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Amantadine analogues – synthesis and biological activity

R. Chayrov[1]*, L. Mukova[2], A. Galabov[2], Y. Mitrev[3], I. Stankova[1]

[1] Department of Chemistry, South-West University "Neofit Rilsky", Blagoevgrad, Bulgaria, [2] Stefan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences,1113 Sofia, Bulgaria

[3]Laboratory "Bulgarian NMR Centre", Institute of Organic Chemistry with Centre of Phytochemistry, 1113 Sofia, Bulgaria

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The biological activity of adamantane derivatives is due to the symmetry and steric bulkiness of the structure and the significant lipophilicity of the rigid hydrocarbon framework. This enables them to penetrate easily through biological membranes. Therefore, modification of organic compounds by an adamantyl radical changes significantly their biological activity, often enhancing it. A large number of strains that are completely resistant to amantadine are currently known. The number of resistant influenza strains increases every year because of spontaneous mutations in the virus genome. This necessitates expanded research on the reasons for the development of resistance and ways of overcoming it by creating new antiviral drugs [1].

Keywords: adamantanes, amino asids, amino acid amides, antiviral activity

INTRODUCTION

Amantadine was a drug that was once used to treat the influenza virus A. The influenza virus A became resist to amantadine due to the misuse of the drug. In 2005 the drug was used in China as a way to treat the avian influenza. The Chinese gave approximately 2.6 billion doses of amantadine to the chickens, which is what caused it to become resistant [2]. The avian flu threat served as a catalyst for scientist to prepare a new antibiotic that could be used to treat the drug-resistant bacterial and viral strains [3]. According to Plumb's Veterinary Drug Handbook, amantadine is safe to use in small animals and equine. It also states that amantadine is still able to treat some influenza viruses, but they have found that the greatest interest in its use is treating chronic pain. The influenza virus it is able to treat is found in equine and is known as equine-2 influenza virus. The handbook states that amantadine is safe for humans and that it is no longer used for fighting the influenza virus but used in Parkinsonian Syndrome [4]. Amantadine is an amino-analogue of adamantane.

Our goal was to modify amantadine with L-Valine, L-Alanine and L-Lys and to investigate their antiviral activity against influenza virus A (H3N2) (Scheme 1). The structures of new analogues were confirmed by NMR and MS analyzes.

EXPERIMENTAL

General information

All amino acids (N-(tert-Butoxycarbonyl)-L-alanine, N-(tert-Butoxycarbonyl)-L-valine and $N^{\alpha,\varepsilon}$-bis-Boc-L-lysine dicyclohexylammonium salt), amantadine hydrochloride, as well as isobutyl chloroformate (IBCF), triethylamine (TEA), were purchased from Sigma Aldrich (FOT, Bulgaria). Dichloromethane was obtained from Fisher Chemical (Bulgaria) and further was distilled. Chloroform was of reagent grade and used without further purification. Thin-layer chromatography (TLC) was conducted on precoated Kieselgel 60F254 plates (Merck, Germany).

The NMR experiments were recorded on Bruker Avance III 600 or Bruker Avance III 400 spectrometer, operating at 600.13 and 400.15 MHz for protons respectively. The measurements in $CDCl_3$ solutions were carried out at ambient temperature (300 K) and tetramethylsilane (TMS) was used as an internal standard. The UV spectra of the compounds were measured with an ''Agilent 8453'' UV-vis spectrophotometer. Electrospray Ionisation (ESI) and EI mass spectra were recorded corresponding on an Esquire 3000 and MAT 8230.

General procedures. Synthesis of 3a - c

The tert-butyloxycarbonyl (Boc) amino acids (Boc-Val-OH, Boc-Ala-OH, Boc-Lys(Boc)-OH) (69 mmol) were dissolved in 2 ml CH_2Cl_2. The solution was cooled to -15ºC, added TEA (69 mmol) and dropwise isobutyl chloroformate (69 mmol). The amantadine (46 mmol) were dissolved in 2 ml mix CH_2Cl_2/$CHCl_3$ in ratio 1:1, added TEA (46 mmol). After 15 min the solutions were mixed and stirred for 1,5h at -15ºC. The mixture was poured into 5% NaHCO3, extracted with CHCI3, washed with brine, dried over Na2SO4 and concentrated in vacio. The residues was purified by

* To whom all correspondence should be sent:
E-mail: rchayrov@swu.bg

TLC on Kieselgel 60F254 using the solvent system chloroform/ methanol (95:5).

The resulting white solid, Boc-Valyl-Amantadine, Boc-Alanyl-Amantadine and oil Boc-Lysinyl(Boc)-Amantadine were dissolved in 2 ml of 50% TFA/ CH_2CI_2 and stirred at 0ºC for 1h to remove the Boc group.

Boc-Valyl-Amantadine (Boc-Val-Am) - M.W. = 350,5;

ESI-MS: 723.4 $[2M+Na]^+$, 389.1 $[M+K]^+$, 373.2 $[M+Na]^+$, 351.2 $[M+H]^+$; **^{1}H NMR** ($CDCl_3$) /600 MHz/ δ (ppm): 0.901 (d, J=6.8 Hz, 3H), 0.937 (d, J=6.5 Hz, 3H), 1.435 (s, 9H), 1.664 (br t, 6H), 1.985 (s, 6H), 2.03 (overlapping, 1H), 2.06 (s, 3H), 3.705 (br t, J= 7.9 Hz, 1H), 5.09 (d, J= 8.2 Hz, 1H), 5.478 (s, 1H).

After removing the Boc group - white powder, 36% yield;

ESI-MS: 523.2 $[2M+Na]^+$, 273.1 $[M+Na]^+$

Boc-Alanyl-Amantadine (Boc-Ala-Am) - M.W. = 322,5;

ESI-MS: 667.2 $[2M+Na]^+$, 361.1 $[M+K]^+$, 345.1 $[M+Na]^+$, 323.1 $[M+H]^+$; **^{1}H NMR** ($CDCl_3$) /600 MHz/ δ (ppm): 1.30, (d, J=7Hz, 3H), 1.44 (s, 9H), 1.66 (s, 6H), 1.973 (s, 6H), 2.065 (s, 3H), 4.024 (br s, 1H, CH), 5.024 (br s, 1H, NH), 5.796 (br s, 1H, NH).

After removing the Boc group – white powder, 58% yield;

ESI-MS: 467.1 $[2M+Na]^+$, 245.0 $[M+Na]^+$

Boc-Lysinyl(Boc)-Amantadine (Boc-Lys(Boc)-Am) - M.W.= 479,7;

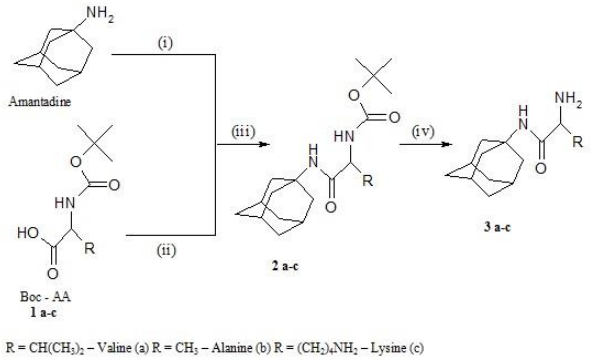
ESI-MS: 981.4 $[2M+Na]^+$, 502.2 $[M+Na]^+$, 480.3 $[M+H]^+$; **^{1}H NMR** ($CDCl_3$) /600 MHz/ δ (ppm): 1.44 (s, 18H, Me), 1.40-1.90 (m, 6H, CH_2), 3.11 (br s, 2H, CH_2), 4.13 (br s, 1H, CH), 4.80 (br s, 1H, NH), 5.44 (br s, 1H, NH).

After removing the Boc groups – white to yellow powder, 28% yield;

ESI-MS: 581.8 $[2M+Na]^+$, 302.1 $[M+Na]^+$.

Viral suspension is from the collection of the section "Virology" of the "Stefan Angelov" Institute of Microbiology, Bulgarian Academy of Sciences.

The virus is cultured in a maintenance environment DMEM (Dulbecco's Modified Eagles's Medium) (Gibco BRL, USA) with 0.5% fetal veal serum, 10 mM HEPES (Merk, Germany) and antibiotics (penicillin 100 UI / mL and streptomycin 100 μg / mL) at 37 º C in the presence of 5% CO2. After seeding in microtitre plates, MDCK cells were incubated at 5 % CO2, 37 °C and 95 % humidity for 48 h. Thereafter, the cell culture medium was aspirated and serially diluted compound concentrations in fresh cell culture medium were added (100 μl/well; 2 parallels/concentration, dilution factor 2). Six untreated wells were used as cell control (negative control). 72 h after compound addition and incubation cell were stained with a crystal violet/methanol solution. After dissolving away the stain, the optical density (OD) of individual wells was determined in a Dynatech microplate Photometer (550 /630 nm) and compared with the mean optical density of the 6 cell controls.



Scheme 1. (i) Et_3N, $CH_2Cl_2/CHCl_3$ 1:1, -15ºC; (ii) Et_3N, IBCF, CH_2Cl_2, -15ºC, 15min; (iii) 1,5h, -15ºC; (iv) TFA/ $CHCl_3$

RESULTS AND DISCUSSION

We presents test results for the ability of the prepared compounds to inhibit virus A/H3N2 strain Aichi and the cytotoxic activity of the compounds on MDCK cell culture.

Antiviral activity of the compounds was studied at a concentration of 5μg/mL. The percent inhibition of the compounds given in Table 1 is the arithmetic mean.

It can be concluded by analyzing the results that the antiviral activity is depends by the length the carbohydrate chain. There is an optimal size and structure.

As we can see in the table branched chain amino acids valine did not show activity, even it's hydrophobic portion together with the lipophilic carbocyclic adamantane created the optimum structure for penetrating the membrane bilayer of the virus capsule and disrupting replication processes valine derivative (3 a) behaved no antiviral activity on A(H3N2) virus. Compound 3 b represented amantadine linked to the amino acid alanine. It slightly inhibited A(H3N2). The moderate inhibition activity of this compound possibly is due to its compact structure, which fit into M2 protein envelope and partially obstructs proton conduction.

Table. 1. Antiviral activity against influenza virus A (H3N2) and cytotoxicity of the amantadine derivatives.

Compounds	Cytotoxicity (CC_{50})	Inhibition (IC_{50})
Val-Am (3 a)	189.02	0
Ala-Am (3 b)	211.16	33.28
Lys-Am (3 c)	206.13	15.32
Amantadine	> 200	n/a

Based on the activity of the ornithine derivative, which Shibnev and etc. report, we synthesized close structural analog – lysinyl-amantadine (3 c). The activity of the compound was slightly less than that of 3 b. Carbohydrate chain is much longer compare to 3 b, but in this derivative are two amino groups, which increase the basic properties and inclination to formation hydrogen bonds with M2 protein [5].

CONCLUSION

We synthesized and tested three compounds of amantadine analogues with the amino acids valine, alanine and lysine. The new analogues 3 a-c were evaluated for their antiviral activity towards influenza virus A(H3N2). This study shows that the antiviral properties of amantadine amino acid derivatives could resuscitation the antiviral properties and the resistance was overcome.

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REFERENCES

1. V. A. Shibnev et al., *Pharm. Chem. J.*, **46**, 1, (2012).
2. R. A. Bright et al., *The Lancet*, **366**, 1175 (2005).
3. X. Chen et al., *Europ. J. of Med. Chem.*, **93**, 182 (2015).
4. D. C. Plumb, *PharmaVet*., **6**, 508 (2005).
5. V. Gaiday et al., *Intern. Conf. on Computational Sci.*, 360 (2008).
6. S. S. Anchuri et al., *Biointerf. Research in Applied Chem.*, **6**, 1491 (2016).

АМАНТАДИНОВИ АНАЛОЗИ – СИНТЕЗ И БИОЛОГИЧНА АКТИВНОСТ

Р. Чайров[1]*, Л. Мукова[2], А. Гълъбов[2], Я. Митрев[3], И. Станкова[1]

[1]Катедра по химия, Югозападен университет „Неофит Рилски", ул. „Иван Михайлов" № 66, 2700 Благоевград, България
[2]Институт по микробиология „Стефан Ангелов", ул. „Акад. Георги Бончев" бл. 26, 1113 София, България
[3]Институт по органична химия с Център по фитохимия, ул. „Акад. Георги Бончев" бл. 9, 1113 София, България

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(Резюме)

Пространственото разположение, значителната липофилност, лесно преминаване през биологичните мембрани на аминокиселините са причините, които оказват влияние върху повишена биологичната активност на техни производни с амантадина.

Известни са голям брой грипни щамове резистентни към амантадина. Техният брой се увеличава всяка година заради спонтанни мутации във вирусния геном. Създаване на нови аминокиселинни аналози на амантадина са възможност за преодоляване на резистентността [1].