Hindawi
Journal of Chemistry
Volume 2022, Article ID 7582587, 11 pages
https://doi.org/10.1155/2022/7582587

Research Article

Hybridization of Aminoadamantanes with Cinnamic Acid Analogues and Elucidation of Their Antioxidant Profile

Maya Chochkova,[1] **Almira Georgieva**,[2] **Tsvetelina Ilieva**,[1] **Madlena Andreeva**,[2] **Georgi Pramatarov**,[2] **Nejc Petek**,[3] **Petranka Petrova**,[1] **Martin Štícha**,[4] **Yavor Mitrev**,[5] **and Jurij Svete**[3]

[1]*Faculty of Mathematics and Natural Sciences, South-West University, Neofit Rilski 66, Ivan Mihailov Str. 2700, Blagoevgrad, Bulgaria*
[2]*Laboratory of Free Radical Processes, Institute of Neurobiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Bl. 23, Sofia 1113, Bulgaria*
[3]*Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna Pot 113, Ljubljana 1000, Slovenia*
[4]*Charles University, Faculty of Science, Section of Chemistry, Hlavova 2030/8 12843, Prague 2, Czech Republic*
[5]*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str. Bl. 9, Sofia 1113, Bulgaria*

Correspondence should be addressed to Maya Chochkova; mayabg2002@yahoo.com

Received 22 August 2022; Revised 14 October 2022; Accepted 21 October 2022; Published 16 November 2022

Academic Editor: Liviu Mitu

A series of seventeen cinnamic acid hybrids (**4a$_i$–c$_i$**) were obtained through an amidation of aminoadamantanes (amantadine, rimantadine, and memantine) with mixed anhydride generated from different substituted cinnamic acid and ethyl chloroformate. ^{1}H NMR, ^{13}C NMR, IR, and HRMS were used for the confirmation of the structures of the synthesized hybrids. Moreover, the antioxidant profiles of amides were estimated as per five different in vitro methods: 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid cation radical (ABTS$^{\bullet+}$), ferric reducing antioxidant power (FRAP), cupric reducing antioxidant capacity (CUPRAC) assay, and inhibition of Fe(III)/asc induced lipid peroxidation (LP) in brain homogenate. For comparison, caffeic acid (CaffA), known as a potent naturally occurring antioxidant, was used as a reference compound in our study. The results revealed that the most prominent antioxidant activity was demonstrated by compound **4b$_2$**, with excellent CUPRAC, FRAP, scavenging ABTS$^{\bullet+}$ potential, and inhibition of Fe/asc–induced LP, followed by **4c$_6$** > **4a$_6$** > **CaffA** > **4c$_5$** and **4a$_5$** > **4a$_7$**. Overall, the results suggest that the hybrids (**4b$_2$**, **4c$_6$**, and **4a$_6$**) consisting of a caffeoyl moiety and lipophilic adamantane core endow the molecules with the higher antioxidant activity than their parent compound (caffeic acid), especially against LP. Thus, these promising antioxidants could have beneficial effects in various pathological conditions, where oxidative stress is implicated.

1. Introduction

Nowadays, natural and synthetical antioxidants are one of the most attractive spheres of influence in biomedical research, especially in the field of oxidative stress-mediated disorders (e.g., neurodegenerative, cancer, and influenza). Indeed, in such pathological conditions, the generation of reactive oxygen species (e.g., superoxide anion, hydroxyl radical, and hydrogen peroxide) exceeds the capacity of endogenous antioxidant systems [1]. It is widely accepted that antioxidants exert their effects by different mechanisms of action: scavenging of radicals, chelation ability towards transition metals (e.g., copper or iron), inhibiting enzymes involved in the overproduction of reactive species, induction of endogenous antioxidant enzymes, and controlling gene expression [2–5].

Phenylpropenoic acids as substituted cinnamic acids (e.g., ferulic, sinapic, and caffeic acids) and their derivatives (cinnamates and cinnamamides), especially those with phenolic hydroxyl groups, are one of the most important classes of exogenous phenolic antioxidants [6–9]. Due to the low toxicity and high bioactivity, hydroxycinnamoyl rest is a privileged scaffold not only in various natural products (such as food additives) but also in modern drug discovery, as drug-like molecules with potential pharmacological activity [10, 11]. However, the poor lipophilicity of phenolic acids often limits their beneficial effects as antioxidants in biological systems [12, 13]. Therefore, to increase the lipophilicity, various structural modifications on the phenolic acid core have been made.

Earlier studies indicated that one possible way for alteration of the lipophilicity of hydrophilic hydroxycinnamic acids was their esterification to lipophilic alcohols [14–17].

Particularly, simple adamantanes functionalization is a promising strategy in enhancing the lipophilicity and stability of drugs [18, 19].

In drug design, the adamantyl skeleton endows the molecules with different "faces" as antivirals, antimalarials, as agents against type 2 diabetes, and against diseases of the central nervous system [18]. Good examples of these are aminoadamantanes, currently used in clinical practice—amantadine, *Am* (antiviral, antiparkinsonian drugs) [20, 21]; rimantadine, *Rim* (antiviral drug) [22]; memantine, *Mem* (used in Alzheimer's disease therapy) [23–26].

For the last fifteen years, the research group has focused the attention on the synthesis of cinnamic acid derivatives, comprising various pharmacophores, and studied them as antioxidants, antiglucosidase inhibitors, antityrosinase inhibitors, and antimicrobials [8, 27–31].

In continuation of our ongoing research project directed toward finding out the "magic" antioxidant with higher lipophilicy, herein, the amide functionalization of substituted cinnamic acids with aminoadamantanes (amantadine, memantine, and rimantadine) has been investigated for their in vitro antioxidant capacity. The antioxidant activity was estimated by applying 5 different tests: 1,1-diphenyl-2-picrylhydrazyl (DPPH·) radical, 2,2′-azinobis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), ferric reducing antioxidant power (FRAP), cupric reducing antioxidant capacity (CUPRAC) assay, and inhibition of Fe (III)/asc induced LP in brain homogenate.

2. Materials and Methods

2.1. Materials. All substituted cinnamic acids, aminoadamantanes (amantadine, rimantadine, and memantine), and triethylamine were purchased from Angene Chemical, whereas ethyl chloroformate was obtained from Sigma Aldrich (FOT, Bulgaria). Thin-layer chromatography (TLC) was conducted on precoated Kieselgel $60F_{254}$ plates (Merck, Germany) with detection by UV absorption (254 nm). Visualization of chromatograms was accomplished with Ce–PMo reagent: 10 g Ce $(SO_4)_2$, 25 g H_3 [P $(Mo_3O_{10})_4$] × H_2O, and 940 mL H_2O, (60 mL conc. H_2SO_4) solution followed by heating. Flash chromatography of the target hybrids was performed on prepackaged BÜCHI FlashPure EcoFlex silica columns.

The solvents were purchased from Thermo Fisher Scientific, Bulgaria, and were used without further purification.

2.2. Instrumentation. The NMR spectra were recorded in deuterated solvents with $(CH_3)_4$ Si as the internal standard on a Bruker Ascend neo NMR 600 instrument (Bruker, Billerica, MA, USA) at 600 MHz for ^{1}H and at 151 MHz for ^{13}C nuclei, respectively, and on Bruker Avance II + spectrometer (14.09 T magnet), operating at 600.01 MHz ^{1}H frequencies, equipped with 5 mm BBO probe with the z-gradient coil. The temperature was maintained at 293 K, using Bruker B-VT 3000 temperature unit with an airflow of 535 L/h. Data for ^{1}H NMR are reported as chemical shifts (δ) in ppm, multiplicity (bs = broad singlet, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant, and integration. Data for ^{13}C are reported as chemical shift (δ) in ppm. IR analysis of amides was performed using a Thermo Scientific Nicolet iS10 FT-IR device with ID5 ATR accessory (diamond crystal). The electrospray mass spectrometry (ESI-MS) experiments were acquired on Bruker Compact QTOF-MS (Bruker Daltonics, Bremen, Germany) and controlled by the Compass 1.9 Control software. The data analysis was performed and the monoisotopic mass values were calculated using Data analysis software v 4.4 (Bruker Daltonics, Germany). The analyses were conducted in the positive and negative ion mode at a scan range from m/z 50 to 1000, and nitrogen was used as nebulizer gas at a pressure of 4 psi and flow of 3 L/min for the dry gas. The capillary voltage and temperature were set at 4500 V and 220°C, respectively.

*2.3. Synthesis of N-Cinnamoyl Adamantane Hybrids (**4a$_i$–c$_i$**) [32].* The corresponding substituted cinnamic acid (12 mmol) was dissolved in THF (30 mL), to which under argon atmosphere were added dropwise at 0°C, Et_3N (2,4 ml, 17.4 mmol) and secondly ethyl chloroformate (1, 5 ml, 15.6 mmol). After 20 min of stirring, the mixture was added a solution of (13.3 mmol) aminoadamantanes (Am, Rim, or Mem) and Et_3N (2.2 ml, 15.5 mmol) in THF (40 mL). The reaction mixture was stirred for 3 h, and after completion of the reaction, the mixture was filtered and then evaporated in a vacuo. The residue was diluted with CH_2Cl_2 (100 mL) and washed with water (5 × 50 mL) and then with 5% $NaHCO_3$ (5 × 50 mL). The organic phase was dried over Na_2SO_4 and further evaporated to dryness. The crude product was purified by flash-chromatography (HE/EtOAc) and then recrystallized from acetonitrile to give the desired hybrids (**4a$_i$–c$_i$**).

The detailed NMR, IR, and HRMS spectra data (see also supplementary information file: APPENDIX_ J_Chem.docx) of the obtained cinnamoyl hybrids (**4a$_i$–c$_i$**) are as follows:

(*E*)–*N*-Cinnamoyl amide of amantadine (CA-Am, 4a_1): White crystals. M.p: 214-215°C. IR (ATR)u_{max}: 3315, 3273, 2902, 2850, 1655, 1617, 1542, 1447, 1358, 1348,

1310, 1221, 979, 764, 724 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ 7.57 (s, 1H), 7.54–7.49 (m, 2H), 7.40 (t, $J = 7.4$ Hz, 2H), 7.37–7.34 (m, 1H), 7.32 (d, $J = 15.7$ Hz, 1H), 6.68 (d, $J = 15.7$ Hz, 1H), 2.07–2.01 (m, 3H), 1.99 (s, 6H), 1.64 (t, $J = 3.0$ Hz, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 164.5, 138.2, 135.6, 129.7, 129.4, 127.8, 124.3, 51.3, 41.5, 36.5, 29.3. HRMS (m/z): 304.1673 $(M + Na)^+$, calculated: 304.1672.

(*E*)–*N*-α-Methylcinnamoylamide of amantadine (α-CH_3-CA-Am, $4a_2$):

White crystals. M.p: 90-91°C. IR (ATR)u_{max}: 3328, 2904, 2848, 1643, 1612, 1523, 1449, 1360, 1343, 1302, 1253, 1146, 772, 703, 694 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ 7.42–7.35 (m, 4H), 7.32–7.27 (m, 1H), 7.17 (s, 1H), 7.06 (s, 1H), 2.06–2.01 (m, 9H), 1.96 (d, $J = 1.5$ Hz, 3H), 1.64 (bs, 6H). ^{13}C NMR (151 MHz, DMSO) δ 169.3, 136.8, 134.3, 131.7, 129.6, 128.8, 127.9, 51.6, 41.3, 36.6, 29.4, 15.0. HRMS (m/z): 318.1831 $(M + Na)^+$, calculated: 318.1828.

(*E*)–*N*-3-Methylcinnamoylamide of amantadine (CA (3-CH_3)-Am, $4a_3$):

White crystals. M.p: 161-163°C. IR (ATR) u_{max}: 3305, 2904, 2847, 1655, 1612, 1538, 1345, 1335, 1309, 1240, 1207, 1008, 985, 778 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ 7.54 (s, 1H), 7.35–7.25 (m, 4H), 7.17 (d, $J = 7.0$ Hz, 1H), 6.66 (d, $J = 15.7$ Hz, 1H), 2.32 (s, 3H), 2.03 (s, 3H), 1.99 (s, 6H), 1.64 (s, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 164.5, 138.5, 138.2, 135.6, 130.3, 129.3, 128.4, 125.0, 124.2, 51.3, 41.5, 36.5, 29.3, 21.4. HRMS (m/z): 318.1830 $(M + Na)^+$, calculated: 318.1828.

(*E*)–*N*-4-Methylcinnamoylamide of amantadine (CA (4-CH_3)-Am, $4a_4$):

White crystals. M.p: 184-185°C. IR (ATR)u_{max}: 3326, 2903, 2846, 1659, 1623, 1570, 1540, 1521, 1452, 1357, 1346, 1309, 1220, 1211, 984, 810 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ 7.51 (s, 1H), 7.41 (d, $J = 8.0$ Hz, 2H), 7.28 (d, $J = 15.7$ Hz, 1H), 7.21 (d, $J = 7.9$ Hz, 2H), 6.61 (d, $J = 15.7$ Hz, 1H), 2.31 (s, 3H), 2.03 (s, 3H), 1.99 (s, 6H), 1.64 (s, 6H). ^{13}C NMR (151 MHz, DMSO) δ 164.64, 139.33, 138.13, 132.87, 129.96, 127.79, 123.26, 51.29, 41.50, 36.53, 29.29, 21.38.^{13}C NMR (151 MHz, DMSO-d_6) δ 164.6, 139.3, 138.1, 132.9, 130.0, 127.8, 123.3, 51.3, 41.5, 36.5, 29.3, 21.4. HRMS (m/z): 318.1834 $(M + Na)^+$, calculated: 318.1828.

(*E*)–*N*-3,4-Diacetylcaffeoylamide of amantadine (CaffA (3,4-Ac_2)-Am, $4a_5$):

White crystals. M.p: 164-165°C. IR (ATR) u_{max}: 3282, 2905, 2853, 1765, 1657, 1615, 1544, 1501, 1425, 1368, 1360, 1245, 1201, 1109, 1009, 989, 898 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ 7.57 (s, 1H), 7.45 (dd, $J = 8.4$, 2.1 Hz, 1H), 7.42 (d, $J = 2.0$ Hz, 1H), 7.33–7.27 (m, 2H), 6.66 (d, $J = 15.7$ Hz, 1H), 2.29 (d, $J = 4.5$ Hz, 6H), 2.03 (s, 3H), 1.99 (s, 6H), 1.64 (s, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.7, 168.6, 164.2, 142.9, 142.7, 136.5, 134.5, 126.1, 125.5, 124.6, 122.6, 51.4, 41.5, 36.5, 29.3, 20.8, 20.8. HRMS (m/z): 420.1789 $(M + Na)^+$, calculated: 420.1781.

(E)–N-Caffeoylamide of amantadine (CaffA-Am, $4a_6$):

Orange solid. IR (ATR) u_{max}: 3322, 3159, 2906, 2851, 1644, 1610, 1560, 1520, 1435, 1371, 1359, 1269, 1240, 1111, 1011, 979, 855, 807 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ 9.30 (s, 1H), 9.08 (s, 1H), 7.43 (s, 1H), 7.13 (d, $J = 15.6$ Hz, 1H), 6.91 (d, $J = 2.1$ Hz, 1H), 6.79 (dd, $J = 8.2$, 2.1 Hz, 1H), 6.73 (d, $J = 8.1$ Hz, 1H), 6.38 (d, $J = 15.6$ Hz, 1H), 2.02 (s, 3H), 1.98 (s, 6H), 1.63 (s, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.0, 147.5, 146.0, 138.7, 127.1, 120.6, 120.6, 116.2, 114.3, 51.2, 41.6, 36.6, 29.3. HRMS (m/z): 336.1574 $(M + Na)^+$, calculated: 336.1570.

(*E*)–*N*-4-Hydroxycinnamoylamide of amantadine (CA (4-OH)-Am, $4a_7$):

Yellow crystals. M.p: 235-237°C. IR (ATR) u_{max}: 3338, 3062, 2905, 2849, 1643, 1607, 1581, 1540, 1515, 1448, 1362, 1349, 1264, 1220, 1170, 1104, 1010, 985, 829 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ 9.77 (s, 1H), 7.43 (s, 1H), 7.34 (d, $J = 8.2$ Hz, 2H), 7.22 (d, $J = 15.6$ Hz, 1H), 6.78 (d, $J = 8.1$ Hz, 2H), 6.46 (d, $J = 15.6$ Hz, 1H), 2.02 (s, 3H), 1.98 (s, 6H), 1.64 (s, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.0, 159.1, 138.3, 129.5, 126.6, 120.8, 116.2, 51.2, 41.6, 36.6, 29.3. HRMS (m/z): 320.1621 $(M + Na)^+$, calculated: 320.16210.

(*E*)–*N*-Cinnamoyl amide of memantine (CA-Mem, $4b_1$):

White crystals. M.p: 170-172°C. IR (ATR)u_{max}: 3253, 3063, 2942, 2896, 2861, 2841, 1653, 1611, 1562, 1450, 1355, 1336, 1292, 1253, 1230, 987, 765, 724, 676 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ 7.60 (s, 1H), 7.53–7.50 (m, 2H), 7.40 (t, $J = 7.2$ Hz, 2H), 7.37–7.34 (m, 1H), 7.31 (d, $J = 15.7$ Hz, 1H), 6.66 (dd, $J = 15.7$, 1.6 Hz, 1H), 2.13–2.06 (m, 1H), 1.83 (bs, 2H), 1.66 (d, $J = 11.8$ Hz, 2H), 1.62 (d, $J = 11.9$ Hz, 2H), 1.34 (d, $J = 11.8$ Hz, 2H), 1.27 (d, $J = 12.3$ Hz, 2H), 1.13 (bs, 2H), 0.83 (s, 6H). ^{13}C NMR (151 MHz, DMSO) δ 164.6, 138.3, 135.6, 129.7, 129.4, 127.8, 124.3, 52.9, 50.8, 47.5, 42.8, 40.0, 32.3, 30.6, 30.0. HRMS (m/z): 310.2165 $(M + H)^+$, calculated: 310.2165; 332.1983 $(M + Na)^+$, calculated: 332.1985.

(*E*)–*N*-Caffeoylamide of memantine (CaffA-Mem, $4b_2$):

Beige solid. M.p: 232-233°C. IR (ATR)u_{max}: 3536, 3340, 2942, 2895, 2861, 2838, 1657, 1584, 1553, 1527, 1446, 1373, 1355, 1333, 1278, 1222, 1190, 1114, 970, 812 cm^{-1}. ^1H (DMSO-d_6) δ (ppm): 9.11 (s, 1H, OH), 9.33 (s, 1H, OH), 7.45 (s, 1H, NH), 7.11 (d, 1H, $J = 15.5$ Hz), 6.90 (d, 1H, $J = 2.0$ Hz), 6.78 (dd, 1H, $J = 8.2$ Hz, $J = 2.0$ Hz), 6.72 (d, 1H, $J = 8$ Hz), 6.36 (d, 1H, $J = 15.5$ Hz), 2.08 (m, 1H), 1.81 (s, 2H), 1.64 (d, 2H, $J = 11.9$ Hz), 1.59 (d, 2H, $J = 11.9$ Hz), 1.33 (d, 2H, $J = 12.1$ Hz), 1.25 (d, 2H, $J = 12.1$ Hz), 1.11 (m, 4H), 0.82 (s, 6H). ^{13}C (DMSO-d_6) δ (ppm): 165.1 (CO), 147.5, 145.9, 138.7(=CH), 127.0, 120.7(Ar-CH), 120.5(=CH), 116.2(Ar-CH), 114.2(Ar-CH), 60.2, 52.8, 50.8 (CH_2), 47.6 (2x CH_2), 42.8(2x CH_2), 40.0 (CH_2), 32.3, 30.6 (2xCH_3), 30.0 (CH). HRMS (m/z): 340.1923 $(M - H)^+$, calculated: 340.1918.

(*E*)–*N*-*α*-Methylcinnamoylamide of memantine (*α*-CH_3-CA-Mem, 4b_3):

White crystals. M.p: 104-105°C. IR (ATR) u_{max}: 3249, 3053, 2942, 2860, 2841, 1645, 1613, 1538, 1497, 1452, 1354, 1338, 1321, 1311, 1297 1278, 1240, 1194, 1143, 922, 772, 712, 693 cm^{-1}.^{1}H (DMSO-d_6) δ (ppm): 7.40 (m, 2H), 7.36 (m, 2H), 7.29 (m, 1H), 7.24 (brs, 1H, NH), 7.05 (brs, 1H, =CH), 2.09 (m, 1H), 1.95 (d, 3H, J = 1.5 Hz), 1.85 (m, 2H), 1.69 (d, 2H, J = 11.8 Hz) 1.64 (d, 2H, J = 12.0 Hz), 1.33 (d, 2H, J = 12.2 Hz), 1.26 (d, 2H, J = 12.2 Hz), 1.12 (m, 2H), 0.83 (s, 6H). ^{13}C (DMSO-d_6) δ (ppm): 169.4 (CO), 136.8 134.3, 131.7 (=CH), 129.6 (ArCH), 128.8 (ArCH), 127.9 (ArCH), 53.2, 50.8(CH_2), 47.35 (2xCH_2), 42.8 (2xCH_2), 39.75 (CH_2), 32.4, 30.6(2xCH_3), 30.0 (CH), 15.0 (CH_3). HRMS (m/z): 324.2316 $(M + H)^+$, calculated: 324.2322; 346.2135 $(M + Na)^+$, calculated: 346.2141.

(*E*)–*N*-3-Methylcinnamoyl of memantine (CA (3-CH_3)-Mem, 4b_4):

White crystals. M.p: 145-146°C. IR (ATR) u_{max}: 3277, 3069, 2944, 2896, 2861, 2839, 1654, 1614, 1548, 1484, 1354, 1334, 1238, 1211, 981, 784, 735, 665 cm^{-1}.^{1}H (DMSO-d_6) δ (ppm): 7.78 (s, 1H, NH), 7.32 (m, 1H), 7.30 (m, 1H), 7.28 (m, 1H), 7.26 (d, 1H, J = 15.7 Hz), 7.16 (brd, 1H, J = 7.1 Hz), 6.65 (d, 1H, J = 15.6 Hz), 2.31 (s, 3H), 2.09 (m, 1H), 1.81 (m, 2H), 1.65 (d, 2H, J = 12.0 Hz), 1.61 (d, 2H, J = 12.0 Hz), 1.33 (d, 2H, J = 12.2 Hz), 1.26 (d, 2H, J = 12.2 Hz), 1.12 (m, 2H), 0.83 (s, 6H, 2xCH_3). ^{13}C (DMSO-d_6) δ (ppm): 164.6 (CO), 138.5, 138.2(=CH), 135.5, 130.4(Ar-CH), 129.3(Ar-CH), 128.4(Ar-CH), 125.00(Ar-CH), 124.1 (=CH), 52.9, 50.7 (CH_2), 47.5 (2xCH_2), 42.8 (2xCH_2), 40.0 (CH_2), 32.3, 30.6 (2xCH_3), 30.0 (CH), 21.4 (CH_3). HRMS (m/z): 324.2321 $(M + H)^+$, calculated: 324.2322; 346.2140 $(M + Na)^+$, calculated: 346.2141.

(*E*)–*N*-*α*-Methylcinnamoylamide of rimantadine (*α*-CH_3-CA-Rim, 4c_1):

White crystals. M.p: 118-119°C. IR (ATR) u_{max}: 3313, 2899, 2884, 2846, 1651, 1614, 1574, 1531, 1494, 1446, 1380, 1363, 1351, 1339, 1271, 995, 698, 687, 685 cm^{-1}.^{1}H NMR (600 MHz, DMSO-d_6) δ 7.49 (d, J = 9.4 Hz, 1H), 7.44–7.37 (m, 3H), 7.31 (tt, J = 6.5, 2.0 Hz, 1H), 7.12 (s, 1H), 3.70 (dq, J = 9.4, 7.0 Hz, 1H), 2.02 (d, J = 1.5 Hz, 3H), 1.94 (s, 3H), 1.68–1.57 (m, 6H), 1.58–1.46 (m, 6H), 1.01 (d, J = 7.0 Hz, 3H).^{13}C NMR (151 MHz, DMSO-d_6) δ 169.2, 136.7, 133.7, 131.8, 129.7, 128.8, 128.0, 52.8, 38.6, 37.2, 36.6, 28.3, 15.2, 14.5. HRMS (m/z): 346.2141 $(M + Na)^+$, calculated: 346.2141.

(*E*)–*N*-2-Methylcinnamoylamide of rimantadine (CA (2-CH_3)-Rim, 4c_2):

White crystals. M.p: 165-166°C. IR (ATR) u_{max}: 3294, 3061, 2900, 2847, 1663, 1651, 1614, 1542, 1487, 1448, 1378, 1354, 1343, 1278, 1226, 1211, 1160, 1115, 978, 756, 738, 673 cm^{-1}.^{1}H NMR (600 MHz, DMSO-d_6) δ 7.72 (d, J = 9.4 Hz, 1H), 7.62 (d, J = 15.6 Hz, 1H), 7.53 (d, J = 7.5 Hz, 1H), 7.27–7.21 (m, J = 2.1 Hz, 3H), 6.68 (d, J = 15.6 Hz, 1H), 3.66 (dq, J = 9.4, 6.9 Hz, 1H), 2.37 (s, 3H), 1.94 (s, 3H), 1.70–1.56 (m, 6H), 1.56–1.45 (m, 6H), 0.99 (d, J = 6.9 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 164.8, 137.1, 136.2, 134.4, 131.1, 129.5, 126.8, 126.3, 124.4, 52.7, 38.5, 37.1, 36.1, 28.3, 19.9, 14.8.HRMS (m/z): 346.2139 $(M + Na)^+$, calculated: 346.2141.

(*E*)–*N*-3-Methylcinnamoylamide of rimantadine (CA (3-CH_3)-Rim, 4c_3):

White crystals. M.p: 148-149°C. IR (ATR) u_{max}: 3291, 2898, 2847, 1654, 1619, 1538, 1446, 1355, 1342, 1239, 1208, 982, 737, 666 cm^{-1}.^{1}H NMR (600 MHz, DMSO-d_6) δ 7.68 (d, J = 9.5 Hz, 1H), 7.40–7.31 (m, 3H), 7.29 (t, J = 7.5 Hz, 1H), 7.18 (d, J = 7.4 Hz, 1H), 6.76 (d, J = 15.8 Hz, 1H), 3.65 (dq, J = 9.3, 6.9 Hz, 1H), 2.33 (s, 3H), 1.94 (s, 3H), 1.70–1.56 (m, 6H), 1.56–1.43 (m, 6H), 0.98 (d, J = 6.9 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 164.8, 138.8, 138.5, 135.6, 130.4, 129.3, 128.3, 125.2, 123.2, 52.7, 38.5, 37.1, 36.1, 28.3, 21.4, 14.8. HRMS (m/z): 346.2137 $(M + Na)^+$, calculated: 346.2141.

(*E*)–*N*-4-Methylcinnamoylamide of rimantadine (CA (4-CH_3)-Rim, 4c_4):

White crystals. M.p: 184-185°C. IR (ATR) u_{max}: 3288, 2906, 2876, 2850, 1654, 1615, 1536, 1445, 1355, 1343, 1215, 1207, 973, 761 cm^{-1}.^{1}H NMR (600 MHz, DMSO-d_6) δ 7.66 (d, J = 9.5 Hz, 1H), 7.44 (d, J = 8.1 Hz, 2H), 7.35 (d, J = 15.7 Hz, 1H), 7.22 (d, J = 7.9 Hz, 2H), 6.70 (d, J = 15.7 Hz, 1H), 3.65 (dq, J = 9.4, 6.9 Hz, 1H), 2.32 (s, 3H), 1.94 (s, 3H), 1.69–1.56 (m, 6H), 1.55–1.45 (m, 6H), 0.98 (d, J = 6.9 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 164.9, 139.4, 138.7, 132.9, 130.0, 127.8, 122.3, 52.6, 38.5, 37.1, 36.2, 28.3, 21.4, 14.8. HRMS (m/z): 346.2146 $(M + Na)^+$, calculated: 346.2141.

(*E*)–*N*-3,4-Diacetylcaffeoylamide of rimantadine (CaffA (3,4-Ac_2)-Rim, 4c_5):

White crystals. IR (ATR) u_{max}: 3362, 2902, 2847, 1774, 1745, 1666, 1629, 1542, 1501, 1429, 1371, 1259, 1245, 1201, 1174, 1108, 1010, 994, 969, 896, 846, 837 cm^{-1}.^{1}H NMR (600 MHz, DMSO-d_6) δ 7.72 (d, J = 9.5 Hz, 1H), 7.48 (dd, J = 8.4, 2.1 Hz, 1H), 7.46 (d, J = 2.0 Hz, 1H), 7.37 (d, J = 15.7 Hz, 1H), 7.31 (d, J = 8.3 Hz, 1H), 6.75 (d, J = 15.8 Hz, 1H), 3.64 (dq, J = 9.4, 6.9 Hz, 1H), 2.29 (d, J = 3.9 Hz, 6H), 1.94 (s, 3H), 1.68–1.56 (m, 6H), 1.54–1.45 (m, 6H), 0.98 (d, J = 6.9 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.7, 168.6, 164.5, 143.0, 142.7, 137.1, 134.5, 126.2, 124.6, 124.5, 122.6, 52.7, 38.4, 37.1, 36.1, 28.3, 20.8, 20.8, 14.7. HRMS (m/z): 448.2096 $(M + Na)^+$, calculated: 448.2094.

(*E*)–*N*-Caffeoylamide of rimantadine (CaffA-Rim, 4c_6):

Orange solid. M.p:171-173°C. IR (ATR) u_{max}: 3327, 3282, 2900, 2848, 1653, 1581, 1510, 1145, 1358, 1281, 1193, 1161, 1112, 974 cm^{-1}.^{1}H NMR (600 MHz, DMSO-d_6) δ 9.32 (s, 1H), 9.08 (s, 1H), 7.56 (d, J = 9.5 Hz, 1H), 7.20 (d, J = 15.6 Hz, 1H), 6.93 (d, J = 2.1 Hz, 1H), 6.82 (dd, J = 8.2, 2.1 Hz, 1H), 6.74 (d, J = 8.1 Hz, 1H), 6.46 (d, J = 15.6 Hz, 1H), 3.64 (dq, J = 9.3, 6.9 Hz, 1H), 1.94 (s, 3H), 1.68–1.55 (m, 6H),

1.55–1.44 (m, 6H), 0.97 (d, $J = 6.9$ Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.3, 147.6, 146.0, 139.3, 127.1, 120.6, 119.6, 116.2, 114.4, 52.5, 38.5, 37.2, 36.2, 28.3, 14.8. HRMS (m/z): 340.1923 $(M\text{-}H)^+$, calculated: 340.1918.

2.4. In Vitro Antioxidant Activity Assays

2.4.1. DPPH Assay. DPPH analysis was carried out according to the method of Brand-Williams et al. [33]. Five hundred μL of the test solution in increasing concentrations (8–500 μM) were added to 500 μL of a freshly prepared solution of 0.1 mM DPPH in methanol. The resulting mixture was incubated in the dark for 30 minutes, and the absorbance was read at 517 nm. A 1 : 1 mixture of DPPH solution and methanol was used as a control sample.

Antioxidant activity was calculated as follows: antioxidant activity (%) = $[(A_{517}$ control $- A_{517}$ sample$)/A_{517}$ control$] \times 100$.

2.4.2. ABTS Assay. The method of Re et al. [34], based on the inhibition of ABTS (2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)) oxidation to a cationic radical ($ABTS^{\bullet+}$) by an antioxidant, was applied. $ABTS^{\bullet+}$ was prepared by mixing 7.0 mM ABTS with 2.45 mM potassium persulfate. The mixture was kept in dark at room temperature for 16 hours before use. The solution was diluted in methanol (2 mL $ABTS^{\bullet+}$ + 58 mL methanol) giving a working solution with absorption at 743 nm about 1.1 ± 0.02. Then, 75 μL of the tested substance was added to 1.425 mL of the working solution. After 15 minutes of incubation at 37°C, the sample was measured at 743 nm against methanol. A blank containing 75 μL of water instead of the test substance also was measured against methanol.

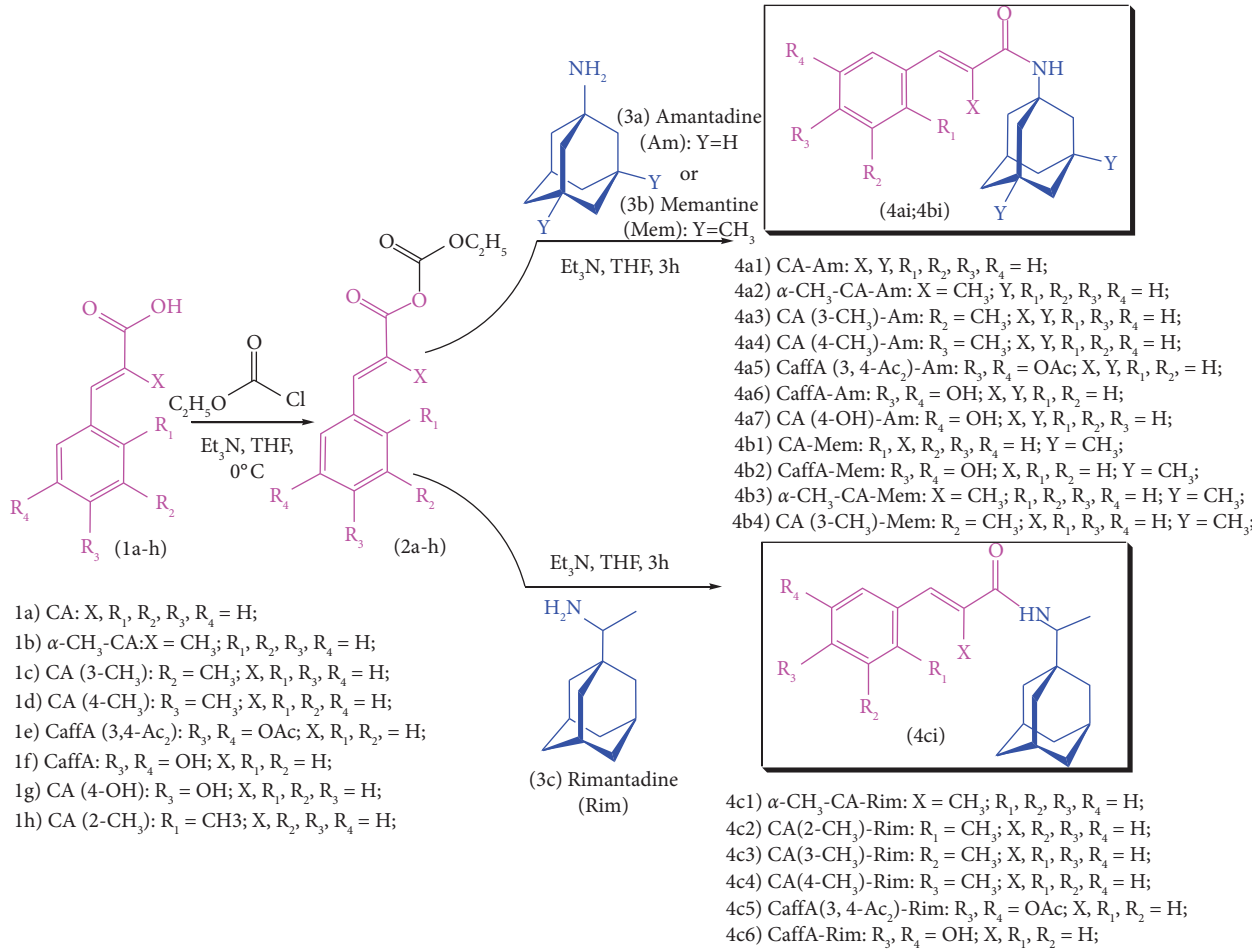
2.4.3. Ferric Reducing Antioxidant Power (FRAP) Assay. FRAP assay was performed according to Benzie and Strain [35] with some modifications. The method is based on the reduction of the colorless Fe (III)-TPTZ complex (ferric-tripyridyltriazine) to a blue-stained Fe (II)-TPTZ complex (ferrous-tripyridyltriazine) at low pH in the presence of a reductant (antioxidant). The following solutions were prepared: (1) 0.03 M acetate buffer, pH 3.6; (2) 1 mM TPTZ (2,4,6-tripyridyl-s-triazine, in 40 mM HCl); (3) 1.5 mM $FeCl_3.6H_2O$ and mixed in the ratio 10 : 1 : 1 (10 parts 0.03 M acetate buffer: 1 part 1 mM TPTZ: 20 parts 1.5 mM $FeCl_3$). The sample containing 1.5 mL of the reaction mixture, 50 μL of the tested substance, and the blank sample containing 1.5 mL of the reaction mixture and 50 μL H_2O were incubated for 4 min at 37°C and after that read at 593 nm. The standard curve of Trolox was prepared, and the results were expressed as μmol Trolox equivalents.

2.4.4. Cupric Reducing Antioxidant Capacity (CUPRAC) Assay. The method of Apak et al. with some modifications was applied [36]. The method is based on the reaction of Cu (II)-neocuproine complex (CUPRAC reagent, Cu (II)-Nc) with an antioxidant, resulting in a yellow-orange product, Cu (I)-neocuproine chelate complex, measurable at 450 nm. The following solutions were prepared: (1) 10 mM $CuCl_2$ in d. H_2O; (2) 1.0 M ammonium acetate buffer; pH 7; (3) 7.5 mM Neocuproine (NC) in 96% ethanol, and they were mixed in 1 : 1 : 1 ratio: 1 part Cu (II) (1): 1 part NC (3): 1 part buffer (2). In a 96-well plate, 0.01 mL of the tested substance in different concentrations were added to 290 mL of the reaction mixture and mixed. After incubation at 50°C for 20 min, the absorption was read at 450 nm against a blank sample (0.01 mL DMSO added to 0.290 mL reaction mixture). The standard curve was prepared with Trolox in concentrations varying in the range from 0.1 mM to 1 mM, and the obtained results were expressed as μM Trolox equivalent.

2.4.5. Inhibition of Fe (III)/asc Induced Lipid Peroxidation in Brain Homogenate. The inhibition of Fe (III)/asc-induced lipid peroxidation (LP) in brain homogenate by the tested substances was estimated by the method of Hunter et al. [37] based on the reaction of thiobarbituric acid with lipid peroxidation end products. In brief, 1 mL of the brain homogenates (1 mg/ml protein) were incubated in the presence of Fe (III)/asc (0.1 mM $FeCl_3$ and 0.5 mM ascorbic acid) and in the absence and presence of increased concentrations of the tested substrates for 30 min at 37°C. The reactions were stopped by the addition of 0.2 ml 2.8% trichloroacetic acid, 0.1 ml 5 M HCl, and 0.6 ml thiobarbituric acid (2% w/v in 50 mM NaOH). Thereafter, the samples were incubated at 100°C for 15 min, and the absorption of the formed color complex, malondialdehyde, was read at 532 nm. The antioxidant activity of the tested substances was expressed as percent inhibition of the process.

3. Results and Discussion

3.1. Chemistry. In the current work, in order to find the specific pharmacophores capable to generate potent antioxidant capacities, a series of different substituted cinnamic amides were synthesized. The *N*-cinnamoylamides (**4a$_i$–c$_i$**) were obtained by following the procedure given in the patent literature [32]. Briefly, as shown in Scheme 1, the amidation of aminoadamantanes (amantadine (**3a**), memantine (**3b**), or rimantadine (**3c**)) was proceeded with various cinnamic acids (**1a–h**) via a mixed anhydride activation steps (**2a–h**) with ethyl chloroformate in THF and a base triethylamine. In the case of caffeoylamides (3-(3′,4′-dihydroxyphenyl)-*N*-adamantyl-propeneamides) (**4a$_6$, 4b$_2$,** and **4c$_6$**), the preliminary acetylation step of caffeic acid (**1e**) was carried out. Since the catechol feature of caffeic acid is susceptible to autoxidation [38], we provided the reaction under an argon atmosphere by refluxing dichloromethane in the presence of acetic anhydride, triethylamine, and dimethylaminopyridine as a catalyst. Indeed, diacetylated caffeic acid was smoothly obtained in high yield (87%) as reported in the literature [32]. Furthermore, the deprotection of acetyl groups of the compounds was accomplished by potassium hydroxide hydrolysis in the medium of THF/MeOH (1 : 1).



Scheme 1: Synthesis of cinnamoylamides of aminoadamantanes (Am, Mem, and Rim).

Except for diacetylated caffeic acid (**1e**), which was crystalized from CH_2Cl_2/HE, the rest of the compounds **4a$_i$–c$_i$** were isolated in pure form after flash-chromatography purification and further recrystallized by acetonitrile in satisfactory yields (Table 1). The structure of the compounds was confirmed by spectral methods, ^{1}H- and ^{13}C NMR, IR, and HRMS spectra. In all cases (except with the *α*-methylcinnamoylamides (4a_2, 4b_3, and 4c_1)), the configuration of the double bond was determined to be *E*-, based on the high value of the ^{1}H vicinal coupling constant (3J~16 Hz). Moreover, the similar trans-configuration was also found in other cinnamic acid amides of aminoadamantanes, previously obtained by us [9, 31].

Detailed characterization data of the amides are listed in Section 2.3.

3.2. In Vitro Antioxidant Activity Capacity. Nowadays, the increase in oxidative stress-related diseases has become a sizable interest worldwide. With respect to the fundamental role of antioxidants to act against oxidation processes through different mechanisms [39], they will be able to prevent or to reduce the harmful impact. Consequently, there is an unremitting pursuit of new exogenous antioxidants that could be used as preventive agents for the treatment of global health problems such as cancer, neurodegenerative disorders, influenza pathogenesis, and others.

Up to now, there is not a general method that can be used for the assessment of antioxidant capacity; therefore, several antioxidant assays were performed on our synthetically obtained cinnamoylamides.

3.2.1. DPPH (1,1-Diphenyl-2-picrylhydrazyl) Scavenging Activity. According to the PubMed database, DPPH·is the most popular free radical for in vitro estimation of antiradical activity. Since it is first used in 1922 until August 2022, there are nearly 21,000 studies applying this stable radical. Due to its simple, high-precision one-step analysis, in our study, we employed the DPPH·method [33] to estimate the radical scavenging activity of the synthesized by us amides (**4a$_i$-c$_i$**), as well as the caffeic acid (CaffA), used as a reference. Among all tested amides, only three of them had DPPH radical scavenging potential (Figure 1), caffeoylamides of amantadine (CaffA-Am, **4a$_6$**) and of rimantadine (CaffA-Rim, **4c$_6$**) and to a lesser extent 4-hydroxycinnamoylamide of amantadine (CA (4-OH)-Am, **4a$_7$**). Meanwhile, compounds **4a$_6$** and **4c$_6$** had a similar pattern of DPPH inhibition as did CaffA. In

Table 1: Synthesized *N*-cinnamoyl adamantane amides.

	Substituted cinnamic acids	Amines	Products	Yields (%)
1a:	Cinnamic acid **(CA)**	**3a**	**$4a_1$: CA-Am**	83
1b:	α-Methylcinnamic acid (α **-CH_3-CA**)	**3a**	**$4a_2$: α-CH_3-CA-Am**	75
1c:	3-Methylcinnamic acid **(CA (3-CH_3))**	**3a**	**$4a_3$: CA (3-CH_3)-Am**	70
1d:	4-Methylcinnamic acid **(CA (4-CH_3))**	**3a**	**$4a_4$: CA (4-CH_3)-Am**	78
1e:	3,4-Diacetylcaffeic acid **(CaffA (3,4-Ac_2))**	**3a**	**$4a_5$: CaffA (3,4-Ac_2)-Am**	65
1f:	Caffeic acid **(CaffA)**	**3a**	**$4a_6$: CaffA-Am**	77
1g:	4-Hydroxycinnamic acid **(CA (4-OH))**	**3a**	**$4a_7$: CA (4-OH)-Am**	42
1a:	**CA**	**3b**	**$4b_1$: CA-Mem**	81
1f:	**CaffA**	**3b**	**$4b_2$: CaffA-Mem**	54
1b:	**α -CH_3-CA**	**3b**	**$4b_3$: α-CH_3-CA-Mem**	59
1c:	**CA (3-CH_3)**	**3b**	**$4b_4$: CA (3-CH_3)-Mem**	55
1b:	**α -CH_3-CA**	**3c**	**$4c_1$: α-CH_3-CA-Rim**	62
1h:	2-Methyl-cinnamic acid **(CA (2-CH_3))**	**3c**	**$4c_2$: CA (2-CH_3)-Rim**	66
1c:	**CA (3-CH_3)**	**3c**	**$4c_3$: CA (3-CH_3)-Rim**	71
1d:	**CA (4-CH_3)**	**3c**	**$4c_4$: CA (4-CH_3)-Rim**	81
1e:	**CaffA (3,4-Ac_2)**	**3c**	**$4c_5$: CaffA (3,4-Ac_2)-Rim**	51
1f:	**CaffA**	**3c**	**$4c_6$: CaffA-Rim**	72

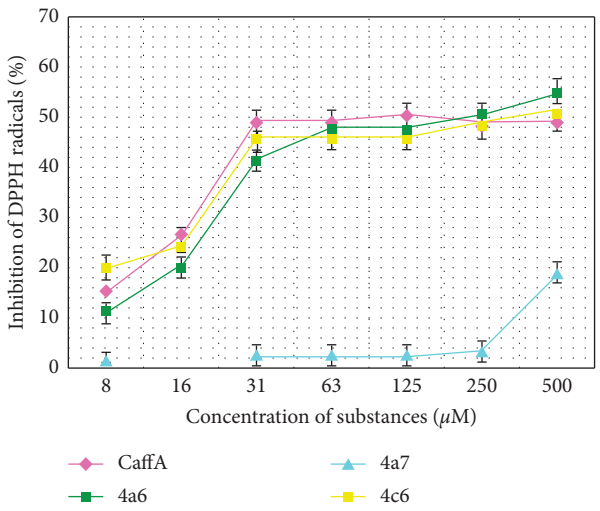


Figure 1: DPPH radicals inhibition (%) of the tested substances, applied in different concentrations (from 8 to 500 μM).

methanolic media, at concentrations above 31 μM, CaffA and amides **$4a_6$** and **$4c_6$** reached almost 50% inhibition of DPPH radicals and retained this effect. Moreover, the greatest value of DPPH inhibition percentages was found for caffeoyl derivatives **$4a_6$** (55.06%) and **$4c_6$** (51.38%) at the highest concentration of 500 μM. Interestingly, comparing the results obtained for *N*-caffeoyl-rimantadine (**$4c_6$**) and caffeic acid by another DPPH methodology [40], previously applied by us [9], a difference in activities was found. Thus, the compounds **$4c_6$** and CaffA at 48 μM in ethanolic media displayed higher %RSA values of 72.58 ± 8.26 and 92.65 ± 2.90, respectively [9], compared to the current methanolic conditions (Figure 1). Indeed, not only the solvent affects the scavenging activity; however, various documented DPPH protocols differ in more than one experimental condition, and the information provided are often contradictory [40].

In contrast to its diphenolic analogue **$4a_6$**, 4-hydroxycinnamoyl amide of amantadine **$4a_7$** being a simple phenol had 2.8 times lower radical quenching ability (about 20%) at the highest concentration measured (500 μM). As expected, the data obtained are in line with literature reports, concerning that removal of the hydroxyl group at the 3-position of the phenyl ring (**$4a_7$**) caused the decrease of activity. Unlike compound (**$4a_7$**), the stabilization of the phenoxyl radical through an intramolecular hydrogen bond can occur in its o-diphenolic counterpart (**$4a_6$**); hence, the catechol moiety has been defined as a key structural feature that is responsible for profound scavenging activity [40, 41].

3.2.2. ABTS Radicals Scavenging Activity. In the current study, for the measurement of the antioxidant activity of cinnamoylamides (**$4a_i$-c_i**) and referent CaffA, the decolorization assay based on the reduction of ABTS radical cation by antioxidants was applied [34]. Amongst the tested compounds, ABTS-antiradical activity was exerted only by amides **$4b_2$**, **$4a_7$**, and **$4c_6$** (Figure 2). The obtained results revealed that these compounds demonstrated dose-dependent inhibitory effects against $ABTS^{\bullet}$. Figure 2 depicts that at 25 μM concentration, *N*-caffeoyl-rimantadine (**$4c_6$**) reached the maximal effect equal to CaffA, 99.29% and 99.17%, respectively. Additionally, a two-fold increase in the latter concentration (at 50 μM) leads N-caffeoyl-memantine (**$4b_2$**) to overtake the maximal effect, whereas 4-hydroxycinnamoyl-amantadine **$4a_7$** demonstrated 83.5% inhibition of $ABTS^{\bullet+}$. Interestingly, the least active antioxidant (**$4a_7$**) toward **DPPH** (Figure 1) seems to be efficient in the case of the ABTS test. Our results are in accordance with Nenadis's observation that an increase in the number of hydroxyl groups in the aromatic ring is not obligatory for the increase of the TE values [42].

3.2.3. FRAP (Ferric Reducing Antioxidant Power). Besides the radical scavenging properties, the antioxidants must also have a key reducing power; therefore, ferric

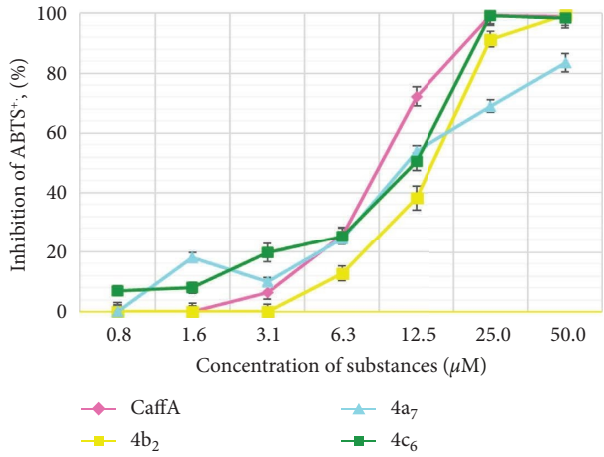


Figure 2: ABTS radicals inhibition (%) of the tested compounds, applied in different concentrations (from 0.8 to 50 μM).

reducing antioxidant power (FRAP) assay was carried out. The results of the tested substances (**4a$_i$-c$_i$**) were expressed as Trolox equivalent (TE) (Figure 3). Again, amongst the active group of compounds, significant FRAP was observed by caffeoylamides **4b$_2$** (9.15 μMTE), followed by **4c$_6$** (7.76 μM TE) and **4a$_6$** (6.1 μM TE) at the maximal tested concentration of 33.3 μM, while 4-hydroxycinnamoyl-amantadine **4a$_7$** (1.1 μM TE) demonstrated the lowest one. It was also noticed that at 4.2 μM concentration, observable FRAP was displayed only by **4a$_6$** and **4c$_6$** with 3.13 and 2.09 μMTE, respectively.

3.2.4. CUPRAC (Cupric Reducing Antioxidant Capacity) Assay. Another antioxidant method based on the cupric reducing antioxidant power of synthesized compounds and CaffA was applied [36]. Our results showed that the tested compounds were expressed as μMTE. After the studies performed in the concentration range from 4.2 to 33.3 μM, the amides that did not show reducing properties were not shown, whereas the others 4a_6, 4a_7, 4b_2, and 4c_6 demonstrated dose-dependent effect (Figure 4). At the highest concentration (33.3 μM), the CUPRAC of these amides and CaffA was as follows: **4c$_6$** = 105.78, CaffA = 101.00, **4b$_2$** = 100.54, **4a$_6$** = 90.26, and **4a$_7$** = 21.67 μM TE, respectively.

3.2.5. Inhibition of Fe(III)/asc Induced LP in Brain Homogenate. The assessment of the ability of synthesized amides (**4a$_i$-c$_i$**) and CaffA to suppress Fe(III)/asc-induced lipid peroxidation (LP) in brain homogenate was tested, according to the method of Hunter et al. [37]. The compounds were evaluated in a concentration range from 0.5 to 31.2 μM (Figure 5). From the results obtained, it is evident that at 31.2 μM concentration, pronounced inhibition was observed for several compounds: **4b$_2$** (85.08%) > **4c$_5$** (82.13%) > **4a$_6$** (82.06%) > **4a$_5$** (81.86%) > **4c$_6$** (80.19%). Moreover, it can be concluded that there is no significant difference in LP inhibition between amides with free phenolic hydroxyl groups (**4a$_6$**, **4b$_2$**, **4c$_6$**) and their diacetylated counterparts **4c$_5$**, **4a$_5$**. The most active one was *N*-caffeoyl-

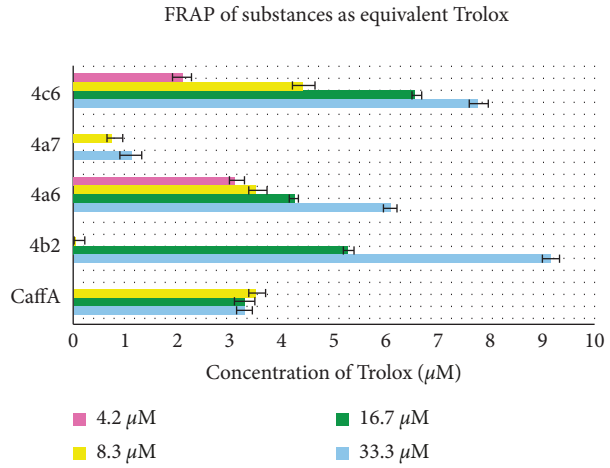


Figure 3: Ferric reducing antioxidant power of the tested substances, applied in different concentrations (from 4 to 33 μM) and expressed as the Trolox equivalent (TE).

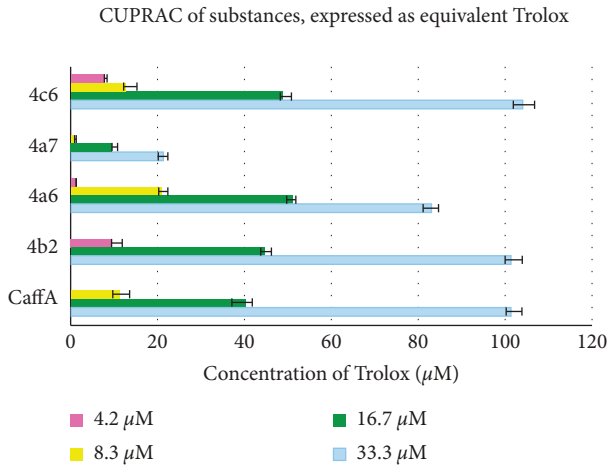


Figure 4: Cupric reducing antioxidant capacity (CUPRAC) of the tested substances applied in different concentrations (from 4 to 33 μM) and expressed as the Trolox equivalent.

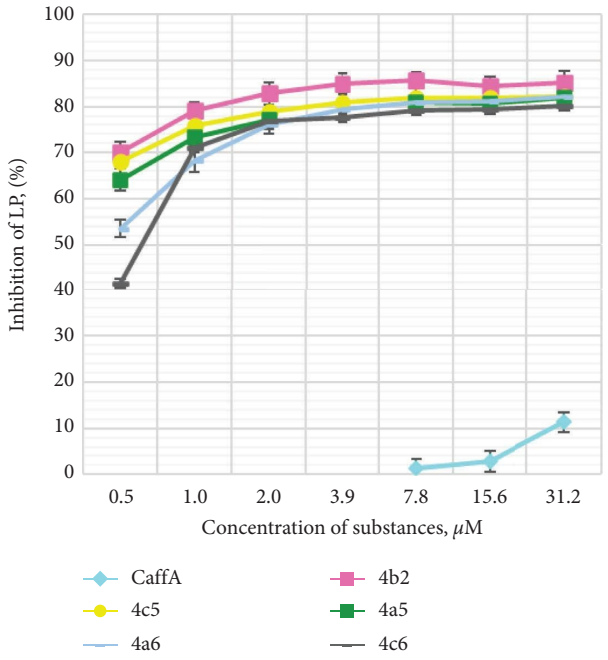


Figure 5: Inhibition of Fe (III)/asc induced lipid peroxidation (LP) in brain homogenate by the tested substances, applied in different concentrations.

memantine (CaffA-Mem, $\mathbf{4b_2}$) with 85.08%, whereas its parent compound CaffA seems to be inactive (11.33%). However, since the antioxidant activity of caffeic acid may provide neuroprotection against H_2O_2-induced toxicity [43], herein, we can assume that the presented remarkable LP inhibition by the lipophilic antioxidant *N*-caffeoylamide of memantine $\mathbf{4b_2}$ could serve as a promising antioxidant in the management of neurodegenerative disorders. In this context, increasing evidence can be found in the literature concerning multitarget agents (e.g., in Alzheimer's disease) based on the adamantane core of memantine and known neuroprotectants as antioxidants [31, 44–47].

4. Conclusions

In the present work, a mixed anhydride method was successfully applied to yield N-cinnamoylamides (**4ai-ci**), composed of substituted cinnamoyl and aminoadamantantyl scaffolds. The antioxidant capacity of synthetically obtained hybrids was analyzed by different methods and radical inhibition. From the overall results, it can be concluded that the most potent antioxidant activity demonstrated compound $\mathbf{4b_2}$, with excellent CUPRAC, FRAP, ABTS potential, and inhibition of Fe/asc–induced LP, followed by $\mathbf{4c_6} > \mathbf{4a_6} > \mathbf{CaffA} > \mathbf{4c_5}$ and $\mathbf{4a_5} > \mathbf{4a_7}$.

Noteworthy, the antioxidant activity of caffeoyl hybrids ($\mathbf{4b_2}$, $\mathbf{4c_6}$, and $\mathbf{4a_6}$) greatly increases against lipid peroxidation in the brain homogenate in comparison to their parent compound, caffeic acid, known as a natural antioxidant. Considering the structure of the compounds under study, it can be assumed that the noticeable antioxidant activity of the caffeoylamides is due to the presence of catecholic moiety in the aromatic rings. Therefore, the strategy of merging of hydrophilic caffeic acid with lipophilic aminoadamantanes could be successfully utilized to modify its solubility in a hydrophobic medium.

Abbreviations

ABTS: 2,2′-Azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)
Ac: Acetyl
Am: Amantadine
CA: Cinnamic acid (3-phenylpropenoic acid)
CaffA: Caffeic acid (3-(3′,4′-dihydroxyphenyl) propenoic acid) or (3,4-dihydroxycinnamic acid)
CUPRAC: Cupric reducing antioxidant capacity assay
DPPH: 1,1-Diphenyl-2-picrylhydrazyl radical
Et3N: Triethylamine; EtOAc-ethylacetate
FRAP: Ferric reducing antioxidant power
HE: Hexane
LP: Lipid peroxidation
Mem: Memantine
Rim: Rimantadine
RSA: Radical scavenging activity
TE: Trolox equivalent
THF: Tetrahydrofuran.

Data Availability

The NMR and HRMS spectra data of all obtained cinnamoyl hybrids ($4a_i$–$4c_i$) are presented in Supporting Information.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

The authors are grateful for the financial support from South-West University "Neofit Rilski", Blagoevgrad, Bulgaria. This work was supported by the Bilateral Project Bulgaria–Russia, contract number (KP-06-Russia-7/27.09.2019).

Supplementary Materials

Supplementary data to this article can be found online at. . . (*Supplementary Materials*)

References

[1] E. Niki, "Do antioxidants impair signaling by reactive oxygen species and lipid oxidation products?" *FEBS Letters*, vol. 586, no. 21, pp. 3767–3770, 2012.
[2] J. K. Jacob, K. Tiwari, J. Correa-Betanzo, A. Misran, R. Chandrasekaran, and G. Paliyath, "Biochemical basis for functional ingredient design from fruits," *Annual Review of Food Science and Technology*, vol. 3, pp. 79–104, 2012.
[3] T. Nguyen, P. J. Sherratt, and C. B. Pickett, "Regulatory mechanisms controlling gene expression mediated by the

antioxidant response element," *Annual Review of Pharmacology and Toxicology*, vol. 43, no. 1, pp. 233–260, 2003.

[4] R. Rodrigo, A. Miranda, and L. Vergara, "Modulation of endogenous antioxidant system by wine polyphenols in human disease," *Clinica Chimica Acta*, vol. 412, no. 5-6, pp. 410–424, 2011.

[5] G. Scapagnini, V. Sonya, A. G. Nader, C. Calogero, D. Zella, and G. Fabio, "Modulation of Nrf2/ARE pathway by food polyphenols: a nutritional neuroprotective strategy for cognitive and neurodegenerative disorders," *Molecular Neurobiology*, vol. 44, no. 2, pp. 192–201, 2011.

[6] H. Sies, "Polyphenols and health: update and perspectives," *Archives of Biochemistry and Biophysics*, vol. 501, no. 1, pp. 2–5, 2010.

[7] M. Sova, "Antioxidant and antimicrobial activities of cinnamic acid derivatives," *Mini Reviews in Medicinal Chemistry*, vol. 12, no. 8, pp. 749–767, 2012.

[8] L. Georgiev, M. Chochkova, I. Totseva et al., "Anti-tyrosinase, antioxidant and antimicrobial activities of hydroxycinnamoylamides," *Medicinal Chemistry Research*, vol. 22, no. 9, pp. 4173–4182, 2013.

[9] M. G. Chochkova, A. P. Georgieva, G. I. Ivanova et al., "Synthesis and biological activity of hydroxycinnamoyl-containing antiviral drugs," *Journal of the Serbian Chemical Society*, vol. 79, no. 5, pp. 517–526, 2014 2014.

[10] J. Guzman, "Natural cinnamic acids, synthetic derivatives and hybrids with antimicrobial activity," *Molecules*, vol. 19, no. 12, pp. 19292–19349, 2014.

[11] N. Gaikwad, S. Nanduri, and Y. Madhavi, "Cinnamamide: an insight into the pharmacological advances and structure–activity relationships," *European Journal of Medicinal Chemistry*, vol. 181, Article ID 111561, 2019.

[12] S. Benfeito, C. Oliveira, P. Soares et al., "Antioxidant therapy: still in search of the 'magic bullet," *Mitochondrion*, vol. 13, no. 5, pp. 427–435, 2013.

[13] T. Silva, C. Oliveira, and F. Borges, "Caffeic acid derivatives, analogs and applications: a patent review (2009–2013)," *Expert Opinion on Therapeutic Patents*, vol. 24, no. 11, pp. 1257–1270, 2014.

[14] A. Gaspar, M. Martins, P. Silva et al., "Dietary phenolic acids and derivatives. Evaluation of the antioxidant activity of sinapic acid and its alkyl esters," *Journal of Agricultural and Food Chemistry*, vol. 58, no. 21, pp. 11273–11280, 2010.

[15] J. C. Menezes, S. P. Kamat, J. A. Cavaleiro, A. Gaspar, J. Garrido, and F. Borges, "Synthesis and antioxidant activity of long chain alkyl hydroxycinnamates," *European Journal of Medicinal Chemistry*, vol. 46, no. 2, pp. 773–777, 2011.

[16] T. L. Serafim, F. S. Carvalho, M. P. M. Marques et al., "Lipophilic caffeic and ferulic acid derivatives presenting cytotoxicity against human breast cancer cells," *Chemical Research in Toxicology*, vol. 24, no. 5, pp. 763–774, 2011.

[17] J. Garrido, A. Gaspar, E. M. Garrido et al., "Alkyl esters of hydroxycinnamic acids with improved antioxidant activity and lipophilicity protect PC12 cells against oxidative stress," *Biochimie*, vol. 94, no. 4, pp. 961–967, 2012.

[18] L. Wanka, K. Iqbal, and P. R. Schreiner, "The lipophilic bullet hits the targets: medicinal chemistry of adamantane derivatives," *Chemical Reviews*, vol. 113, no. 5, pp. 3516–3604, 2013.

[19] J. Liu, D. Obando, V. Liao, T. Lifa, and R. Codd, "The many faces of the adamantyl group in drug design," *European Journal of Medicinal Chemistry*, vol. 46, no. 6, pp. 1949–1963, 2011.

[20] W. L. Davies, R. R. Grunert, R. F. Haff et al., "Antiviral activity of 1-adamantanamine (amantadine)," *Science*, vol. 144, pp. 862-863, Article ID 3620, 1964.

[21] R. S. Schwab, A. C. England, D. C. Poskanzer, and R. R. Young, "Amantadine in the treatment of Parkinson's disease," *JAMA, the Journal of the American Medical Association*, vol. 208, no. 7, pp. 1168–1170, 1969.

[22] A. Tsunoda, H. F. Maassab, K. W. Cochran, and W. C. Eveland, "Antiviral activity of alpha-methyl-1-adamantanemethylamine hydrochloride," *Antimicrobial Agents and Chemotherapy*, vol. 5, pp. 553–560, 1965.

[23] J. Kornhuber, K. Schoppmeyer, and P. Riederer, "Affinity of 1-aminoadamantanes for the σ binding site in post-mortem human frontal cortex," *Neuroscience Letters*, vol. 163, no. 2, pp. 129–131, 1993.

[24] H. S. Chen, J. W. Pellegrini, S. K. Aggarwal et al., "Open-channel block of N-methyl-D-aspartate (NMDA) responses by memantine: therapeutic advantage against NMDA receptor-mediated neurotoxicity," *Journal of Neuroscience*, vol. 12, no. 11, pp. 4427–4436, 1992.

[25] M. Weller, F. Finiels-Marlier, and S. M. Paul, "NMDA receptor-mediated glutamate toxicity of cultured cerebellar, cortical and mesencephalic neurons: neuroprotective properties of amantadine and memantine," *Brain Research*, vol. 613, no. 1, pp. 143–148, 1993.

[26] J. Miguel-Hidalgo, X. Alvarez, R. Cacabelos, and G. Quack, "Neuroprotection by memantine against neurodegeneration induced by β-amyloid (1–40)," *Brain Research*, vol. 958, no. 1, pp. 210–221, 2002.

[27] M. Spasova, V. Kortenska-Kancheva, I. Totseva, G. Ivanova, L. Georgiev, and T. Milkova, "Synthesis of cinnamoyl and hydroxycinnamoyl amino acid conjugates and evaluation of their antioxidant activity," *Journal of Peptide Science*, vol. 12, no. 5, pp. 369–375, 2006.

[28] V. Kancheva, M. Spasova, I. Totseva, and T. Milkova, "Study on the antioxidant activity of N-hydroxycinnamoyl-amino acid conjugates in bulk lipid autoxidation," *Rivista Italiana Delle Sostanze Grasse*, vol. 83, no. 4, Article ID 163, 2006.

[29] M. Spasova, S. Philipov, L. Nikolaeva-Glomb, A. Galabov, and T. Milkova, "Cinnamoyl-and hydroxycinnamoyl amides of glaucine and their antioxidative and antiviral activities," *Bioorganic & Medicinal Chemistry*, vol. 16, no. 15, pp. 7457–7461, 2008.

[30] M. G. Chochkova, P. P. Petrova, B. M. Stoykova et al., "Structure-activity relationships of n-cinnamoyl and hydroxycinnamoyl amides on α-glucosidase inhibition," *Journal of Chemistry*, vol. 2017, pp. 1–5, 2017.

[31] M. Chochkova, H. Jiang, R. Kyoseva et al., "Cinnamoyl-memantine hybrids: synthesis, X-ray crystallography and biological activities," *Journal of Molecular Structure*, vol. 1234, Article ID 130147, 2021.

[32] H. S. Baek, J. W. You, G. W. Nam et al.: Hydroxycinnamic Acid Derivatives and Preparation Method Thereof and Cosmetic Composition Containing it.

[33] W. Brand-Williams, M.-E. Cuvelier, and C. Berset, "Use of a free radical method to evaluate antioxidant activity," *LWT - Food Science and Technology*, vol. 28, no. 1, pp. 25–30, 1995.

[34] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans, "Antioxidant activity applying an improved ABTS radical cation decolorization assay," *Free Radical Biology and Medicine*, vol. 26, no. 9-10, pp. 1231–1237, 1999.

[35] I. F. Benzie and J. J. Strain, "The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the

FRAP assay," *Analytical Biochemistry*, vol. 239, no. 1, pp. 70–76, 1996.

[36] R. Apak, K. Güçlü, M. Özyürek, and S. E. Karademir, "Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method," *Journal of Agricultural and Food Chemistry*, vol. 52, no. 26, pp. 7970–7981, 2004.

[37] F. E. Hunter Jr, J. Gebicki, P. Hoffsten, J. Weinstein, and A. Scott, "Swelling and lysis of rat liver mitochondria induced by ferrous ions," *Journal of Biological Chemistry*, vol. 238, no. 2, pp. 828–835, 1963.

[38] N. Kerry and C. Rice-Evans, "Peroxynitrite oxidises catechols to o-quinones," *FEBS Letters*, vol. 437, no. 3, pp. 167–171, 1998.

[39] F. Shahidi and Y. Zhong, "Lipid oxidation and improving the oxidative stability," *Chemical Society Reviews*, vol. 39, no. 11, pp. 4067–4079, 2010.

[40] N. Nenadis and M. Tsimidou, "Observations on the estimation of scavenging activity of phenolic compounds using rapid 1, 1-diphenyl-2-picrylhydrazyl (DPPH•) tests," *Journal of the American Oil Chemists' Society*, vol. 79, no. 12, pp. 1191–1195, 2002.

[41] F. Shahidi, P. K. Janitha, P. D. Wanasundara, P. Janitha, and P. Anasundara, "Phenolic antioxidants," *Critical Reviews in Food Science and Nutrition*, vol. 32, no. 1, pp. 67–103, 1992.

[42] N. Nenadis, L.-F. Wang, M. Tsimidou, and H.-Y. Zhang, "Estimation of scavenging activity of phenolic compounds using the $ABTS^{\bullet+}$ assay," *Journal of Agricultural and Food Chemistry*, vol. 52, no. 15, pp. 4669–4674, 2004.

[43] C.-H. Jeong, H. R. Jeong, G. N. Choi, D.-O. Kim, U. Lee, and H. J. Heo, "Neuroprotective and anti-oxidant effects of caffeic acid isolated from Erigeron annuus leaf," *Chinese Medicine*, vol. 6, no. 1, pp. 25–29, 2011.

[44] M. Benchekroun, A. Romero, J. Egea et al., "The antioxidant additive approach for Alzheimer's disease therapy: new ferulic (lipoic) acid plus melatonin modified tacrines as cholinesterases inhibitors, direct antioxidants, and nuclear factor (erythroid-derived 2)-like 2 activators," *Journal of Medicinal Chemistry*, vol. 59, no. 21, pp. 9967–9973, 2016.

[45] M. Rosini, E. Simoni, R. Caporaso et al., "Merging memantine and ferulic acid to probe connections between NMDA receptors, oxidative stress and amyloid-β peptide in Alzheimer's disease," *European Journal of Medicinal Chemistry*, vol. 180, pp. 111–120, 2019.

[46] G. Marotta, F. Basagni, M. Rosini, and A. Minarini, "Memantine derivatives as multitarget agents in Alzheimer's disease," *Molecules*, vol. 25, no. 17, Article ID 4005, 2020.

[47] E. Fornasari, L. Marinelli, A. Di Stefano et al., "Synthesis and antioxidant properties of novel Memantine derivatives," *Central Nervous System Agents in Medicinal Chemistry*, vol. 17, no. 2, pp. 123–128, 2017.