Proceeding

Synthesis, Antibacterial and Antifungal Activities of Hybrid Molecules Based on Alzheimer Disease Drugs and Bearing an Amino Acid Fragment †

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† Presented at the 23rd International Electronic Conference on Synthetic Organic Chemistry, 15 November- 15 December 2019; Available Online: https://sciforum.net/conference/ecsoc-23.

Received: 10 November 2019;

Abstract: The objective of the current study was to develop novel compounds – memantine derivatives with antimicrobial activity designed for application in treatment of bacterial and fungal infections in patients suffering Alzheimer disease. To realize this objective a series of six memantine hybrid molecules were synthesized, characterized by ^1H NMR, ^{13}C NMR, MS, X-ray and tested for their biological properties as anti-Alzheimer agents and their antimicrobial potential.

Keywords: memantine; hybrid molecules; Alzheimer's disease; antibacterial activity; antifungal activity; X-Ray

1. INTRODUCTION

There have been links between Alzheimer's disease (AD) and infections that cause a long-term activation of the immune system, a process known as chronic inflammation. Some of the infections that are thought to be linked to Alzheimer's include herpes, pneumonia and infection with bacteria [1]. In the last few decades neonatal sepsis and meningitis (NSM) remain a leading cause of infant mortality and morbidity, despite the use and availability of antibiotics. Bacterial *E. coli* is the most common Gram-negative pathogen causing NSM [2]. Regardless of the remarkable successes, there is still an unmet need for new effective therapeutic approaches to treat the disease. On the other hand, chronic viral, bacterial and fungal infections might be causative factors for the inflammatory pathway in AD [3]. However, it is not yet clear whether these infections might trigger Alzheimer's or cause it to get worse. It could be that people with Alzheimer's are more susceptible to picking up infections. The blood-brain barrier protects the brain by controlling what substances can pass from the blood into brain tissue. In Alzheimer's disease, the blood-brain barrier is damaged, particularly in the brain region affected by Alzheimer's. Evidences suggest that inflammation, the Alzheimer's hallmark amyloid protein (APP?) and the ApoE4 gene, which are all linked to Alzheimer's disease, can contribute to the breakdown of the blood-brain barrier. Once it has been weakened, bacteria, viruses, and other harmful substances can get through BBB into the brain more easily. This may explain why certain viruses and bacteria, such as herpes and spirochetes, are more common in the brains of people with AD [4]. Using the blood-brain barrier (BBB) and RNA-sequence models, *Nie, P., et al.* undertook a drug repositioning study to identify unknown antimicrobial activities for known drugs. The authors demonstrated for the first time that memantine (MEM), a drug approved by the FDA for the treatment of Alzheimer's disease, can very effectively block E. coli-caused bacteremia and meningitis in a mouse model of NSM. MEM was able to synergistically improve the antibacterial activity of ampicillin in neonatal mice with bacteraemia and meningitis and also significantly regulate anti-inflammatory factors.

In the present study we report on the design, synthesis and antimicrobial properties of new memantine derivatives functionalized with amino acids e.g. peptidomimetics. The results of the study suggest that some of the memantine analogues reveal a potential for application as antimicrobial agents [5-10].

2. MATERIALS AND METHODS

General

Unless otherwise stated, the starting materials, reagents, and solvents were obtained from commercial purchase and used as supplied without further purification. Analytical thin-layer chromatography (TLC) was run on Merck silica gel 60 F-254, with detection by UV light (254 nm). Memantine hydrochloride (MH) was purchased from Sigma-Aldrich (USA), amino acids were purchased from Bachem (Germany).

^{1}H and ^{13}C spectra were recorded on Bruker Avance II+ spectrometer (14.09 T magnet), operating at 600.11 MHz ^{1}H frequencies, equipped with 5 mm BBO probe with z-gradient coil. The temperature is maintained at 293 K, using Bruker B-VT 3000 temperature unit with airflow of 535 L/h. All chemical shifts are reported in parts per million (ppm), referenced against teramethylsylane (TMS, 0.00 ppm) or using the residual solvent signal (7.27 ppm for $CDCL_3$ of 2.5 ppm for DMSO). 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 mono-isotopic mass values were calculated using Data analysis software v 4.4 (Bruker Daltonics, Germany). The analyses were conducted in the positive 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. An external calibration for mass accuracy was carried out by using of sodium formate calibration solution. The precursor ion of each compound was selected, and ESI–MS/MS analysis was performed by collision-induced dissociation (CID); nitrogen was the collision gas, and the collision energy varied from 5 to 40 eV. MSn experiments were conducted on an ion trap instrument Esquire 3000 (Bruker Daltonics, Germany) and controlled by the Esquire Control 5.3.11 software. ESI-MS data were collected in positive-ion mode at a scan range from m/z 50 to 500. In all ESI-MS measurements, the nebulizer gas pressure was 124.1 kPa at a flow rate of 5 L min-1; the desolvation temperature was 300 °C and capillary voltage was adjusted to 4000 V. The sample solutions were delivered to nebulizer by a syringe pump (Cole Parmer, USA) at a flow rate 3 μL min-1.

Synthesis

General procedure: The compounds **1 a-e** were synthesized with memantine according to a known procedure (Scheme 1). The compounds **1 a-e** (3 mmol), DIPEA (3.1 mmol) was added to a solution of TBTU (3 mmol) in CH_2Cl_2 (15 ml). After stirred the mixture was treated with memantine (3 mmol) along with DMAP (3 mmol). This mixture was stirred at RT for 3 h, and then evaporated to dryness. The residues was purified by collumn chromatography using chloroform/methanol (95:5). The Boc- protected group were removed by TFA (5 ml) at 0ºC for 1h. After that TFA.AA-memantine derivatives were treated with ammonia solution.

Described compounds **3a-3d** were characterized recently by Stankova et al. *(Amino acids in press).*

Boc-Gly-Thz-memantine: [1]H (DMSO-d6) δ (ppm): 0.84 (s, 6H, CH3), 1.14 (m, 2H), 1.28 (d, 2H, J=12.2 Hz), 1.36 (d, 2H, J=12.4 Hz,), 1.41 (s, 9H), 1.69 (m, 2H), 1.88 (m, 2H), 2.12 (m, 1H), 4.39 (d, 2H, J=6Hz), 7.25 (s, 1H, NH), 7.81 (t, J= 6 Hz, 1H), 8.06 (s, 1H). [13]C (DMSO-d6) δ(ppm): 171.9 (CO-amide), 159.9(CO-BOC), 156.2 (Cquat-Thz), 150.6(Cquat-Thz), 123.6(CH-Thz), 79.9 (Cquat), 53.1(Cquat-BOC), 50.6(CH2), 47.4 (CH2), 42.7 (CH2), 42.4 (CH2), 32.4 (Cquat), 30.5(2xCH3), 30.0(CH), 28.6(2xCH3 - BOC). Yield: 65 %. ESI-MS: Molecular formula: $C_{22}H_{33}N_3O_3S$; M_{exact}: 419,59, M_{found} [M+H] 420,63.

Fmoc-Thz-Thz-Mem: [1]H (DMSO-d6) δ (ppm): 0.84 (s, 6H, CH3), 1.16 (m, 2H), 1.29 (d, 2H, J=12.2 Hz), 1.37 (d, 2H, J=12.4 Hz), 1.79 (m, d, 2H, J=11.6 Hz), 1.68 (m, d, 2H, J=11.6 Hz), 1.93 (m, 2H), 2.12 (m, 1H), 4.26 (t, 1H, J=6.5Hz), 4.42 (d, 2H, J=6.2Hz), 4.53 (d, 2H, J=6.7 Hz), 7.34 (t, J= 7.5Hz, 2H), 7.43 (t, J= 7.5Hz, 2H), 7.45 (s, 1H), 7.72 (d, J=7.7 Hz, 2H), 7.91 (d, J= 7.7 Hz, 2H), 8.20 (s, 1H), 8.35 (s, 1H). Yield: 65 %. ESI-MS: Molecular formula: $C_{34}H_{34}N_4O_3S_2$; M_{exact}: 610,80, M_{found} [M+H] 611,76.

X-ray crystallography

Sample crystallization

In order to obtain single crystals with suitable quality the dry powder substances of Beta-Alanine-Memantine ($C_{17}H_{27}F_3N_2O_3$) and 4-F-Phenilalanine-Memantine ($C_{23}H_{32}F_4N_2O_4$) were dissolved in ethanol, methanol and acetone at room temperature. Large crystals (0.3 x 0.2 x 0.15 mm^3) suitable for single crystal X-ray studies formed within 2-3 days by slow evaporation. Crystals were obtained from the three solvents used, but best quality crystal grew in acetone conditions.

Data collection and crystal structure refinement

A single crystal was mounted on a glass capillary and all data were collected at room temperature (290 K) on an Oxford diffraction Supernova diffractometer using Mo-Kα radiation (λ = 0.71013 Å) from micro-focus source. The determination of unit cell parameters, data integration, scaling and absorption corrections were carried out using the CrysalisPro [11-12]. The phases were obtained by direct methods with ShelxS-2018. The refinement of the structure involved several cycles of refinement using full-matrix least-squares on F^2 with the ShelxL-2018 package [13]. The N hydrogen atoms were positioned from difference Fourier map while all other hydrogen atoms were placed at idealized positions. The non-hydrogen atoms were refined anisotropically and hydrogen atoms were refined using the riding model. Visual representation of the two molecules present in the asymmetric unit were carried out using *ORTEP* [14] while three-dimensional packing and hydrogen bonding interactions were drawn using Mercury [15] **.** The data in the Crystallographic Information File (coordinates, structure factors, etc.) have been verified using IUCr checkCIF/PLATON [16].

Thermal analyses

Differential thermal analysis (DTA) and thermo gravimetric measurements (TG) were carried out simultaneously in a thermal analyzer Stanton Redcroft STA780 at the following conditions: heating rate of 5 °C.min^{-1}, dry argon as a carrier gas (~30 ml.min^{-1}) and sample weight of 10-11 mg, in corundum crucible.

Biological methods

Agar disk-diffusion method

Agar disk-diffusion testing developed in 1940 [17] is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. Nowadays, many accepted and approved standards are published by the Clinical and Laboratory Standards Institute (CLSI) for bacteria and yeasts testing [18]. In this well-known procedure, agar plates are inoculated with a standardized inoculum of the test microorganism. Then, filter paper discs (about 6 mm in diameter), containing the test compound at a desired concentration, are placed on the agar surface. The Petri dishes are incubated under suitable conditions. Generally, antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism and then the diameters of inhibition growth zones are measured.

Standard Antibacterial In Vitro Metrics. Minimum inhibitory concentration were investigated by Abedon [19]

Time-kill test for evaluation of bacteriostatic/bactericide activity [20].

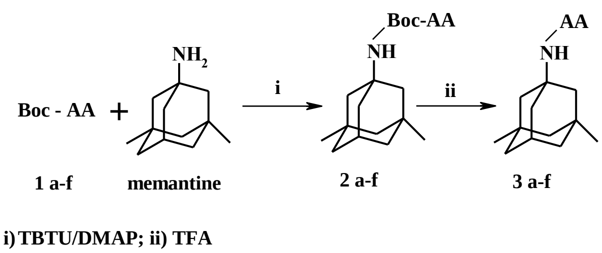
Test cultures were prepared as described above with an initial concentration of 0.1, 0.3 and 0.5 McF. To each culture was added 0.5 ml of 1 mM solution of test compound. Samples were incubated at 37 ° C. OD_{585} measurements were taken at 2, 3, 4, 5 and 6 hours after incubation. In the samples CFU/ml must be determine and compare to control data in same hour. Results are the following:

- if the $x_i > x_k$ – the compound stimulates microorganisms to grow;
- if the $x_i = x_k$ – the compounds does not affect the microorganisms;
- if x_i = CFU/ml before the incubation – the compound has a bacteriostatic effect;
- if the $x_i < x_k$ – the compound has a bactericide effect.

3. RESULTS AND DISCUSSION

Synthesis of novel compounds – memantine hybrid molecules, with antimicrobial activity designed for application in treatment of bacterial and fungal infections in patients suffering dementia of Alzheimer's type.

The peptide bond between the memantine and amino acids and peptidomimetics was formed using the TBTU coupling reagent [21]. The protected groups were removed in CH_2Cl_2/TFA at 0 °C (Scheme 1).



Scheme 1. Synthesis of memantine derivatives substituted with amino acid and peptidomimetics **3 a-f**. **where**: *Glycyl-memantine (**3 a**), 4-F-Phenylalanyl-memantine (**3 b**), Valyl-memantine (**3 c**), β-Alanyl-memantine (**3 d**), Gly-Thiazolyl-memantine (**3 e**), Gly-Thiazolyl-Thiazolyl (3 **f**).*

X-ray study

Single crystal X-ray study showed that the compounds crystallize in a centrosymmetric manner (SG $P2_1/c$, No 14) for the Beta-Alanine-Memantine and in a non-centrosymmetric manner (SG $P2_12_12_1$, No 19) for the 4-F-Phenilalanine-Memantine. The asymmetric units of the two studied compounds are shown in **Fig. 1 and Fig. 2.**

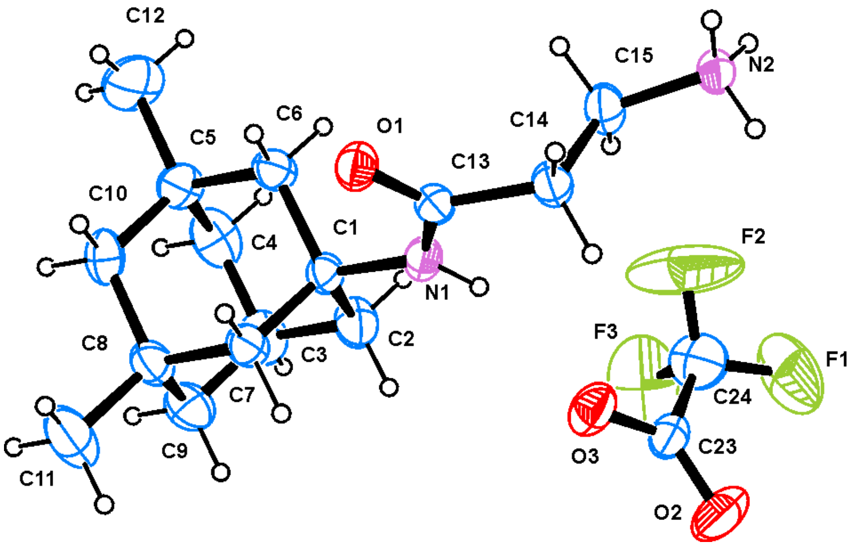


Figure 1. ORTEP view of the *β*-Alanine-Memantine asymmetric unit along with atom numbering scheme. Atomic displacement parameters (ADP) are drawn at the 50% probability while hydrogen atoms are shown as spheres with arbitrary radii.

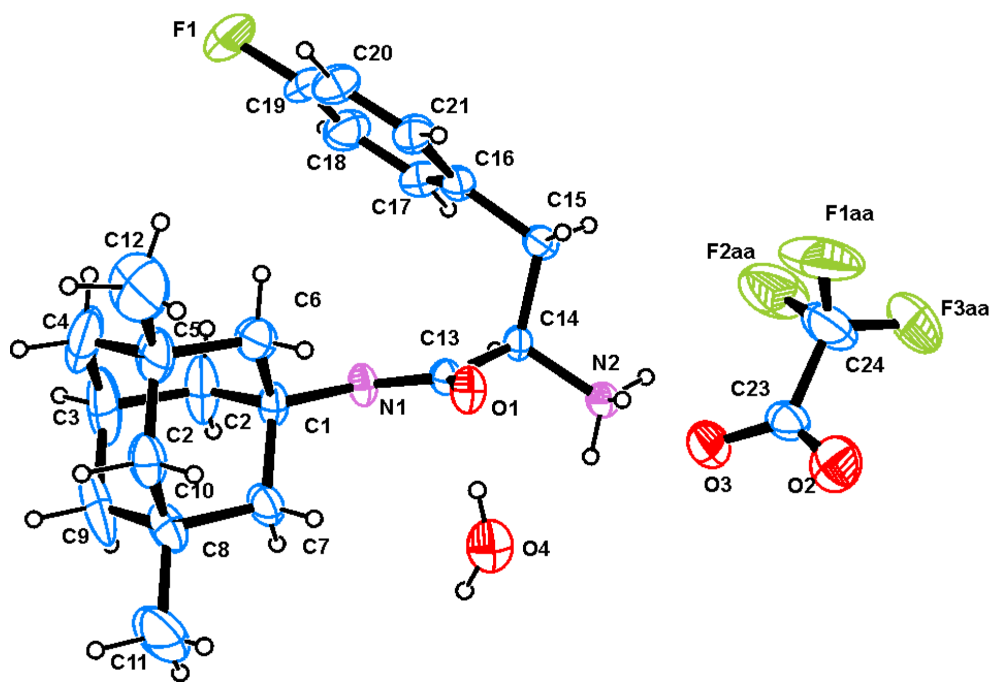


Figure 2. View of the 4-F-Phenilalanine-Memantine asymmetric unit and the applied numbering scheme. Atomic displacement parameters (ADP) are drawn at the 50% probability while hydrogen atoms are shown as spheres with arbitrary radii.

The bond lengths and angles of the two molecules are comparable (**Table 1 and Table 2)**. The aromatic ring system present in the 4-F-Phenilalanine-Memantine molecule is essentially planar with *rmsd* of 0.002Å. The overlay of the two molecules shows that the memantine geometry is highly conserved.

Table 1. Selected geometric parameters for β-Alanine-Memantine and 4-F-Phenilalanine-Memantine. Bond Lengths [Å]

Bond	Length [Å] for *β*-Ala-memantine	Length [Å] for 4-F-Phe-memantine
O1 – C13	1.219(5)	1.229(7)
N1 – C1	1.485(5)	1.485(9)
N1 – C13	1.336(6)	1.312(8)
C13 – C14	1.513(6)	1.522(9)
C15 – C14	1.478(6)	1.529(9)
C1 – C7	1.523(6)	1.500(10)
C1 – C2	1.523(7)	1.538(11)
C1 – C6	1.516(6)	1.520(11)
C2 – C3	1.534(7)	1.547(14)
C4 – C3	1.517(9)	1.530(2)
C3 – C9	1.502(9)	1.500(2)
C5 – C4	1.524(8)	1.531(17)
C5 – C12	1.529(8)	1.517(16)
C7 – C8	1.538(6)	1.520(11)
C10 – C8	1.527(7)	1.508(12)
C11 – C8	1.539(8)	1.544(15)

Table 2. Selected geometric parameters for *β* -Ala-Memantine and 4-F-Phe-Memantine. Bond Angles/°

Angle	Angle [°] for *β*-Ala-memantine	Angle [°] for 4-F-Phe-memantine
C13 – N1 – C1	126.6(4)	128.0(5)
O1 – C13 – N1	123.4(4)	126.3(6)
O1 – C13 – C14	120.8(4)	118.0(6)
N1 – C13 – C14	115.7(4)	115.6(5)
N1 – C1 – C7	110.3(4)	112.1(6)
N1 – C1 – C2	107.3(4)	104.7(6)
N1 – C1 – C6	110.8(4)	111.8(6)

The observed difference is related to the β-Alanine-Memantine and 4-F-Phenilalanine-Memantine orientation (**Fig. 3**).

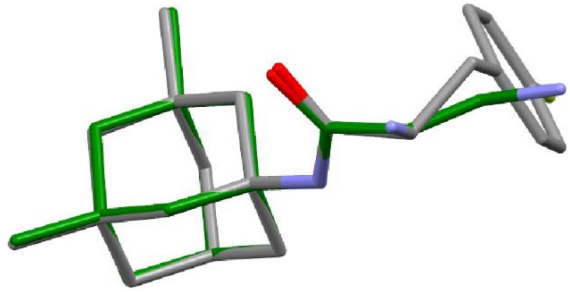


Figure 3. Overlay of the molecules of 4-F-Phenilalanine-Memantine and Beta-Alanine-Memantine based on the identical memantine moiety.

Having in mind the similarity (bond lengths and angles) one should expect a similar hydrogen bonding interactions as the side chain difference provides the main difference between the structures. Indeed in both structures intermolecular N-H···O hydrogen bonds (**Table 3**) stabilizes the three-dimensional packing of the molecules. The crystal structure of 4-F-Phenilalanine-Memantine and *β*-Alanine-Memantine also reveals the presence of Trifluoroacetic acid (TFA) that produces the zwitterionic structure. One solvent water molecule is present in the 4-F-Phenilalanine-Memantine.

Table 3. Hydrogen Bonds geometry (Å, °) **A**) for β -Alanine-Memantine and **B**) for 4-F-Phenilalanine-Memantine.

A) β-Alanine-Memantine

D -	H…	A	d(D-H)/Å	d(H...A)/Å	d(D...A)/Å	D-H...A/°
N2 -	H2A	O1[1]	0.89	1.87	2.725(5)	161.1
N2 -	H2B	O15[2]	0.89	1.92	2.725(5)	149.3
N2 -	H2C	O4[3]	0.89	1.93	2.788(5)	162.3
N3 -	H3	O4	0.86	2.12	2.908(5)	151.5
C6 -	H6A	O1	0.97	2.61	3.169(6)	117
C8 -	H8B	F2	0.97	2.56	3.467(9)	155.9
C14 -	H14B	O1	0.97	2.45	3.047(6)	119.5

B) 4-F-Phenilalanine-Memantine

D -	H…	A	d(D-H)/Å	d(H...A)/Å	d(D...A)/Å	D-H...A/°
N2 -	H2A	O11[1]	0.89	2.15	2.916(9)	144.3
N2 -	H2B	O8[2]	0.89	1.91	2.766(8)	161.2
N2 -	H2C	O8	0.89	2.04	2.868(10)	153.9
N3 -	H3	O4[1]	0.86	2.1	2.942(8)	164.5
C7 -	H7	O4[1]	0.98	2.57	3.441(9)	147.3
C23 -	H23B	O1	0.97	2.5	3.071(10)	117.8

Thermal behavior

The thermal behavior of the F-Phenilalanine-Memantine and β-Alanine-Memantine compounds is investigated between room temperature and 250 °C using DTA-TG-DTG analysis (**Fig. 4**).

For the β-Alanine-Memantine (**Fig. 4A**), DTA measurement shows a slow endothermic effect at 150 – 170°C without loss of mass on the TG curve. Such kind of *endo* effect can be related to the melting point of the compound. A second *endo* effect is detected at higher temperatures 200–250°C. The second effect is accompanied by mass losses and can be associated decomposition of β-Alanine-Memantine.

Similarly for the 4-F-Phenilalanine-Memantine (**Fig. 4B**), DTA measurement shows two endothermic effects: the first is in the range 150 – 170 °C and the second 200 – 250 °C. The first *endo* effect is accompanied by ~3% weight losses which corresponds to a loss of a water molecule and is in agreement with the crystal structure. The second DTA effect is associated with pronounced weight losses and is the melting and decomposition of the 4-F-Phenilalanine-Memantine. After the melting a pronounced weight losses is started due to the volatility. The two processes – melting and decomposition – cannot be clearly separated, because the they seem to occur almost simultaneously.

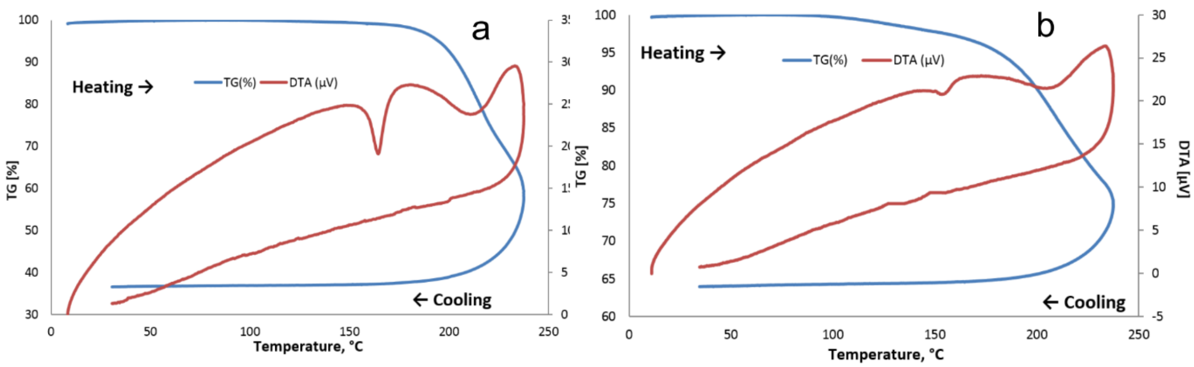


Figure 4. DTA-TG curves: **a)** β-Alanine-Memantine; **a)** 4-F-Phenilalanine-Memantine

Biological part

The new memantine analogues were investigated for antibacterial activity against model Gram-positive and Gram-negative bacteria and activity against yeast microorganisms - **Gram-positive** bacteria - *Staphylococcus aureus NBIMCC (6538), Bacillus megaterium*, **Gram-negative** bacteria **-** *Escherichia coli (NBIMCC 3397)*, *Salmonella enterica (NBIMCC 869)* and **yeasts -** *Rhodotorula sp. (16-25)*, *Candida lusitniae (74-4)*. Antibacterial activity of investigated compounds are presented in **Table 4.** Two concentrations were prepared: 1 mM and 10 mM. The efficacy of the test compounds was determined by measuring the inhibition zone (in mm) and compared with the controls.

The compound 4-F-Phe memantine shows highest antibacterial activity against both type Gram bacteria. Its inhibition effect is similar with this of commercially available tetracycline (30 μg/disk). Antibacterial activity of compounds Val-memantine and Thz-memantine is lower and variable between 10 and 16 mm inhibition area. Compound β-Ala-memantine demonstrate activity only against *S. enterica*, while compounds Gly-memantine and Fmoc-Thz-Thz-memantine are both inactive. All tested compounds were dissolved in DMSO. This solvent, 4-F-L-Phenylalanine, tetracycline and the memantine (Nemdatine 10 mg tablets) were included as controls.

Comparing analysis of antimicrobial activity shows that compound Val-memantine has greater activity against Gram (-) bacteria. Among Gram (+) bacteria, only *Bacillus megaterium* growing was affected.

Table 4. Antibacterial activity of chemical derivatives of memantine (10 mM) against Gram (+) and Gram (-) bacteria. The area of inhibition are presented in mm.

Compound № (10 mM)	Gram (-) bacteria		Gram (+) bacteria		
	S. enterica	***E. coli***	***St. aureus***	***B. megaterium***	***St. epidermidis***
Gly-memantine	n/a	n/a	n/a	n/a	n/a
4-F- Phe memantine	20	22	21	22	24
Val-memantine	11	11	n/a	16	n/a
β-Ala-memantine	16	n/a	n/a	n/a	n/a
Thz-memantine	n/a	13	10	16	n/a
Fmoc-Thz-Thz-memantine	n/a	n/a	n/a	n/a	n/a
Memantine .HCl	n/a	n/a	n/a	n/a	n/a
4-F-L-Phenylalanine	n/a	n/a	n/a	n/a	n/a
DMSO	n/a	n/a	n/a	n/a	n/a
Tetracycline	26	26	28	34	n/a

Compound β-Ala-memantine shows activity against *Salmonella enterica*, while Thz-memantine affects mostly Gram (+) bacteria with the exception of *Staphylococcus epidermidis*, which growing was not influenced. It is obviously that there is selectivity of inhibition among diverse memantine analogues. The exeption is 4-F- Phe memantine, which affect growing of all tested strains.

The investigation of 1 mM compounds concentration inhibitory effect was observed only at Gram (+) bacteria *St. aureus* and *B. megaterium* by compound 4-F- Phe memantine .

Yeasts

Yeasts are widespread in nature. Often they cause of mycosis in individuals with immunocompromised status. The main cause are *Candida* and *Rhodotorula*. Recent studies indicate that the incidence of mycosis caused by *Rhodotorula* is between 0.5% and 20.3% in the US and Europe. The investigated isolates were resistant to the antibiotic fluconazole.

Table 5. Antifungal activity of chemical derivatives of memantine (10 mM) against model yeast microorganisms. The area of inhibition are presented in mm.

Compound № (10 mM)	*Rhodotorula sp.*	*Candida lustiniae*	*Sacch. cerevisiae*
Gly-memantine	n/a	n/a	14
4-F- Phe-memantine	26	23	21
Val-memantine	n/a	n/a	n/a
β-Ala-memantine	n/a	n/a	n/a
Thz-memantine	n/a	n/a	n/a
Fmoc-Thz-Thz-memantine	n/a	n/a	n/a
Memantine .HCl	n/a	n/a	n/a
4-F-L-Phenylalanine	n/a	n/a	n/a
DMSO	n/a	n/a	n/a
Nystatin	26	24	n/a

The antifungal activity data is presented on **Table 5**. The compound 4-F- Phe memantine 2 is the only one active against all tested yeast microorganisms. Its effect is similar to well-known antifungal medication

Nystatin. Compound Gly-memantine demonstrate activity only against *Sacch. cerevisiae*. Experimental data shows that the memantine derivatives have antimicrobial effect against model prokaryote microorganisms and tested eukaryote cultures as well.

Quantitative analysis of antimicrobial activity: determination of minimum inhibitory concentration (MIC).

The results from the MIC test are presented in **Tables 6**.

Table 6. Minimal inhibitory concentration values of memantine analogues.

	Gram (-) bacteria		Gram (+) bacteria		Yeasts	
Compound № (μM)	*S. enterica*	*E. coli*	*St. aureus*	*B. megaterium*	*Rhodotorula sp. 16-25*	*Candida lustiniae 74-4*
Gly-mem (3a)	1.25	n/a	n/a	1.25	n/a	0.3125
4-F-Phe mem (3b)	0.156	1.25	1.25	0.625	0.078	0.078
Val-mem (3c)	1.25	5	10	2.5	n/a	0.3125
β-Ala-mem (3d)	n/a	10	n/a	n/a	n/a	0.078
Thz-mem (3e)	5	5	10	10	1.25	0.3125

Antibacterial activity against Gram (+) and Gram (-) bacteria could be evaluated by the following way:

- ✓ *E. coli* - №3b > №3c, №3e;
- ✓ *S. enterica* - №3b > № 3e> №3c;
- ✓ *St. aureus* - №3b > №3e > №3c;
- ✓ *B. megaterium* - №3b > №3c > №3e;
- ✓ *St. epidermidis* - №3b > №3c, №3e.

Compound Gly-memantine is active against *Salmonella enteria* and *B. megaterium* with MIC value 1.25 mM. Reagarding β-Ala-memantine , we observed low antimicrobial activity (MIC=10 mM) against *E. coli*. Compound 4-F-Phe-memantine demonstrate highest antimicrobial potential against all tested microorganisms. The MIC values are in wide range from 1.25 mM to 0.078 mM. Compound Thz-memantine is also active against all tested microorganism at higher concentrations, compared to compound 4-F-Phe memantine . Val-memantine shows activity, but at high concentrations.

Time-kill test for evaluation of bacteriostatic/bactericide activity

Time-kill test of 4-F-Phe-memantine, conducted with the most sensitive bacterial species *Salmonella enterica* shows bactericidal effect and 100% inhibition of bacterial growth after 7 hours of cultivation in the presence of the test substance (**Figure 5**). For the same period, the control reached a cell density above 6 x 10^8 CFU/ml.

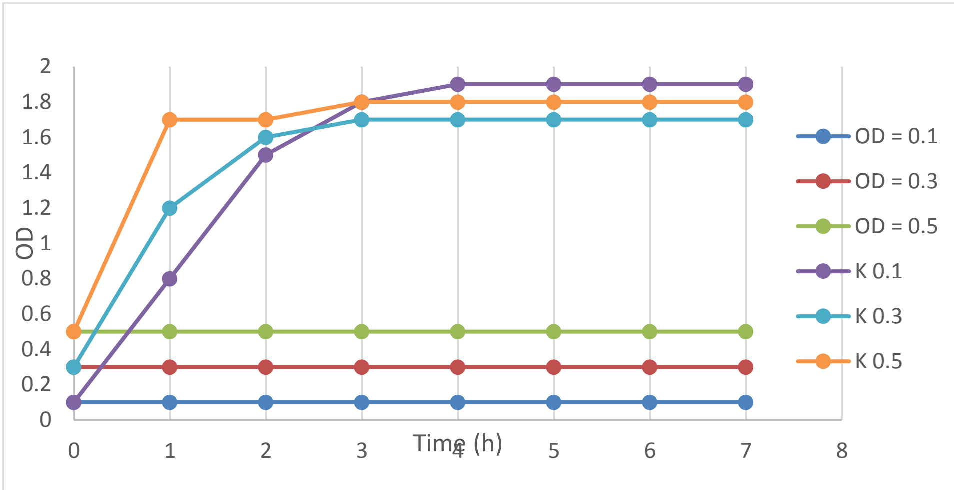


Figure. 1 Dynamics of *Salmonella enterica* growh in presence of 1 mM 4-F-Phe-memantine.

4-F-Phe-memantine showed a strong inhibitory effect on the growth of filamentous fungi (**Table 7**). It is in the range of 8% for *Penicillium chaviforme* and 62.9% for *Fusarium graminearum*.

Table 7. Radial speed of growth inhibition growth rate (Kr) of filamentous fungi in presence of 4-F-Phe-memantine **3b**

Hours	***Penicillium chaviforme d (mm)***				***Fusarium graminearum d (mm)***			
	K	**Topsin**	**DMSO**	**3b**	**K**	**Topsin**	**DMSO**	**3b**
0	0	0	0	0	0	0	0	0
24	5,6	0/0	9,8	7,6	10/7	15/16	20/22	7,7
48	11,12	0/0	14/13	11,10	26/26	39/39	39/39	35/35
72	16/16	0/0	13/14	15/14	52/49	44/44	70/62	43/49
98	21/21	0/0	23/23	20/19	71/70	49/45	83/82	46/50
120	25/25	0/0	27/27	24/22	85/85	50/50	85/85	51/55
Kr (mm/h)	19,7	0	17,5	15,7	84,9	49,8	84,8	19,8

CONCLUSION

The investigated memantine analogues with amino acids exhibit inhibitory effects on individual test microorganisms. Val-memantine shows greater efficacy against Gram-negative bacterial employees, and toward Gram-positive conditions that raises a plant only on the Bacillus megatermium. β-Ala-memantine has an effect exclusively on Salmonella enterica plants, and Gly-Thiazole has a good inhibitory effect against Gram-positive bacteria.

The 4-F-Phe-memantine being effective on all the strains required. The inhibitory effect is commensurate with this time to control the commercially available tetracycline and nystatin. The hybrid 4-F-Phe-memantine is the most promising for possible application as a new anti-infective host-directed therapeutic agent against clinically significant conditionally pathogenic bacteria in patients suffering from moderate to severe dementia of Alzheimer's type.

Author Contributions: R.T.- synthesis and purification of compounds, A. T. - synthesis and purification of compounds, Hristina Sbirkova-Dimitrova - X-ray, Boris Shivachev – interpretation X-ray , Anna V. Kujumdzieva – antimicrobial activity, Trayana Nedeva – interpretation antimicrobial activity, I. S. – interpretation of structure/activity relationship

FUNDING: We gratefully acknowledge the financial support from Bulgarian Science Fund (Project M23/8) and South-West University „Neofit Rilski" (Project RPY-A3/19).

CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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