

SYNTHESIS AND ANTIOXIDANT ACTIVITY OF SOME AMINOADAMANTANE DERIVATIVES

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Received 20 September 2023

Accepted 23 May 2024

DOI: 10.59957/jctm.v59.i4.2024.15

ABSTRACT

Chronic fatigue syndrome (CFS) and neurocognitive deficits are a major problem of modern society. The etiology of CFS remains unclear, however, a large number of recent studies have shown oxidative stress may be involved in its pathogenesis. Fatigue is frequent and important in the lives of Parkinson's disease (PD) patients. 1-aminoadamantane (amantadine and memantine) derivatives has been proposed to be useful in the treatment of Parkinson's and Alzheimer's diseases. Its beneficial effect has been related to its novel properties as an N-methyl-D-aspartate receptor (NMDAR) blocker which can neutralize the effect of glutamate at striatal and subthalamic levels. We synthesized and evaluated the antioxidant activity of eleven new aminoadamantane derivatives. Their antioxidant activity has been evaluated using different (superoxide anion, hydroxyl) radical generating systems. The compound with s highest anti-oxidative capacity contain 2-(Benzhydrylsulfinyl) substituent.

Keywords: amantadine, rimantadine, memantine, antioxidant activity, fatigue.

INTRODUCTION

Fatigue, or paucity of physical and mental drive, affects about 80 % of patients with multiple sclerosis (MS). MS-related fatigue is understood to be related to axonal injury secondary to glutamate toxicity as manifested by the reduction in N-acetylaspartate levels seen in multivoxel spectroscopic studies. Several non-pharmacologic modalities (cognitive behavioral therapy, exercise, and energy conservation) and pharmacologic agents (modafinil, amantadine, methylphenidate, and aspirin) have shown to decrease fatigue symptoms using several scales [1]. Widely accepted is that the activation of the dopaminergic and glutaminergic systems in PD and dopamine/glutamate interactions, yields the production of reactive oxygen species (ROS), such as hydroxyl radical ($\cdot\text{OH}$), superoxide ($\text{O}_2^{\cdot-}$) and

H_2O_2 . Consequently, ROS are in excess compared to the antioxidant capacity of neural cells, thereby produce oxidative stress (OS), damage and cells death [2]. Excitotoxicity is a pathological process in which neuronal death occurs due to the over stimulation of NMDAR, an event that causes an excessive influx of calcium ions into neuronal cells [3]. Linking some aminoadamantanes, such as memantine with bioactive payload able to tackle $\text{A}\beta$ /ROS/neuroinflammation could be a promising pathway to deepen knowledge in NMDAR-mediated neurotoxicity [4].

We decide to combine three aminoadmantanes (amantadine, rimantadine and memantine) with: amino acids Sarcosine (N-methylglycine) and N,N-dimethylglycine (DMG); small peptides Gly-Gly and Gly-Gly-Gly; drug modafinil. All stuctures are shown Fig. 1. These compounds were included in other our investigations [5, 6].

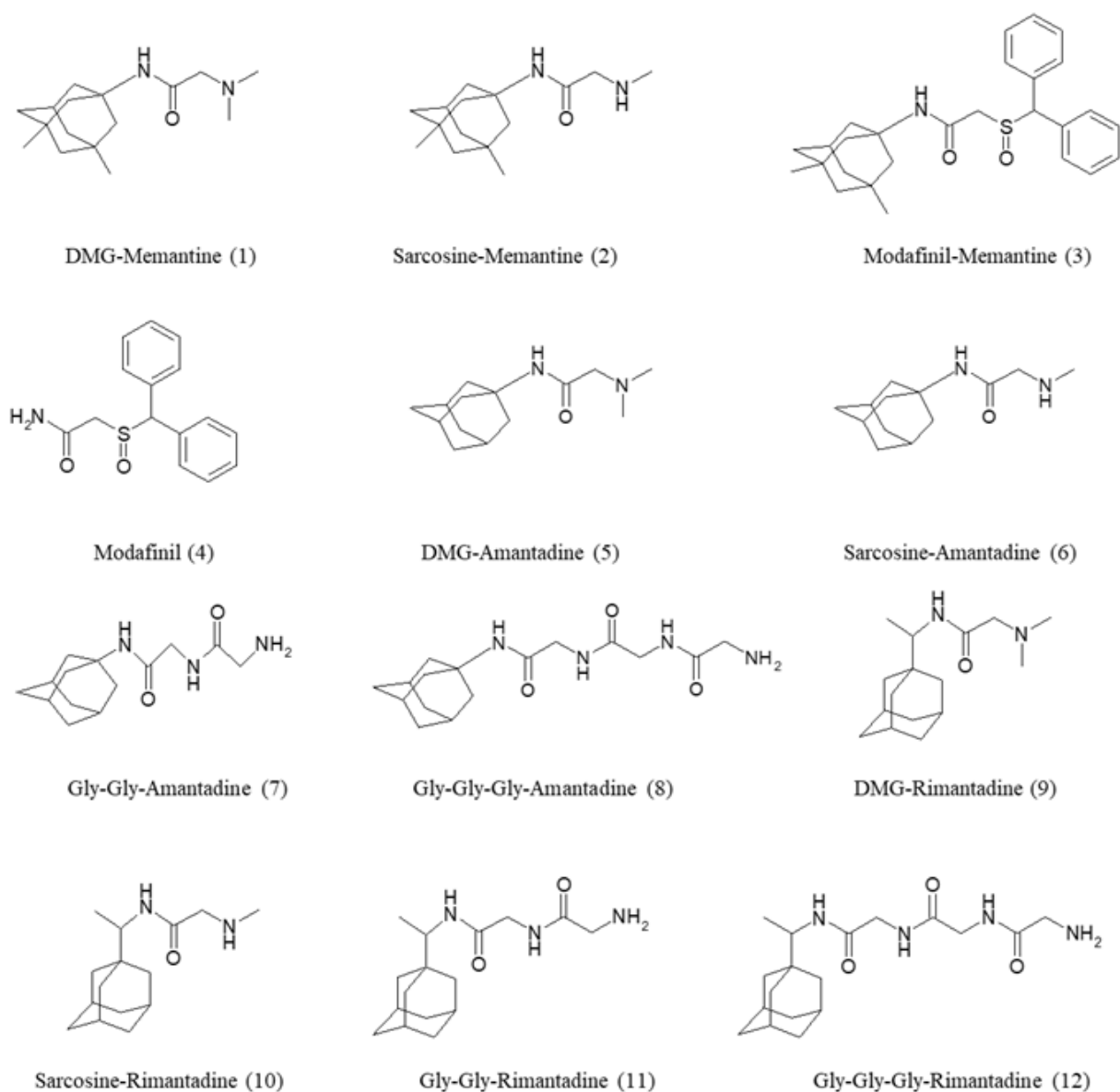


Fig. 1. Chemical structures of the the synthesized aminoadantane derivatives.

EXPERIMENTAL

Materials and methods

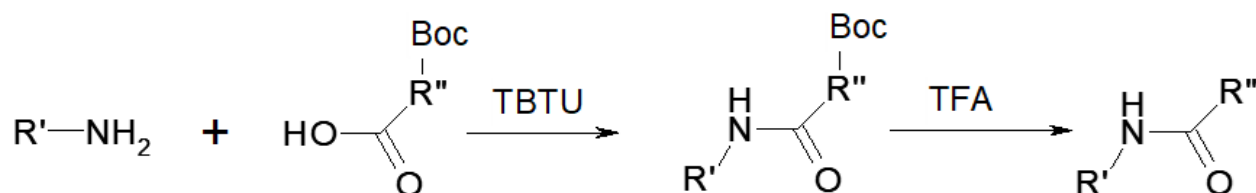
Amantadine, Rimantadine, Memantine, amino acids, peptides, TBTU coupling reagent, triethylamine (TEA), trifluoroacetic acid (TFA) and all necessary solvents for the synthesis were purchased from Sigma-Aldrich. Aluminum TLC plates, silica gel coated with fluorescent indicator F_{254} are from Merck. All NMR spectra are recorded on Bruker AVANCE 500 MHz NMR spectrometer in DMSO- d_6 . MS spectra are recorded on

Bruker Esquire 3000 Plus Ion Trap Mass Spectrometer in ESI mode.

Chemical synthesis

Amide bond formation (Scheme 1), for all compounds was conducted according to method described in [7].

Amantadine, resp. Rimantadine or Memantine (8 mmol) was dissolved in 5 mL Dichloromethane (DCM), TEA (8 mmol) was added to the solution. In separate flask the carboxyl component* (8.8 mmol) was dissolved in 10 mL DCM. To the mixture was



R' = memantine; amantadine; rimantadine.

R'' = *N,N'*-dimethylglycine; sarcosine; Gly-Gly; Gly-Gly-Gly.

Scheme 1. Synthesis of Amantadine/Rimantadine/Memantine derivatives via TBTU as a coupling reagent.

added TEA (8.8 mmol) and TBTU (12.3 mmol). Both solutions were mixed after 30 min. The reaction mixture was stirred at room temperature under nitrogen for 24 h. The reaction was monitored by TLC. After 24 h the reaction mixture was washed by 5 % NaHCO₃ solution twice, then dried with anhydrous Na₂SO₄. Sarcosine and the peptides necessitated Boc-protection group removing (DCM/TFA 50:50 for 3 h) before any further purification. The obtained compound was purified by flash chromatography on silica gel column with elution system CHCl₃/MeOH 97:3 or Hex/EtOAc 5:4 ratio, depending on the polarity of the compounds.

*As a carboxyl component in the case with compound Modafinil-Memantine 2-Benzhydrylsulphonylacetic acid was used.

Antioxidant study

Methods

The 12 compounds (11 newly synthesized and Modafinil (4) for comparison) were tested for their antioxidant activity in the following concentrations 15.63, 31.25, 62.5, 125, 250, 500, and 1000 μM using different radical generating systems.

ABTS assay

The method is described in [8]. ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) is oxidized with potassium persulfate to give the cationic radical (ABTS •+), which has a maximum absorption at 743 nm (acquires a bluish-green colour). In the presence of hydrogen-donor antioxidants, the solution was decolorized and the absorbance at 743 nm (A743) decreased. ABTS •+ was prepared by mixing ABTS (7.0

mM in double distilled H₂O) with potassium persulfate (2.45 mM) in ddH₂O). The mixture was allowed to stand in the dark at room temperature for 12 - 16 h before use. The solution was diluted in methanol (2 mL ABTS •+ + 58 mL methanol). The absorption at 743 nm of the resulting working solution to be 1.1 ± 0.02. 1.425 mL of ABTS •+ working solution was added to a sample of 75 μL of the test substance, after 15 minutes of incubation at 37°C measure A743 against methanol. A blank containing 75 μL of water instead of the test substance also was measured against methanol.

DPPH assay

DPPH analysis was carried out by the method of Brand-Williams et al. [9]. First, 500 μL of the test solution was added to 500 μL of a freshly prepared solution of 0.1 mM DPPH in methanol and the resulting mixture was incubated in the dark for 30 minutes. Then the absorbance at a wavelength of 517 nm (A517) was measured. A 1:1 mixture of DPPH solution and methanol was used as a control sample.

Antioxidant activity was calculated as: Antioxidant activity (%) = [(A517 control - A517 probe) / A517 control] x 100).

FRAP assay

FRAP analysis was performed according to Benzie et al. [10]. The following solutions was prepared: 1) 0.03 M acetate buffer, pH 3.6; 2) 1.0 mM TPTZ (2,3,5-Triphenyltetrazolium chloride, in 40 mM HCl; 3) 1.5 mM FeCl₃·6H₂O. Thereafter the solutions were mixed in the following ratio: 10 parts 0.03 M acetate buffer (1): 1 part 1.0 mM TPTZ (2): 20 parts 1.5 mM

FeCl₃ (3). To 1.5 mL of the reaction mixture was added 50 µL of the sample. 50 µL H₂O was placed in the blank instead of the test substance. After incubation for 4 min at 37°C, absorbance was measured at 593 nm (A593).

CUPRAC assay

The reaction of the Cu²⁺-neocuproin (NC) complex with antioxidants results in a change in the color of the solution from blue to yellow-orange due to the formation of Cu⁺-neocuproin chelate with an absorption maximum at 450 nm.

The CUPRAC analysis was performed according to Apak, R. et al. [11]. The following solutions were prepared: 1) 10 mM CuCl₂ in ddH₂O; 2) 1.0 M ammonium acetate buffer; pH 7.0; 3) 7.5 mM neocuproin (NC) in 96 % ethanol. The total reaction mixture was prepared in the following order: 1 part CuCl₂ (1): 1 part NC (3): 1 part buffer (2). X mL of the test substance was added to Eppendorf tubes with a cap and the volume was adjusted to 0.550 mL by adding H₂O. 1.5 mL of the total reaction mixture was then added. After incubation at 50°C for 20 minutes, absorbance was measured at 450 nm against a blank (1.5 mL total reaction mixture and 0.550 mL H₂O).

Metal Chelating assay

The method was described by Dinis et al. [12]. A total of 0.2 mL of the sample solution, 0.74 mL of 0.1 M acetate buffer (pH 5.25), and 0.02 mL of 2 mM ferrous sulfate solution in 0.2 M hydrochloric acid were mixed for 10 - 15 s. Afterward, 0.04 mL ferrozine solution 5 mM (MW 492.46) was added, and the absorbance of the solution was measured after keeping it for 10 min in the absence of light, against a blank prepared under similar conditions. The metal chelating capacity was determined for the initial extract and the investigated fraction using the following formula: Activity (%) = 100 (Ac - As)/(Ac), where Ac is the absorbance of the control solution and As is the absorbance of the sample solution.

NBT test (Superoxide anion radical generating system)

For the generation of superoxide anion radicals (O₂⁻) was used the method of Beauchamp and Fridovich [13]. The radicals were generated photochemically in a medium containing 50 mM potassium phosphate buffer, pH 7.8; 1.17x10⁻⁶ M riboflavin; 0.2 mM methionine; 2x10⁻⁵ M KCN, and 5.6x10⁻⁵ M nitro-blue tetrazolium

(NBT). The NBT reduction by O₂⁻ to a blue formazan product, in the absence (control) and presence of increasing concentrations of the tested substances, was measured at 560 nm. The antioxidant capacity of the derivatives was expressed as a percentage of the control.

Deoxyribose test (Hydroxyl radical generating system)

For the generation of hydroxyl radicals (•OH) was applied the method of Halliwell et al. [14]. The method is based on the measurement of the amount of thiobarbituric acid-reactive substances resulting from deoxyribose degradation that serves as a measure to produce hydroxyl radicals. Hydroxyl radicals were generated in a system containing 10 mM potassium phosphate buffer, pH 7.4; 0.1 mM FeSO₄, 0.5 mM H₂O₂ and 2 mM deoxyribose. After 30-min incubation at 37°C in the absence (control) and presence of increasing concentrations of the tested substances, the reactions were stopped by the addition of 0.2 mL 2.8 % trichloroacetic acid, 0.1 mL 5 N HCl, and 0.2 mL thiobarbituric acid (2 % w/v in 50 mM NaOH). The samples were incubated at 100°C for 15 min for colour development and after cooling the absorbance was read at 532 nm. The antioxidant capacity of the derivatives was expressed as a percentage of the control.

RESULTS AND DISCUSSION

FRAP (ferric reducing antioxidant power)

From all tested 12 compounds in all concentrations only compound 3 (Modafinil-Memantine) showed a ferric reducing effect although very low.

DPPH radical scavenging activity

From all tested 12 compounds, a DPPH radical scavenging potential was observed in 6 compounds in total. Compound 12 has the highest activity, followed by compounds 7, 8, and 11 in higher tested concentrations - 1000 µM, 500 µM, 250 µM and 125 µM (Table 1). On Fig. 2 the compounds with highest activity are compared with the compound with the lowest activity.

NBT- test (O₂⁻ scavenging activity)

From all tested 12 substances O₂⁻ radical scavenging capacity was demonstrated from compounds 1 and 2 (about 25 % in concentrations of 250 and 500 µM),

Table 1. Compounds with 4 highest DPPH inhibition values in different concentrations.

Compounds	DPPH inhibition, %			
	125 μM	250 μM	500 μM	1000 μM
Gly-Gly-Amantadine (7)	5.32 \pm 0.35	12.20 \pm 0.89	17.28 \pm 0.23	29.49 \pm 0.51
Gly-Gly-Gly-Amantadine (8)	9.49 \pm 0.25	14.23 \pm 0.25	16.27 \pm 0.98	28.13 \pm 0.50
Gly-Gly-Rimantadine (11)	10.67 \pm 0.98	10.84 \pm 0.76	18.64 \pm 0.85	24.40 \pm 0.82
Gly-Gly-Gly-Rimantadine (12)	12.71 \pm 0.90	14.01 \pm 0.80	19.49 \pm 1.53	34.23 \pm 1.09

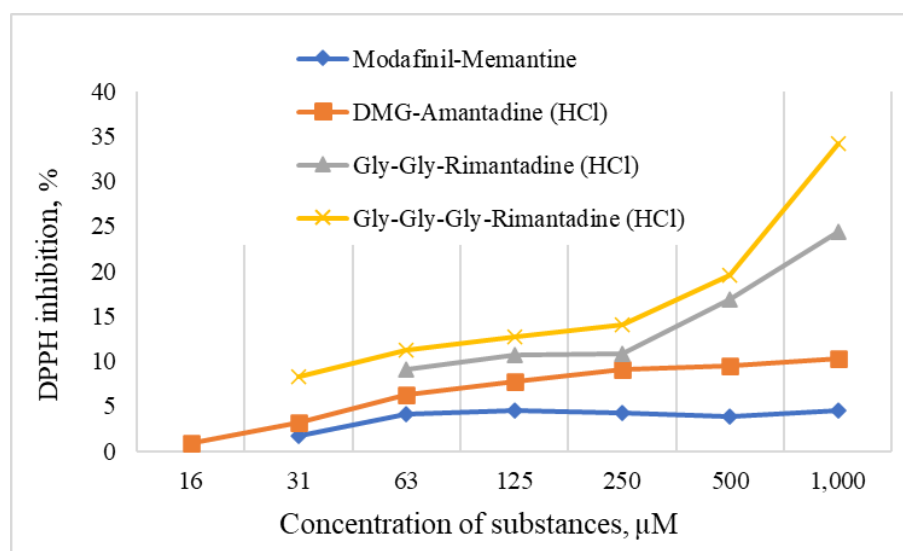


Fig. 2. Compounds with the lowest and the highest DPPH radical scavenging activity.

followed by compounds 7, 8, and 9 (about 18 % in concentrations of 250 μM). The results are presented on Fig. 3.

DR - test ($\bullet\text{OH}$ scavenger activity)

All tested substances, shown on Fig. 4 do not show $\bullet\text{OH}$ scavenger activity.

ABTS \bullet^+ assay

All tested substances didn't have antiradical activity, except compounds 3 and 7, which showed a weak ability (7.69 and 11.70 % inhibition, respectively) to scavenge ABTS \bullet^+ radicals at the maximal tested concentration of 1 mM (Fig. 5).

Fe (II) chelating ability

Iron (II) ions react with ferrozine to form a pink complex

with maximum absorption at 562 nm (Fig. 6). Therefore, the presence of a chelating agent in the reaction medium will result in a decrease in the measured absorbance. At a concentration of 1000 μM , compounds 1, 2, 8, and 9 inhibited the formation of Fe(II)-ferrozine complex by 15.3, 16.28, 20.98, and 42.62 %, respectively.

CUPRAC assay

From all tested substances, compounds 3, 7, 8, 11, and 12 showed Cu (II) reducing activities. The highest reducing potential was demonstrated by compound 7, followed by 3, 8, 11, and 12 (Fig. 7).

We conduct AChE inhibition test in mice brain homogenate. The main purpose of this investigation was to find any correlation between antioxidant activity and AChE inhibition. The results are shown in Table 2. In treated animals, the activity of AChE was not significantly decreased in comparison with the control group.

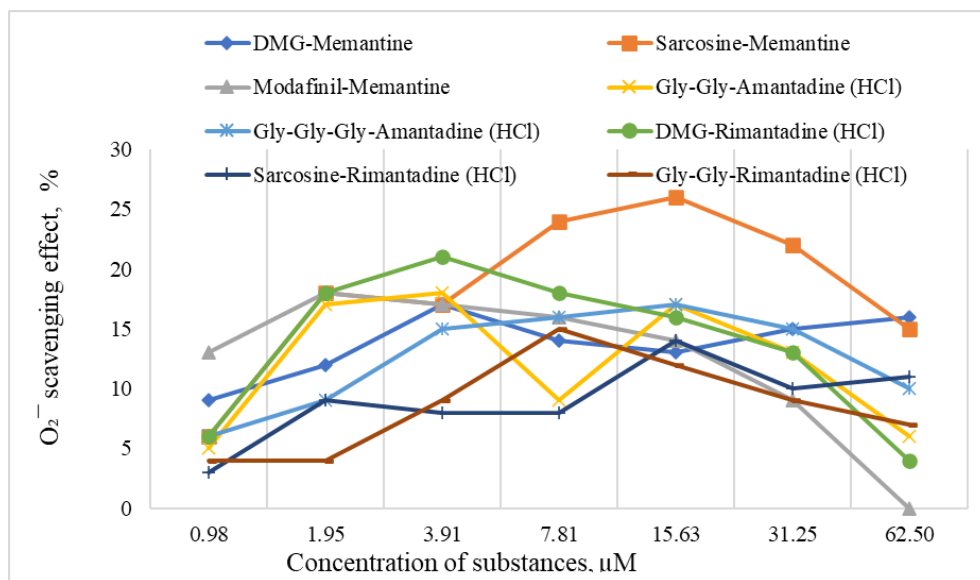


Fig. 3. NBT- test (O_2^- scavenging activity).

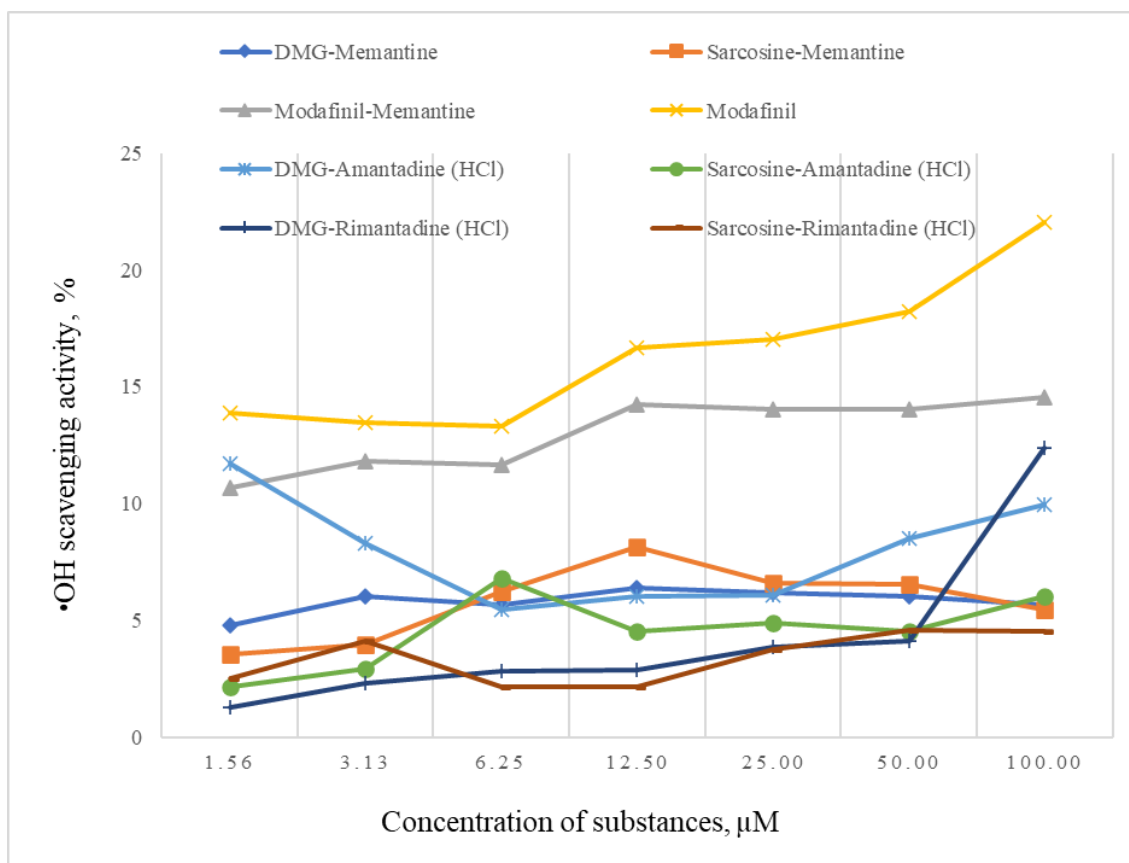


Fig. 4. DR - test ($\bullet OH$ scavenging activity).

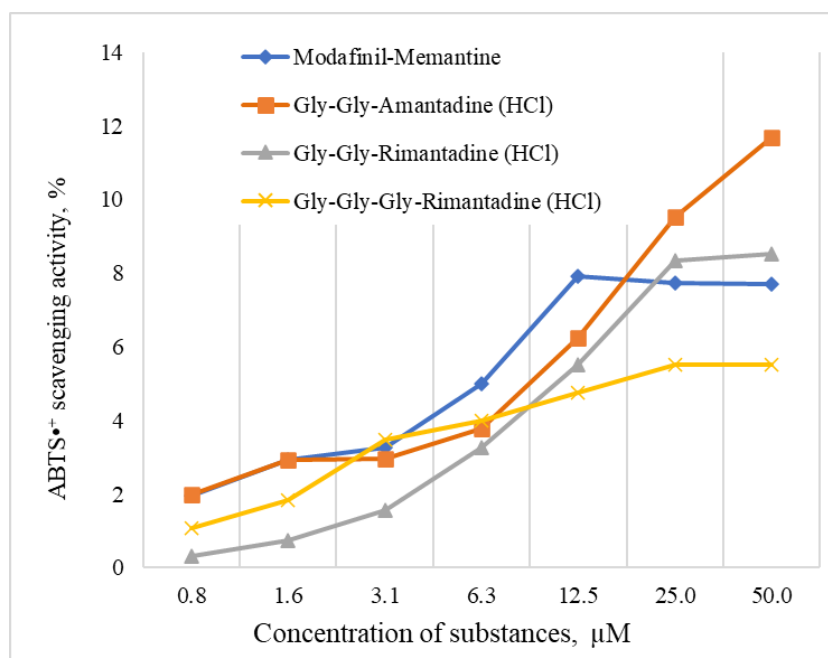


Fig. 5. ABTS•+ assay.

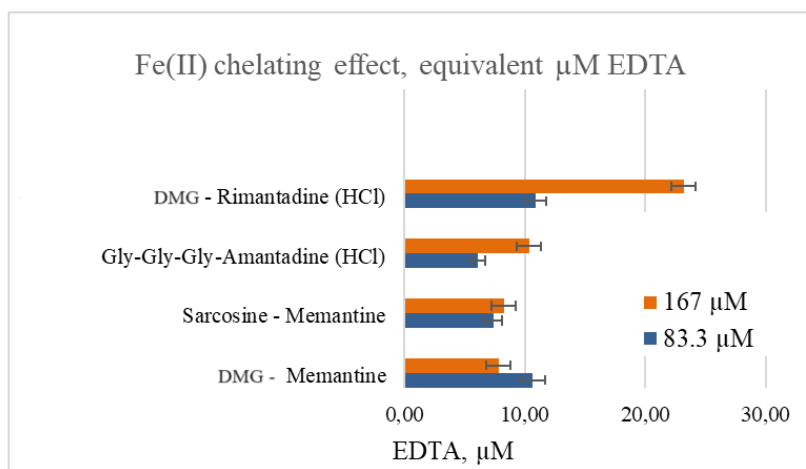


Fig. 6. Fe (II) chelating ability.

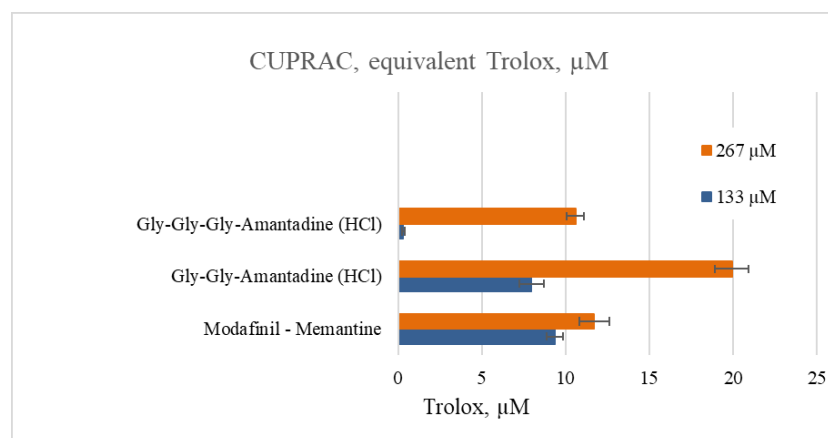


Fig. 7. CUPRAC assay.

Table 2. *In vitro* inhibition of AChE in mice brain homogenate.

Substance	μM	AChE inhibition vs controls, %
Sarcosine-Memantine (2)	15.63	8.6
	62.5	8.6
Modafinil-Memantine (3)	15.63	2.6
	62.5	16.1
Modafinil (4)	15.63	13.9
	62.5	15.4
DMG-Amantadine (HCl) (5)	15.63	1.9
	62.5	1.9
Sarcosine-Amantadine (HCl) (6)	15.63	4.9
Gly-Gly-Amantadine (HCl) (7)	15.63	19.9
Sarcosine-Rimantadine (HCl) (10)	15.63	13.1

CONCLUSIONS

After conducting a battery of 7 assays screening for the antioxidant activity of the 12 tested compounds, it could be resumed that the most promising antioxidant activity had compound 7, followed by compounds 8 > 12 > 11 > 9 > 1 > 2 > 3. It could be assumed that the better antioxidant activity of the substances (compounds 7, 8, and 11, 12) is due to the presence of glycine residues in the molecule.

Acknowledgments

The work is realized thank to financial support of South-West University "Neofit Rilski".

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