SYNTHESIS, ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF HYBRID MOLECULES BASED ON ALZHEIMER DISEASE DRUGS AND BEARING AN AMINO ACID FRAGMENT

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Introduction

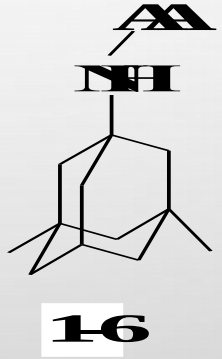
- Wide spread use of antibiotics is rising the incidence of infections with antibiotic resistance
- Combat of antimicrobial resistance crisis encompasses synthesis of natural derivatives of well-known drugs
- Memantine (MEM) is FDA and EU approved drug for treatment of patients with moderate to severe dementia of Alzheimer's type
- MEM could efficiently block *E. coli*-caused bacteremia and meningitis in a mouse model
- MEM could be used as a host-directed antimicrobial agent

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.

Chemistry

A series of memantine derivatives incorporating amino-acid residues have been synthesized by TBTU as a coupling reagent (Fig.1). The synthesized memantine analogues were confirmed by 1H NMR, 13C NMR, and ESI–MS spectrometry.



Where: *Glycine (**1**), 4-F-Phenylalanine (**2**), Valine (**3**), β-Alanine (**4**), Gly-Thiazole (**5**), Gly-Thiazolyl-Thiazole (**6**).*

Fig.1. Structures of the new memantine analogues

Crystal structure determination

Two of the synthesized compounds - **Beta-Alanine-Memantine and 4-F-Phenilalanine-Memantine** - were recrystallized from acetone by slow evaporation and their crystal structures were determined (Figure 2) with Single crystal X-ray diffraction (SCXRD) experiment.

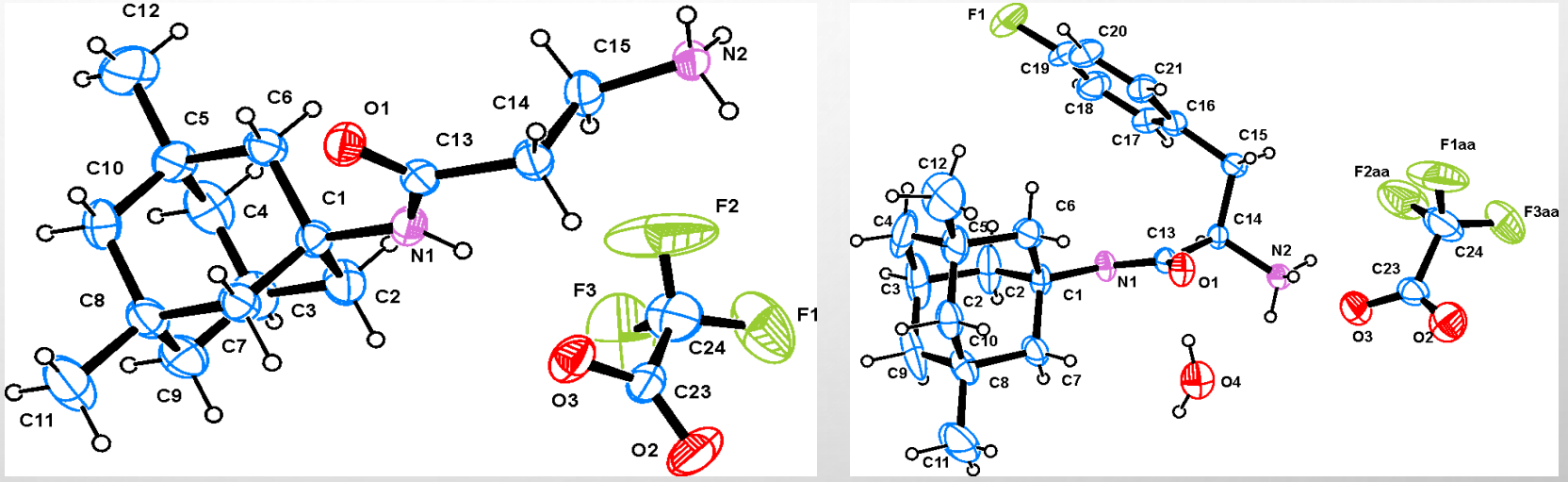


Fig. 2 ORTEP views of the molecules in the asymmetric unit (ASU) of **Beta-Alanine-Memantine** (left) **4-F-Phenilalanine-Memantine** (right)

Crystal structure determination

Overlaying of the molecules of **Beta-Alanine-Memantine** and **4-F-Phenilalanine-Memantine** (Figure 3) showed that they have almost similar orientation with slight deviation due to the different AA substitutent. Both compounds have one acceptor (C=O group) and two donors (N-H and NH_3 groups) of hydrogen atoms that benefit the formation of intermolecular hydrogen bonding interactions which are one of the main factors for the stabilization of the crystal structures.

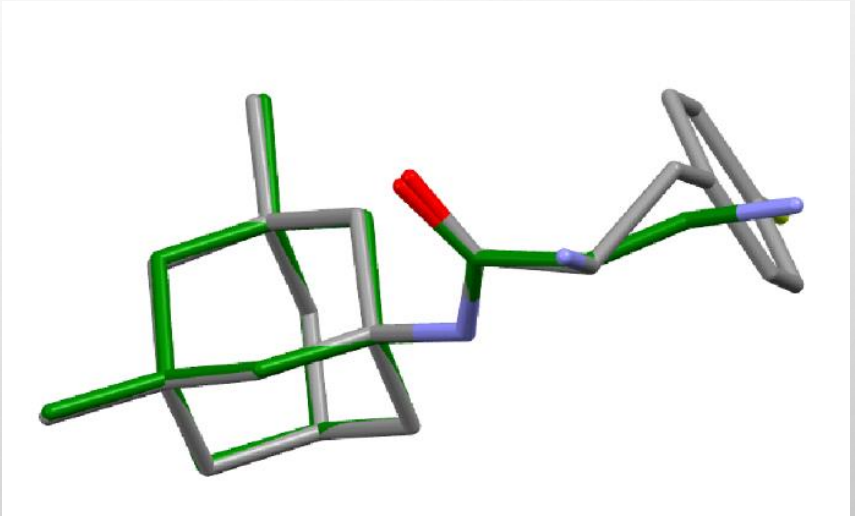


Fig. 3. Overlay of the molecules of **Beta-Alanine-Memantine** (green) and **4-F-Phenilalanine-Memantine** (gray) based on their identical memantine moiety

Thermal properties

Differential thermal (DTA) and thermogravimetric (TGA) analyzes of the two compounds in corundum crucibles were performed (Figure 4). The compounds are stable up to around 20°C after the melting point (around 160°C for **Beta-Alanine-Memantine** and 155 °C for **4-F-Phenilalanine-Memantine).** After that point drastic weight losses due to thermal decomposition are recorded (60% for BAM and 35% for 4FPM). The reverse cooling process is not accompanied by visible weight change due to adsorbtion/adsorption of moisture etc.

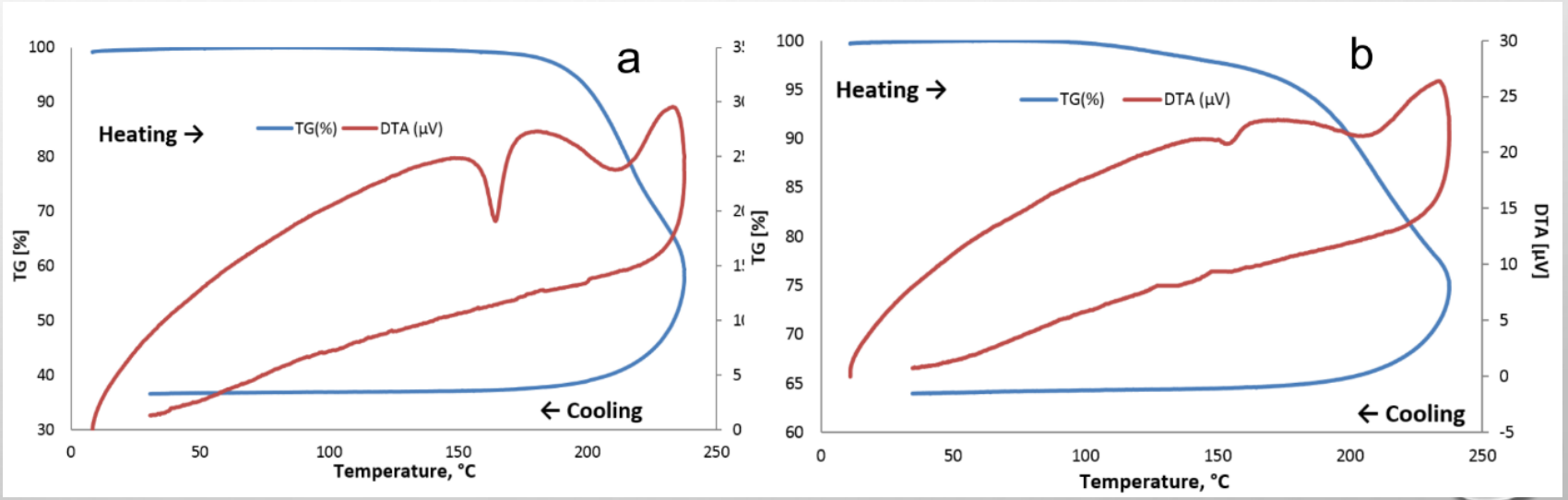


Figure 4. DTA-TG curves: **a)** Beta-Alanine-Memantine; **b)** 4-F-Phenilalanine-Memantine

BIOLOGICAL PART

Test microorganisms:

- *Escherichia coli* (NBIMCC 3397)
- *Salmonella enterica* (NBIMCC 8691)
- *Staphylococcus aureus* (NBIMCC 6538)
- *Bacillus megaterum* (BF 145)
- *Candida lusitaniae* (BF 74-4)
- *Rhodotorula sp.* (BF 16-25)
- *Fusarium graminearum* (NBIMCC 2294)
- *Penicillium claviforme* (BT136)

Table 1.Evaluation of compounds (**1-6**) inhibitory potential: qualitative assay

Disk diffusion susceptibility test						
	Inhibition area (mm)					
Memantine derivatives	**1**	**2**	**3**	**4**	**5**	**6**
Bacteria						
GRAM negative						
Escherichia coli (NBIMCC 3397)	no	22	11	no	13	no
Salmonella enterica (NBIMCC 8691)	no	20	11		16	no
GRAM positive						
Staphylococcus aureus (NBIMCC 6538)	no	21	no	no	10	no
Bacillus megaterium (BF 145)	no	22	16	no	16	no
Yeasts						
Candida lusitaniae (BF 74-4)	no	23	no	no	no	no
Rhodotorula sp. (BF 16-25)	no	26	no	no	no	no

As shown in Table 1. memantine with 4-F-Phenylalanine (**2**) showed activity against all tested bacteria and yeasts

Memantine analogues with Valine (**3**), β-Alanine (**4**) and Gly-Thiazole (**5**) are active against most bacterial strains.

Table 2. Investigation of Minimal Inhibitory Concentration

	GRAM -		GRAM +	
Memantine derivatives (10 mM)	*E. coli* *NBIMCC 3397*	*S. enterica* NBIMCC 8691	*B. megaterium* BF 145	*St. aureus* NBIMCC 6538
N1	> 10	> 10	1.25	> 10
N2	1.25	0.165	0.625	1.25
N3	5	1.25	2.5	10
N4	10	> 10	> 10	> 10
N5	5	5	10	10
N6	> 10	> 10	> 10	> 10
Inhibitory effect	N2→N5&N3	N2→N3→N5	N2→N3→N5	N2→N3&N5

The results demonstrated that memantine derivative with 4-F-Phenylalanine (2) have the lowest values of MIC - 0.165 mM against *S. enterica* (NBIMCC 8691) and MBC - 0.3 mM against *S. enterica* (NBIMCC 8691).

Table.3. Inhibition of radial growth rate (Kr) of filamentous fungi by MEM derivatives

Hour	*Penicillium chaviforme* (mm)				*Fusarium graminearum* (mm)			
	K	**Topsin**	**DMSO**	**N2**	**K**	**Topsin**	**DMSO**	**N2**
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**

Memantine-4-F-Phenylalanyl (2) strongly inhibits the Kr of Fusarium graminearum (NBIMCC 2294)

Conclusion

- The investigated compounds **1-5** exhibit inhibitory effects on individual test microorganisms. Val-memantine ***(3)*** 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 ***(4)*** has an effect exclusively on Salmonella enterica plants, and Gly-Thiazole ***(5)*** has a good inhibitory effect against Gram-positive bacteria.
- The 4-F-Phe-memantine ***(N2)*** 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 ***(N2)*** 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.

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