatory to use external antioxidants as a diet and/or medication to prevent or at least delay the development of the diseases mentioned above.[31,32,64,65,67,69,70] It has been documented that the lichen metabolite, usnic acid (**1**) has some pharmacological properties closely related to oxidative stress.[19,20,25,28,31,70–74]

The DPPH assay evaluates the capacity of antioxidants to scavenge free radicals by donating hydrogen, while the ABTS assay assesses antioxidant activity through a single-electron transfer mechanism. Previous research has demonstrated a lack of direct correlation between the outcomes of these two assays, suggesting that they evaluate antioxidant properties through different mechanisms. The DPPH and ABTS tests differ in their sensitivity to various types of antioxidants, and therefore, it is necessary to apply both methods to comprehensively assess the antioxidant potential of samples.[38,39,43–45] Thus, in the present research, the ABTS and DPPH radical scavenging potentials of the Schiff base derivatives **2**–**5** and usnic acid (**1**) were evaluated for the first time and the results are presented in Tables 3 and 4. As can be seen from these tables, usnic acid (**1**) and the synthesized Schiff bases **2**–**5** strongly scavenged the ABTS radicals with very low IC_{50} values (IC_{50} = 0.002–0.41 mM and 0.001–0.18 mg/mL). In particular, the ABTS radical scavenging activities of the Schiff bases **3** (IC_{50} = 0.002 mg/mL, 0.05 mM) and **4** (IC_{50} = 0.001 mg/mL, 0.002 mM) were found to be higher than the com-

Table 3. Radical scavenging effects of usnic acid and the synthesized Schiff base molecules

Treatments	Conc. (mg/mL)	DPPH Abs±SS	Scavenging (%)	Conc. (ppm)	ABTS Abs±SS	Scavenging (%)
Control	-	**0.502±0.007f**	-	-	**0.747±0.010f**	-
	1.25	0.344±0.002e	31.47*	31.25	0.447±0.006e	40.16*
	2.5	0.332±0.004d	33.86*	62.5	0.354±0.002d	52.61*
Usnic acid (**1**)	5	0.317±0.008c	36.85*	125	0.284±0.006c	61.98*
	10	0.299±0.008b	40.44*	250	0.050±0.005b	93.19*
	20	0.245±0.006a	51.20*	500	0.000±0.000a	100*
Control	-	**0.450±0.006e**	-	-	**0.878±0.007e**	-
	0.312	0.419±0.003d	6.89	62.5	0.613±0.005d	30.18*
	0.625	0.357±0.004c	20.67*	125	0.511±0.006c	41.80*
2	1.25	0.174±0.007b	61.33*	250	0.315±0.009b	64.12*
	2.5	0.000±0.000a	100*	500	0.000±0.000a	100*
Control	-	**0.443±0.004e**	-	-	**0.420±0.005e**	-
	1.25	0.286±0.006d	35.44*	6.25	0.203±0.011d	51.67*
	2.5	0.266±0.005c	39.96*	12.5	0.155±0.006c	63.10*
3	5	0.236±0.006b	46.73*	25	0.103±0.008b	75.48*
	10	0.199±0.005a	55.08*	50	0.000±0.000a	100*
Control	-	**0.437±0.002e**	-	-	**0.455±0.010f**	-
	0.125	0.346±0.004d	20.82*	1.25	0.231±0.010e	49.23*
	0.25	0.261±0.002c	40.27*	2.5	0.207±0.006d	54.51*
4	0.5	0.189±0.003b	56.75*	5	0.188±0.003c	58.68*
	1	0.000±0.000	100*	10	0.118±0.006b	74.07*
				20	0.005± 0.001a	98.90*
Control	-	**0.515±0.005e**	-	-	**0.601±0.006e**	-
	10	0.367±0.006d	26.41*	12.5	0.513±0.002d	14.64
	20	0.367±0.008c	28.74*	25	0.472±0.006c	21.46*
5	40	0.348±0.007b	32.43*	50	0.358±0.006b	38.60*
	80	0.305±0.011a	40.78*	100	0.190±0.006a	68.38

* Abs: Absorbance. SD: Standard deviation. *: Statistically different from control application ($p < 0.05$). The different letters in the lines are statistically different according to the Duncan test

mercial antioxidants, BHA (IC_{50} = 0.05 mg/mL, 0.28 mM) and trolox (IC_{50} = 0.07 mg/mL, 0.28 mM). The current results also demonstrate that the groups newly bound to usnic acid (**1**) provide an enhancing effect on the ABTS radical scavenging activity when compared to the ABTS radical scavenging activities of the synthesized ligands **2**–**5** and usnic acid (**1**). However, Table 4 shows that the synthesized compounds **2**–**5** and usnic acid (**1**) exhibited reduced DPPH radical scavenging activities in contrast to their ABTS radical scavenging activities. As indicated by Table 4, both usnic acid (**1**) (IC_{50} = 54.91 mM and 18.89 mg/mL) and the Schiff bases **2**–**5** (IC_{50} = 0.96–293.22 mM and 0.42–125.50 mg/mL) acted as weaker DPPH radical scavenging agents when compared to the commercial antoxidants, BHA (IC_{50} = 0.83 mM and 0.16 mg/mL) and trolox (IC_{50} = 0.48 mM and 0.12 mg/mL). On the other hand, the compounds **2**–**4** exhibited higher DPPH radical scavenging activities with lower IC_{50} values (0.42–7.30 mg/mL, 0.96–16.78 mM) compared to usnic acid (**1**) (Table 4). In accordance with the previous report,[6] these present results demonstrate that the new hydroxyphenylimino groups bound to usnic acid (**1**) have an enhancing effect on the DPPH radical scavenging activity. Nontheless, the compound **5** displayed a weaker DPPH radical scavenging effect with IC_{50} = 293.22 mM, 125.50 mg/mL than usnic acid (**1**) (IC_{50} = 18.89, 54.91 mM). These findings conclude that the carbonyl group in the usnic acid (**1**) is more effective in DPPH radical scavenging than the –CH=N– (azomethine) group. In conclusion, as can be seen from Table 4, the ligands **2**–**4** can be proposed as potent radical scavenging agents. However, further studies are needed to evaluate their safety and toxicities.

Another method to assess an agent's potential for antioxidant activity is the FRAP method, which is based on the reduction of iron(III) ions to iron(II) ions. According to this method, the high absorbance due to the high concentration of iron(II) measured in the medium indicates a high reduction potential.[75]

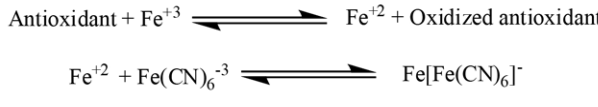


Table 4. IC_{50} and IC_{90} values for radical scavenging activities of the all treatments

Treatments	DPPH				ABTS			
	IC_{50}		IC_{90}		IC_{50}		IC_{90}	
	(mg/mL)	(mM)	(mg/mL)	(mM)	(mg/mL)	(mM)	(mg/mL)	(mM)
BHA*	**0.16**	**0.83**	**0.28**	**1.55**	**0.05**	**0.28**	**0.09**	**0.50**
Trolox*	**0.12**	**0.48**	**0.22**	**0.88**	**0.07**	**0.28**	**0.13**	**0.52**
Usnic acid (**1**)	18.89	54.91	58.55	170.20	0.065	0.18	0.238	0.68
synthesized Schiff bases								
2	**1.23**	**2.82**	**2.07**	**4.75**	**0.18**	**0.41**	**0.43**	**0.98**
3	7.30	16.78	25.64	58.94	**0.002**	**0.05**	**0.04**	**0.09**
4	**0.42**	**0.96**	**0.88**	**2.02**	**0.001**	**0.002**	**0.016**	**0.04**
5	125.50	293.22	323.54	755.93	**0.07**	**0.16**	**0.13**	**0.30**

*The data was acquired from the previous reports published by our research group.[6]

Hereof, in the current investigation, the reducing powers of usnic acid (**1**) and the synthesized Schiff bases **2**–**4** were also evaluated via the FRAP method and the results are shown in Figure 1. As shown in this figure, the synthesized ligands **2**–**5** and usnic acid (**1**) showed lower reducing power than BHA and trolox. However, the compound **4** displayed the highest and noteworthy reducing power among the tested compounds.

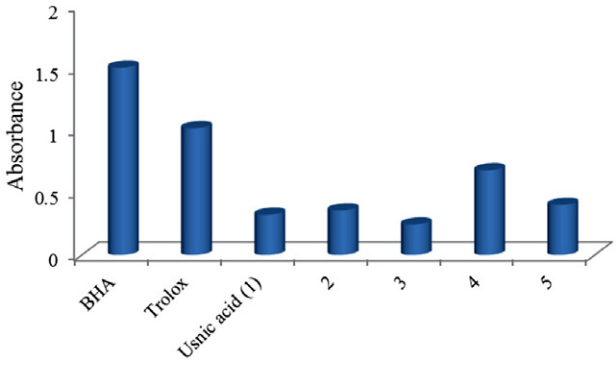


Figure 1. The reducing powers of the compounds **1**–**5**

3. 2. 3. Antidepressant and anti-Parkinson's Disease Activities

Depression or major depressive disorder is a common and serious mental illness that negatively affects how you feel, the way you think, and how you act. Depression causes feelings of sadness and/or a loss of interest in activities you once enjoyed. Depression can lead to various emotional and physical problems such as feeling sad, weight loss or gain due to a lifestyle change, sleeping problems, increased fatigue, purposeless physical activities, feeling guilty and worthless, indecision, and thoughts of death or suicide.[76–78] Moreover, it can be seen in all age and gender groups, and many patients are not aware of it.[76–79] The syndromes observed in depressed patients are closely related to the decrease in the levels of some biogenic amine neuromediators such as serotonin, dopamine and noradrenaline in the central nervous system.[77,78,80,81] It has also been shown that the levels of biogenic amines, serotonin, noradrenaline, norepinephrine, dopamine, catecholamines, homovalinic acid, and 5-OH indole acetic acid present in the blood, urine and brain fluids of patients suffering from depression are outside the normal range.[77,78,80,81] Monoamine oxidases (MAO-A and MAO-B) in the cells are the enzymes responsible for catalyzing the oxidative deamination of the neurotransmitters and their levels increase with age in humans.[78,82–85] It is well known that the decrease in the levels of neurotransmitters in the central nervous system causes the depressive syndroms as a result of increased activity of MAO's, in particular MAO-A.[81–83] Hereof, in the present study, the inhibitory effects of different concentrations of the synthesized compounds **2**–**5** and usnic acid (**1**) were tested for the first time on MAO-A to reveal new potential antidepressant agent(s) (Table 5). The inhibitory potentials of the compounds were also compared with those of the positive control, chlorgyline HCl (a selective MAO-A inhibitor). The IC_{50} and IC_{90} values for each treatment are also presented in Table 6. As can be seen from Table 6, chlorgyline with an IC_{50} value of 1.29 mg/mL (4.18 mM) was found to be a stronger inhibitor than usnic acid (**1**) (IC_{50} = 5.70 mg/mL, 16.57 mM) and the synthesized compounds **2**–**5** (IC_{50} = 3.18–14.83 mg/mL, 7.31–34.10 mM). However, among the tested compounds, compound **4** with an IC_{50} value of 3.18 mg/mL (7.31 mM) exhibited the strongest antidepressant effect by displaying a remarkable inhibitory effect after clorgyline on MAO-A enzyme activity. Considering the IC_{50} values presented in Table 6, the inhibition effects of the Schiff bases **2**–**5** were found to be lower than that of usnic acid (**1**) except for compound **4**. These can be accounted for by the fact that the interaction with MAO-A is altered by the new groups bound to usnic acid (**1**).

We now know that MAO-B-catalyzed bioreactions raise the amount of H_2O_2 in cells.[86,87] H_2O_2 is a neurotoxic substance that is involved in the pathogenicity of a number of illnesses, including depression, social anxiety,

Parkinson's, and Alzheimer's disease.[86–88] Thus, selective MAO-A inhibitors are frequently used in the treatment of neurodegenerative diseases such as depression and social anxiety, while selective MAO-B inhibitors are usually preferred in the treatment of Parkinson's, and Alzheimer's diseases.[79,83,86–91] In the current study, the inhibitory effects of the synthesized compounds **2**–**5** and usnic acid (**1**) at different concentrations were investigated for the first time on MAO-B and their inhibition effects were also compared with a selective MAO-B inhibitor, pargyline HCl (Table 5). The IC_{50} and IC_{90} values for each treatment were also calculated (Table 6). As shown in this table, usnic acid (**1**) and its derivatives **2**–**5** acted as weak inhibitors with very high IC_{50} values of 24.31–94.87 mg/mL or 55.86–264.80 mM as compared with pargyline (IC_{50} = 5.06 mg/mL, 25.86 mM). Moreover, none of the tested compounds acted as selective inhibitors against MAO's and they were found to be stronger inhibitors against MAO-A in comparison to

Table 5. Antidepressant and anti-Parkinson's disease properties of usnic asid (**1**) and the synthesized Schiff bases **2**–**5**

Treatments	MAO-A Conc. (mg/mL)	Abs±SS (%)	Inh (mg/mL)	Conc. (%)	MAO-B Abs±SS	Inh
Enzyme + substrate	-	**0.474±0.004e**	-	-	**0.486±0.004e**	-
	1.25	0.456±0.004d	3.80	20	0.438±0.009d	9.88
Enzyme + substrate + **1**	2.5	0.408±0.010c	13.92*	40	0.389±0.005c	19.96*
	5	0.250±0.001b	47.26*	80	0.230±0.003b	52.67*
	10	0.033±0.003a	93.04*	160	0.081±0.001a	83.33*
Enzyme + substrate	-	**0.562±0.007e**	-	-	**0.309±0.003e**	-
	10	0.269±0.005d	52.14*	20	0.285±0.004d	7.77
Enzyme + substrate + **2**	20	0.204±0.004c	63.70*	40	0.263±0.004c	14.89*
	40	0.033±0.005b	94.13*	80	0.123±0.00b	36.23*
	80	0.000±0.000a	100*	120	0.024±0.003a	92.23*
Enzyme + substrate	-	**0.693±0.002e**	-	-	**0.374±0.006e**	-
	5	0.479±0.001d	30.88*	5	0.361±0.008de	3.21
Enzyme + substrate + **3**	10	0.440±0.001c	36.51*	10	0.332±0.010d	11.23*
	20	0.242±0.001b	65.08*	20	0.270±0.008c	27.81*
	40	0.007±0.006a	98.99*	40	0.154±0.012b	58.82*
	80	0.000±0.000a	100*			
Enzyme + substrate	-	**0.496±0.010d**	-	-	**0.356±0.012f**	-
	0.625	0.468±0.001cd	5.64	2.5	0.339±0.005e	4.76
	1.25	0.446±0.002c	10.08*	5	0.322±0.001d	9.55
Enzyme + substrate + **4**	2.5	0.284±0.001b	42.74*	10	0.300±0.004c	15.73*
	5	0.094±0.003a	81.04*	20	0.171±0.003b	51.97*
	40	0.078±0.002a	78.09*			
Enzyme + substrate	-	**0.498±0.007e**	-	-	**0.372±0.005e**	-
	5	0.387±0.003d	22.29*	10	0.342±0.003d	8.06
Enzyme + substrate + **5**	10	0.285±0.005c	42.77*	20	0.306±0.002c	17.74*
	20	0.072±0.002b	85.54*	40	0.207±0.001b	44.35*
	40	0.000±0.000a	100*	80	0.077±0.001a	79.30*
Enzyme + substrate	-	**0.587±0.013f**	-			
	0.312	0.512±0.005e	12.78*			
	0.625	0.458±0.008d	21.98*			
Enzyme + substrate +	1.25	0.261±0.003c	55.54*			
clorgyline	2.5	0.044±00004b	92.50*			
	5	0.000±0.000a	100.00*			
Enzyme + substrate				-	**0.431±0.004e**	-
				2.5	0.270±0.003d	37.35*
Enzyme + substrate + pargyline				5	0.215±0.002c	50.12*
				10	0.115±0.003b	73.32*
				20	0.000±0.000a	100.00*

Abs: Absorbance. SD: Standard deviation.
*:Statistically different from control application ($p < 0.05$). The different letters in the lines are statistically different according to the Duncan test.

MAO-B**.** These results point out that usnic acid (**1**) and the synthesized Schiff bases **2**–**5** do not have anti-Parkinson's disease activity due to their weak and non-selective inhibitory effects on MAO-B activity. Nevertheless, new 4-hydroxyphenylimino and 3-hydroxyphenylimino groups bound to usnic acid (**1**) (IC_{50} = 91.09 mg/mL, 264.80 mM) via an azomethine bond significantly increased the inhibition effect of the compounds **4** (IC_{50} = 34.31 mg/mL, 78.87 mM) and **3** (IC_{50} = 24.31 mg/mL, 55.86 mM) on the MAO-B activity.

It has been reported in the literature that pargyline and chlorgyline are potent inhibitors with extremely low IC_{50} values.[6,28,92,93] However, in the current study, the IC_{50} values were calculated as higher values for chlorgyline (IC_{50} = 1.29 mg/mL, 4.18 mM) and pargyline (IC_{50} = 5.06 mg/mL, 25.86 mM). This could be accounted for by variations in the assaying techniques employed.[6,28,92,93] Likewise, previously, 0.34 mg/mL and 1.25 mM IC_{50} values for chlorgyline were determined using a different assay method.[6]

nausea, vomiting, agitation, diarrhea, loose stools, nighttime vivid dreams, dehydration, skin rash, bradycardia, peptic ulcer, seizures, weight loss, rhinorrhea, salivation, muscle cramps, and fasciculations.[98,100,103,104] Therefore, further studies focused on new cholinesterase inhibitors that are safer and have fewer side effects are still important for human health.

Hence, in the present work, the inhibition effects of usnic acid (**1**) and the Schiff bases **2**–**5** at different concentrations were evaluated on AChE and BChE activities for the first time to discover new potentially neuroprotective compounds (Table 7). The IC_{50} and IC_{90} calculated for usnic acid (**1**), the Schiff bases **2**–**5** and commercial anticholinesterases, neostigmine and galantamine are also presented in Table 8. As shown in this table, the IC_{50} values for usnic acid (**1**) and the compounds **2**–**5** were determined to be very high with values 54.64–688.69 mM (23.77–294.76 mg/mL) and 24.81–56.12 mM (10.79–24.41 mg/mL) when compared with the inhibitory effects of neostigmine (IC_{50} = 2.87 mM, 0.64 mg/mL) and galantamine (IC_{50} = 16.63

Table 6. IC_{50} and IC_{90} values for the antidepressant, anti-Parkinson's disease effects of the treatments

Treatments	MAO-A				MAO-B			
	IC_{50} (mg/mL)	(mM)	IC_{90} (mg/mL)	(mM)	IC_{50} (mg/mL)	(mM)	IC_{90} (mg/mL)	(mM)
Chlorgyline HCl	**1.29**	**4.18**	**2.37**	**7.68**	-	-	-	-
Pargyline HCl	-	-	-	-	**5.06**	**25.86**	**10.42**	**53.24**
Usnic acid (**1**)	**5.70**	**16.57**	**9.56**	**27.79**	91.09	264.80	166.45	483.87
Synthesized Schiff bases								
2	9.23	21.22	37.45	86.09	94.87	218.09	159.91	367.60
3	14.83	34.10	34.78	79.75	34.31	78.87	59.48	136.73
4	**3.18**	**7.31**	**5.42**	**12.46**	24.31	55.86	43.90	100.92
5	11.62	27.15	21.09	49.28	49.82	116.40	88.82	207.52

3. 2. 4. Neuroprotective Effects

Alzheimer's disease is the most common dementia disease in older adults with a prevalence of 10% after the age of 65.[94,95] Physical and mental behavioral disorders such as language, writing and reading difficulties, and memory loss are observed in Alzheimer's disease patients due to the gradual loss of cells in some parts of the brain.[66,96–98] The loss of cholinergic neurotransmitters, acetylcholine (ACh) and butyrylcholine (BCh) in the brain is known to be one of the main causes of Alzheimer's disease.[96–100] There is also evidence that the brain tissues of Alzheimer's disease patients have higher concentrations of the enzymes AChE and BChE, which use ACh and BCh, respectively, as substrates.[98,99,101] Nowadays, the enhancement of cholinergic neurotransmission by the inhibition of cholinesterases is the main approach in the symptomatic treatment of Alzheimer's disease and dementia.[96,98–102] The most widely used agents as cholinesterase inhibitors are donepezil, rivastigmine, and galantamine; however, they are associated with some side effects like appetite loss,

mM, 4.78 mg/mL).[6] These results conclude that neither usnic acid (**1**) nor its derivatives **2**–**5** have any noteworthy neuroprotective potential as compared with commercial anticholinesterases. However, our results demonstrated that the new functional groups added to usnic acid (**1**) affect the neuroprotective activities of the synthesized compounds **2**–**5** (Tables 7 and 8). In particular, hydroxyphenylimino ligand derivatives **2**–**4** of usnic acid acted as stronger AChE enzyme inhibitors with 23.77–45.00 mg/mL or 103.44–54.64 mM of IC_{50} values than usnic acid (**1**) (IC_{50} = 94.03 mg/mL, 273.34 mM). On the other hand, the ligand **5** displayed a much weaker inhibitory effect with IC_{50} = 294.76 mg/mL or 688.69 mM against the AChE enzyme than usnic acid (**1**). Similar results for the treatments were also obtained against BChE activity (Table 8). As can be seen from Table 8, the inhibition effects of the synthesized compounds **2**–**5** with lower IC_{50} values (IC_{50} = 10.79–24.41 mg/mL, 24.81–56.12 mM) were found to be higher than that of usnic acid (**1**) (IC_{50} = 38.61 mg/mL, 112.24 mM).

Table 7. Neuroprotective effects of usnic acid (**1**) and the synthesized Schiff bases **2**–**4**

Treatments	AChE Conc. (mg/mL)	AChE Abs±SS	AChE Inh (%) (mg/mL)	BChE Conc.	BChE Abs±SS	BChE Inh (%)
Enzyme + substrate	-	**0.498±0.003e**	-	-	**0.374±0.003e**	-
	20	0.462±0.007d	7.23	10	0.332±0.009d	11.23*
Enzyme + substrate +	40	0.428±0.004c	14.06*	20	0.248±0.006c	33.69*
Usnic acid (**1**)	80	0.302±0.003b	39.36*	40	0.169±0.005b	54.81*
	160	0.036±0.002a	92.77*	80	0.010±0.002a	97.33*
Enzyme + substrate	-	**0.688±0.008f**	-	-	**0.390±0.006f**	-
	10	0.517±0.015e	24.85*	5	0.345±0.014e	11.54
	20	0.395±0.009d	42.58*	10	0.287±0.010d	26.41*
Enzyme + substrate + **2**	40	0.279±0.006c	59.44*	20	0.204±0.014c	47.69*
	80	0.086±0.003b	87.5*	40	0.090±0.006b	76.92*
	160	0.020±0.005a	97.07*	80	0.000±0.000a	100*
Enzyme + substrate	-	**0.423±0.006e**	-	-	**0.378±0.012e**	-
	20	0.218±0.007d	48.46*	10	0.318±0.005d	17.20*
Enzyme + substrate + **3**	40	0.157±0.003c	62.88*	20	0.202±0.005c	46.50*
	80	0.017±0.006b	95.98*	40	0.052±0.005b	86.24*
	160	0.000±0.000a	100*	80	0.000±0.000a	100*
Enzyme + substrate	-	**0.398±0.005e**	-	-	**0.336±0.007e**	-
	20	0.317±0.009d	20.35*	5	0.239±0.009d	27.98*
Enzyme + substrate + **4**	40	0.235±0.008c	40.95*	10	0.188±0.008c	44.04*
	80	0.020±0.002b	94.98*	20	0.037±0.006b	88.98*
	160	0.000± 0.000a	100*	40	0.000±0.000a	100*
Enzyme + substrate	-	**0.474±0.006d**	-	-	**0.370±0.004f**	-
	40	0.452±0.013d	4.64	5	0.333±0.008e	10.00*
Enzyme + substrate + **5**	80	0.412±0.010c	13.08*	10	0.285±0.011d	22.92*
	160	0.256±0.004b	24.89*	20	0.204±0.004c	44.60*
	320	0.214±0.006a	54.85*	40	0.075±0.005b	78.67*
				80	0.000±0.000a	100*

Abs: Absorbance. Conc.: Concentration. Inh.: Inhibition. SD: Standard deviation. *: Statistically different from enzyme + substrate applications ($p < 0.05$). The different letters in the lines are statistically different according to the Duncan test

Table 8. IC_{50} and IC_{90} values for the neuroprotective effects of the all treatments

Treatments	AChE IC_{50} (mg/mL)	AChE IC_{50} (mM)	AChE IC_{90} (mg/mL)	AChE IC_{90} (mM)	BChE IC_{50} (mg/mL)	BChE IC_{50} (mM)	BChE IC_{90} (mg/mL)	BChE IC_{90} (mM)
Neostigmine*	**0.64**	**2.87**	**2.42**	**10.84**	**0.03**	**0.10**	**0.06**	**0.20**
Galantamine*	**4.78**	**16.63**	**15.31**	**53.34**	**0.31**	**1.08**	**0.94**	**3.28**
Usnic acid (**1**)	94.03	273.34	157.83	458.81	38.61	112.24	71.91	209.04
Synthesized Schiff bases								
2	33.26	76.46	80.41	184.85	23.91	54.97	45.95	105.63
3	23.77	54.64	69.99	160.90	23.25	53.45	41.16	94.62
4	45.00	103.44	76.77	176.48	10.79	24.81	20.49	47.10
5	294.76	688.69	520.62	1216.40	24.41	56.12	45.11	103.70

*The data was acquired from the previous reports published by our research group.[6]

4. Conclusions

In the current study, the Schiff base derivatives **2**–**5** of a famous lichen metabolite, usnic acid (**1**) were synthesized via the condenzation reaction with 4-aminophenol, 3-aminophenol, 2-aminophenol and 4-aminomorpholine. The antidiabetic, antioxidant, antidepressant, anti-Parkinson's disease and neuroprotective activities of the com-

pounds were also evaluated for the first time. Our results conclude that the compound **4** was found to be a drug candidate molecule for further investigations due to its potent antidiabetic and antioxidant potentials, besides its noteworthy antidepressant effect.

Acknowledgements

The authors would like to more thanks Dr. Cavit Kazaz (Atatürk University) and Dr. Tuba Aydın (Ağrı Ibrahim Çeçen University) for acquiring NMR spectra of the synthesized compounds.

Reseach Funding

The authors are grateful to the Scientific Research Project Governing Unit (BAPYB) of Gaziantep University (Project No. FEF.DT.22.11) for financial support of this research.

Conflicts of interes

The authors wish to confirm that there are no known conflicts of interest associated with this publication.

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Povzetek

Schiffove baze imajo zaradi prisotnosti azometinske (–C=N–) skupine mnoge farmakološke aktivnosti. Usninska kislina je najbolj znan metabolit lišajev; vsebuje dve karbonilni skupini iz katerih je mogoče s primarnimi amini sintetizirati Schiffove baze. V okviru te študije smo iz usninske kisline (**1**) pripravili znane Schiffove baze **2**–**5** z namenom raziskati njihove antidiabetične ter nevrozaščitne lastnosti, antioksidativne aktivnosti in lastnosti delovanja proti depresiji ter Parkinsonovi bolezni. Med preizkušanimi spojinami, je spojina **4** izkazala najmočnejše delovanje proti diabetesu in depresiji, saj je inhibirala delovanje α-glikozidaze, α-amilaze in encima MAO-A. Poleg tega so se vse spojine izkazale kot dobri lovilci radikalov ABTS in DPPH; aktivnost spojin **3** in **4** za lovljenje radikalov ABTS je bila celo večja od aktivnosti komercialnih antioksidantov, kot sta BHA in troloks. Nobena od preizkušanih spojin pa ni pokazala občutnega delovanja proti Parkinsonovi bolezni in niti ni izkazala nevrozaščitnega delovanja. Zaključimo lahko, da bi spojina **4** zaradi svojega antioksidativnega delovanja ter delovanja proti diabetesu in depresiji lahko bila kandidatka za nadaljnje študije.