RESEARCH ARTICLE

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The One Pot Synthesis and Antioxidant Activity Determination of Novel Molecules

Zuhal Gerçek, Erol Erçağ, and Handenur Yılmaz

ABSTRACT

Alzheimer's disease affects many people today. For this reason, the economic and social burdens of the disease on society are also increasing. Although the cause of the disease has not been fully clarified yet, many factors are suggested in this regard. One of the most striking among these is brain deformations as a result of oxidative stress. In this study, three new molecules, (E/Z) - (4-fluorophenylthio) -N-(4-methoxybenzylidene) (4-methoxyphenyl) methenamine (NS-1), (E/Z) - (4-fluorophenylthio) -N-(4-hydroxybenzylidene) (4-hydroxyphenyl) methenamine (NS-2), and (E/Z) - (4-fluorophenylthio) -N-(2-pyyrolidene) (2-pyrrole) methenamine (NS-3) were synthesized and their antioxidant properties were evaluated using CUPRAC method and Trolox equivalent antioxidant capacity coefficients were determined. The antioxidant capacity of NS-3, was found to be considerably higher than torolox, 1.5. The Cyclic voltammetry (CV) of compounds was achieved within the potential range -1.4V to 0.95 V with a 50 mV s^{-1} scan rate. 82.15 µA at 456 mV and -929 mV for 10^{-4} M NS-3 reduction peaks with current values of -104.9 µA were found. The molecule NS-3 can be a potential candidate for antioxidants that could be used to slow the progression of Alzheimer's disease.

Keywords: Alzheimer's Disease, Antioxidant, Cyclic Voltammetry, Oxidative Stress, Torolox Coefficient.

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I. Introduction

Around 100 years ago people lived to the age of 30 on average**.** Improved economic conditions, changes in diet and lifestyle, adjustments in work and social factors, as well as improved public health strategies and regulation of medical care, increase the average life expectancy to 71.5 years today [1]. Prolongation of life leads to common chronic diseases of older people such as cancer, cardiovascular diseases, and Alzheimer's diseases, are becoming much more prevalent.

Alzheimer's disease (AD) was first defined by Dr. Alois Alzheimer in 1907 [2], [3]. Today, Alzheimer's disease (AD) is the third most expensive disease in the United States, costing society approximately $100 billion each year [4].

Although the cause of AD is still a mystery, many theories have been proposed for the causes of it [5], [6]. For example, acceleration of aging, anatomical pathways degenerations, mitochondrial and immune system dysfunctions, environmental and genetic factors, b-amyloid formation can be counted as the factors that lead to the disease [7]-[12]. Although the disease is still not curable with current clinical therapy, the treatment with glutamate receptor-selective drugs, antioxidants, inhibitors of nitric oxide synthase, calcium channel antagonists, receptor or enzyme inhibitors, and growth factors are promising approaches for slowing disease.

Oxidative stress which can be defined as the imbalance between the antioxidant mechanism and the intracellular production of free radicals/reactive oxygen species (ROS), is a potential source of damage to vital molecules such as DNA, lipids, sugars, and proteins within cells. Its role in the pathogenesis and pathophysiology of cardiovascular, neurodegenerative, oncological, inflammatory, etc. diseases have been argued in the literature [13], [14].

Animal and human studies in the literature show the important role of oxidative damage occurring before symptoms occur and both b-amyloid-containing plaques and neurofibrillary tangles form in Alzheimer's disease [11], [15]. Therefore, the use of antioxidants can prevent or slow the progression of Alzheimer's disease.

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Defense and repair systems present in all organisms naturally are insufficient to entirely prevent oxidative damage [16]. Antioxidants, on the other hand, can help the human body prevent or reduce oxidative damage. Today, a large number of antioxidants, either synthesized or isolated from natural resources, can be found in markets. They exhibit good antioxidative activity against DPPH, ABTS, hydroxyl radicals, and so on [17].

In this study, three novel compounds, namely (E/Z) - (4-fluorophenylthio) -N-(4-methoxybenzylidene) (4-methoxyphenyl) methenamine (NS-1), (E/Z) - (4-fluorophenylthio) -N-(4-hydroxybenzylidene) (4-hydroxyphenyl) methenamine (NS-2), and (E/Z) - (4-fluorophenylthio) -N-(2-pyyrolidene) (2-pyrrole) methenamine (NS-3) were synthesized by one-pot reaction in order to combat the effects of oxidative stress in AD (Fig. 1). Their antioxidant activities were investigated using the CUPRAC method, and TEAC coefficients were calculated. The Cyclic voltammetry (CV) of compounds was achieved within the potential range -1.4V to 0.95 V with 50 mV s^{-1} scan rate.

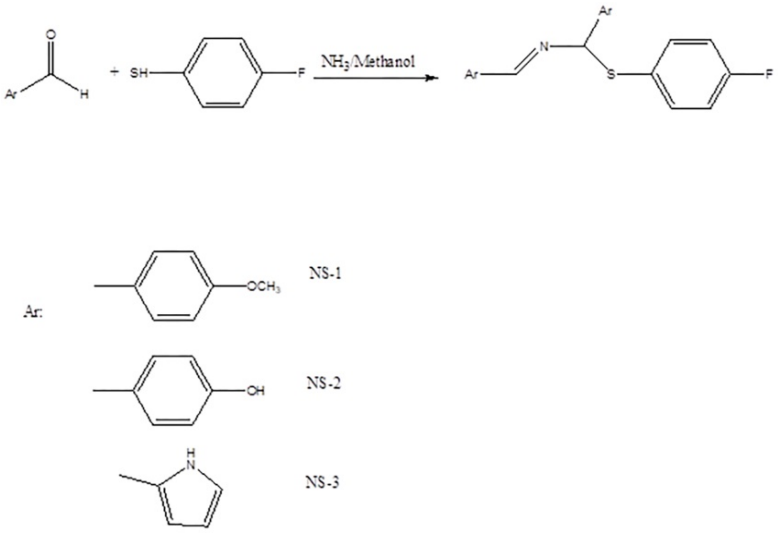

Fig.1. The synthesis of compounds.

II. Experimental

All reagents and solvents were of commercial origin and used without further purification. ^{1}H- NMR and ^{13}C-NMR spectra were recorded with a Bruker Ultra Shield Plus ultra-long-hold-time spectrometer with DMSO-d_6 as the solvent. All chemical shifts are given relative to tetramethylsilane. Mass data were obtained by Water Xevo TQD system.

A. The Chemical Syntheses

The general synthesis procedure was same as in literature [18]. Experimental results are summarized in Table I.

TABLE I: Experimental Results

Molecule	Shape	Melting point (mp)		^{1}H NMR (DMSO-d_6, 600 MHz) δ (ppm)	^{13}C NMR (DMSO-d_6, 150 MHz) δ (ppm)
(E/Z) - (4-fluorophenylthio) -N-(4-methoxybenzylidene) (4-methoxyphenyl) methenamine (NS-1)	yellow solid	58-59 °C	75% yield	7.94 s, 1H, 7.61 d, 2H, J: 8.7 Hz, 7.48 d, 2H, J: 8.7 Hz, 7.42 m, 2H, 7.1 t, 2H, J: 8.8 Hz, 6.9 d, 2H, J: 8.7 Hz, 6.92 d, 2H, J:8.7 Hz, 5.9 s, 1H, 3.76 s, 3H, 3.73 s, 3H.	163.2, 162.0, 161.5, 160.0, 159.3, 136.4, 136.3, 132.0, 130.3, 129.8, 129.0, 128.4, 116.4, 116.3, 114.6, 114.2, 78.9, 55.7, 55.5
(E/Z) - (4-fluorophenylthio) -N-(4-hydroxybenzylidene) (4-hydroxyphenyl) methenamine (NS-2)	white solid	85-86 °C	62% yield	10.6 broad s, 2H, 9.76 s, 2H, 7.73 d, 2H, J: 8.5 Hz, 7.54-7.52 m, 2H, 7.22 t, 2H, J: 8.8 Hz, 6.90 d, 2H, J:8.5 Hz	191.3, 163.7, 163.1, 161.5, 132.5, 131.8, 131.2, 131.2, 128.8, 117.1, 116.9, 116.4, 116.2, 116.0, 114.8
m/z: M^{+}: 353.41 (molecular weight: 352.08).					
(E/Z) - (4-fluorophenylthio) -N-(2-pyyrolidene) (2-pyrrole) methenamine (NS-3)	reddish brown solid	78-79 °C	70% yield	9.2 d, 1H, J: 2.4 Hz, 8.9 d, 1H, J: 2.3 Hz, 8.2 m, 3H, 8.0, d, 1H, J: 8 Hz, 7.9 m, 5H, 7.3 m, 3H	156.5, 156.1, 155.4, 154.4, 148.6, 147.9, 139.9, 137.5, 137.4, 136.5, 124.5, 124.4, 124.2, 124.1, 121.8
m/z: M^{+}: 299.04 (molecular weight: 298.02).					

B. Antioxidant Assay

1) CUPRAC method

The method involves mixing the antioxidant solution directly with solutions of $CuCl_2$, neocuproine, and ammonium acetate at pH 7, and measuring the absorbance at 450 nm after 30 min.

1 mL $CuCl_2$ (0.01 M) + 1 mL Neokuproin (Nc) (7.5 mM) + 1 mL NH_4Ac (1 M) + x mL sample + (1.1–x) mL MeOH (V_{total}: 4.1 mL; t: 30 min.; T: 25 °C; λ: 450 nm)

Trolox equivalent antioxidant capacity (TEAC) was calculated according to (1).

$$TEAC\ coefficent = \varepsilon sample/\varepsilon TR \quad (1)$$

where (ε_{TR} = 16000 L/mol.cm)

C. Cyclic Voltametric Measurements

The Cyclic voltammetry (CV) of compounds was performed within the potential range -1.4V to 0.95 V with 50 mV s^{-1} scan rate using a glassy carbon electrode (GCE) as working electrode platinum (Pt) as the auxiliary electrode an Ag/AgCl electrode as a reference electrode.

5 ml of each substance was taken to contain 0.1 M Tetrabutylammonium bromide. Measurements were made at a concentration of 10^{-4} M.

III. Results And Discussion

Presented molecules were synthesized by a one-pot reaction of different aldehydes with 4-florobenzenethiol. They were purified by recrystallization with methanol. Therefore, the syntheses were performed by using green chemistry. The characterization of molecules was done by ^{1}H NMR, ^{13}C NMR, and mass spectroscopy.

^{1}H NMR spectrum of NS-1 contains a singlet at 7.9 ppm for imine (–HC=N-) proton, aromatic hydrogens appear between 7.6-6.9 ppm. A singlet at 5.9 for CH-S hydrogen and singlets at 3.7 ppm for OCH_3 groups confirm the structure. At ^{13}C NMR spectrum signals are in accordance with the proposed structure.

^{1}H NMR spectrum of NS-2 contains a typical broad singlet for the OH group at 10.6 ppm. Signal of imine (–HC=N-) proton shifts to 9.7 ppm. ^{13}C NMR spectrum also confirms the structure. The molecular weight m/z data is M^+: 353.41 (molecular weight: 352.08).

In the ^{1}H NMR spectrum of NS-3, multiplets at 8.2 ppm and 7.3 ppm belong to two pyrrole rings. ^{13}C NMR spectrum contains typical signals between 156-154 ppm. Its mass spectrum also confirms the structure: m/z: M^+: 299.04 (molecular weight: 298.02).

The results of the novel molecules according to the CUPRAC method are given in Table II, Table III, and Table IV. (2), (3), (4) shows calibration equation of absorption values of NS-1, NS-2 and NS-3 versus concentration, respectively.

$$y = 17579\,x - 0.005;\ r = 0.9999 \quad (2)$$

$$y = 406\,x + 0.006;\ r = 0.9879 \quad (3)$$

$$y = 23673x + 0.002;\ r = 0.9988 \quad (4)$$

TABLE II: NS-1 Absorption Values and Concentrations In Methanol

V (mL)	A_{450}	C (M)
0.05	0.3640	2,08 x 10^{-5} M
0.1	0.7230	4,16 x 10^{-5} M
0.15	1.0900	6.24 x 10^{-5} M
0.2	1.4605	8.32 x 10^{-5} M

TABLE III: NS-2 Absorption Values And Concentrations In Methanol

V (mL)	A450	C (M)
0.25	0.0550	1.25 x 10^{-4} M
0.50	0.1175	2.5 x 10^{-4} M
0.75	0.1598	3.75 x 10^{-4} M
1	0.2099	5 x 10^{-4} M

TABLE IV: NS-3 Absorption Values and Concentrations In Methanol

V (mL)	A450	C (M)
0.1	0.2091	0.897 x 10^{-5}M
0.2	0.4376	1.79 x 10^{-5} M
0.3	0.6199	2.69 x 10^{-5} M
0.4	0.8569	3.59 x 10^{-5} M

TABLE V: CALIBRATION EQUATIONS, LINEAR OPERATING RANGES AND TROLOX EQUIVALENT ANTIOXIDANT CAPACITY (TEAC) COEFFICIENTS OBTAINED ACCORDING TO THE CUPRAC METHOD

	Equation	Linear operating ranges	TEAC coefficient
NS-1	$y = 17579\,x - 0.005$	(2.08-8.32) 10^{-5} M	1.11
NS-2	$y = 406\,x + 0.006$	(1.25 – 5) 10^{-4} M	0.03
NS-3	$y = 23673x + 0.002$	(0.897 – 3.59) 10^{-5} M	1.50

Trolox equivalent antioxidant capacity (TEAC) coefficients obtained according to the CUPRAC Method is shown as (5).

$$y = 1.58 \times 10^4 c - 0.01 \quad (5)$$

The TEAC coefficient expresses the antioxidant capacity of 1 mM antioxidant solution, which is equivalent to 1 mM concentration of trolox (water-soluble vitamin E analogue) selected as the reference standard substance. The TEAC coefficient of trolox is assumed to be 1.00, and the TEAC coefficients of other compounds are given in trolox equivalents. Accordingly, it was observed that NS-2 (TEAC < 0.1) practically did not show antioxidant capacity. NS-3 shows the highest antioxidant capacity. NS-1 has TEAC coefficient of about 1. The antioxidant capacity it showed was found to be very close to the antioxidant capacity of trolox.

A. Cyclic Voltammogram of NS-1 in Methanol

As a result of cyclic voltammetric measurements made between (-1.4 V) and (0.95 V) potential range,as shown in Fig. 2, a reduction peak with a current value of -25.11 µA at 496 mV was found for 10^{-4} M NS-1.

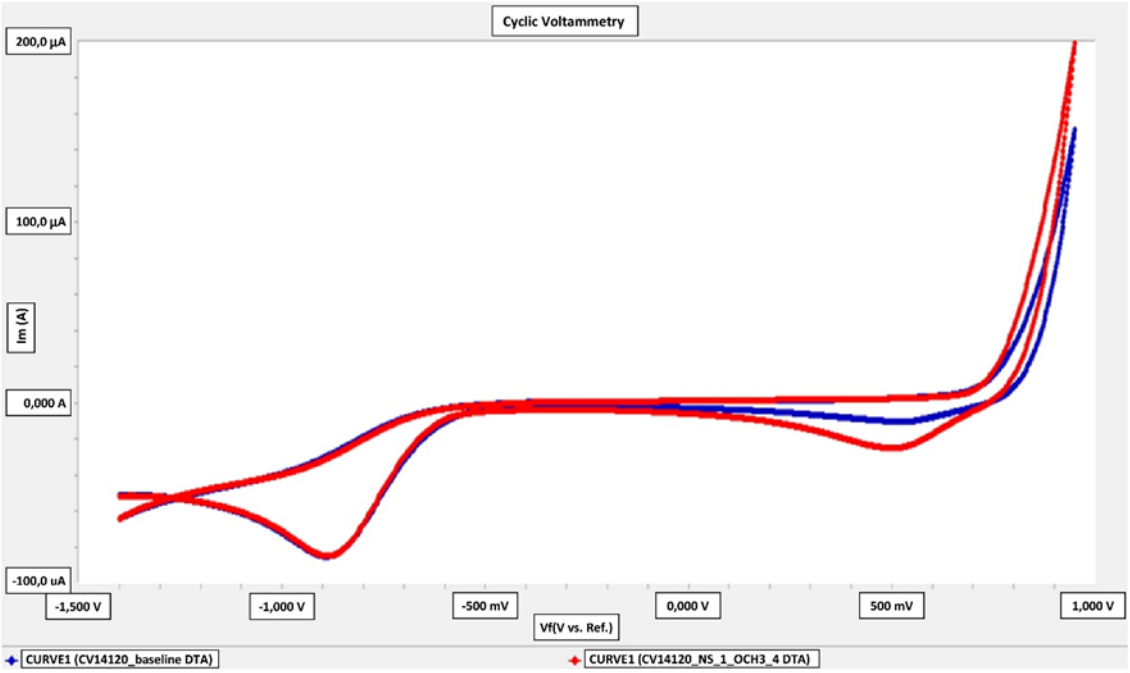

Fig. 2. Cyclic voltammogram of NS-1 in methanol. Baseline (Blue) and 10^{-4} M NS-1 (red) cyclic voltammograms.

B. Cyclic Voltammogram of NS-2 in Methanol

Cyclic voltammetric measurements made between (-1.4 V) and (0.95 V) potential range, gave a reduction peak with a current value of -17.65 µA at 460 mV was found for 10^{-4} M NS-2 (Fig. 3).

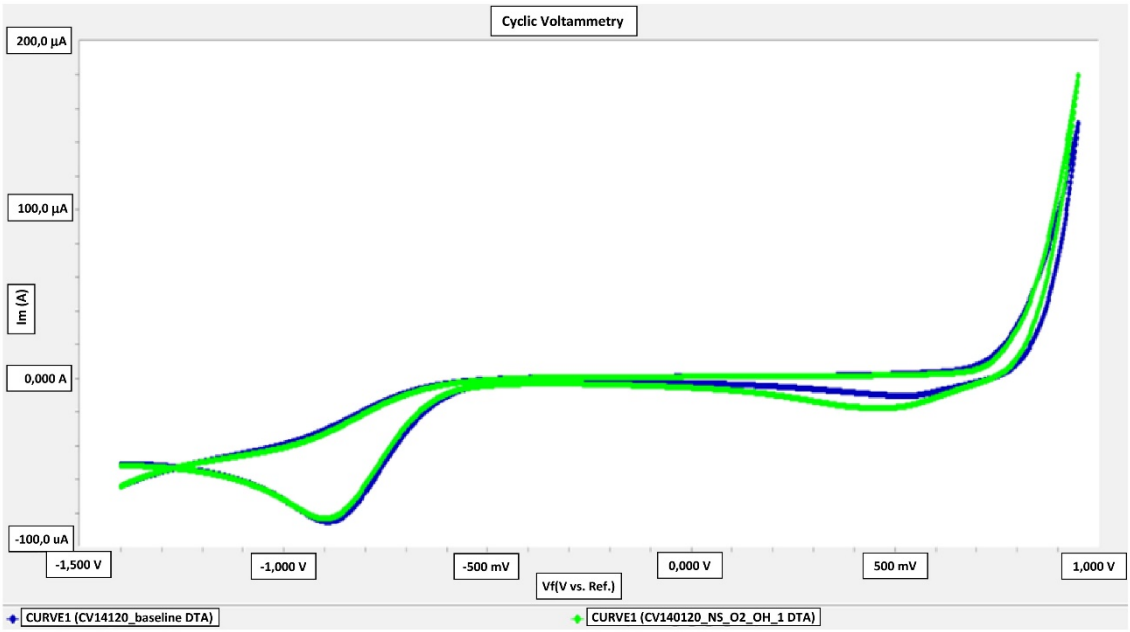

Fig. 3. Cyclic voltammogram of NS-2 in methanol Baseline (Blue) and 10-4 M NS-2 (green) cyclic voltammograms.

C. Cyclic Voltammogram of NS-3 in Methanol

As a result of cyclic voltammetric measurements made in the potential range (-1.4 V) to (0.95 V), -82.15 µA at 456 mV and -929 mV for 10^{-4} M NS-3 reduction peaks with current values of -104.9 µA were found (Fig. 4).

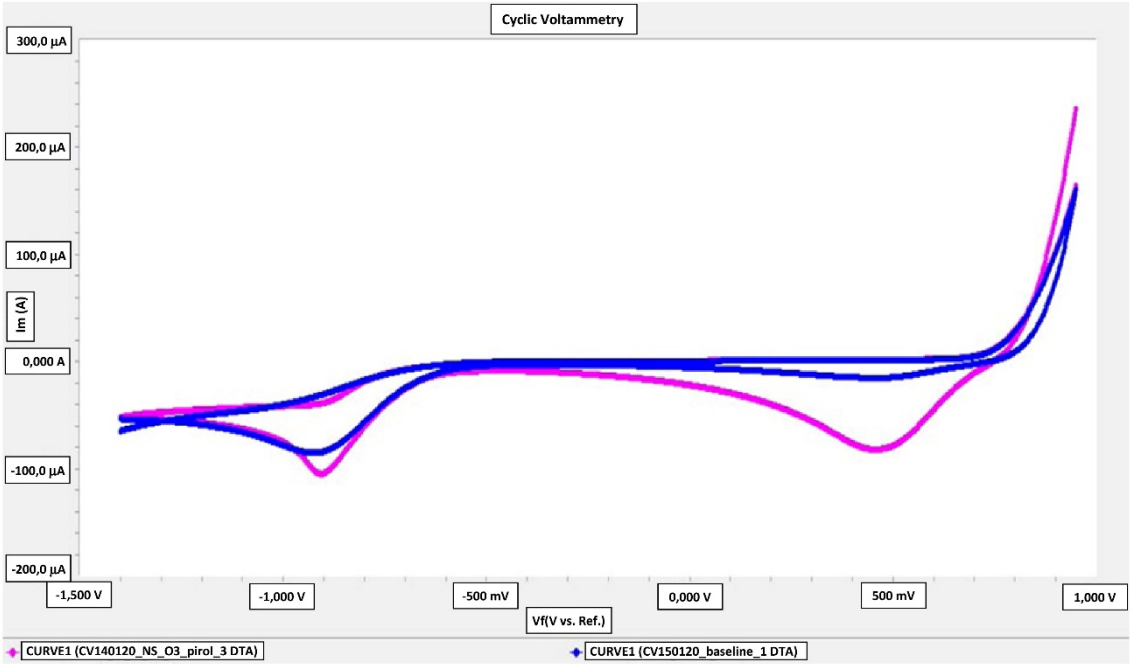

Fig. 4. Cyclic voltammogram of NS-3 in methanol. Baseline (Blue) and 10-4 M NS-3 (pink).

IV. Conclusion

In this study, three new molecules, (E/Z) - (4-fluorophenylthio) -N-(4-methoxybenzylidene) (4-methoxyphenyl) methenamine (NS-1), (E/Z) - (4-fluorophenylthio) -N-(4-hydroxybenzylidene) (4-hydroxyphenyl) methenamine (NS-2), and (E/Z) - (4-fluorophenylthio) -N-(2-pyyrolidene) (2-pyrrole) methenamine (NS-3) were synthesized. By CUPRAC method, the antioxidant capacity of NS-3, was found to be considerably higher than torolox, 1.5. The Cyclic voltammetry (CV) of compounds was achieved within the potential range -1.4V to 0.95 V with a 50 mV s^{-1} scan rate. 82.15 µA at 456 mV and -929 mV for 10^{-4} M NS-3 reduction peaks with current values of -104.9 µA were found. These results show that since all compounds have antioxidant capacity, the molecule NS-3 can be a potential candidate for antioxidants that could be used to slow down the progression of Alzheimer's disease.

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