

Solid-Phase Synthesis and *In Vitro* Cytotoxicity of New Peptide Functionalized Cyclencarboxymethylen and L-DOPA Hybrids

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Abstract

The new small peptide functionalized cyclen and DOPA derivatives were synthesized: cyclen-HisHis, cyclen-AspHis, cyclen-GluHis, DOPA-HisHis were prepared. The solid-phase synthesis strategy was used for preparation of new compounds. Synthesized cyclen- and DOPA-oligopeptide hybrid conjugations were purified by HPLC and analyzed using MALDI-TOF MS spectrometer. The toxic effect was determined against mammalian cells human embryonic kidney cell line HEK293T ATCC®CLR-11268TM for each new compound. The inhibition effect of all tested cyclen-dipeptides on kidney cells was approximately about 30% after 24 hours. The minimal rate of toxicity against human liver cells showed all tested dipeptides with DOPA, their inhibition effect was maximal 10%. The acute inhibition effect of sample DOPA-GluHis-DOPA was 14% immediately after adding. The antioxidant and anticancer activity studies will be next part of the ongoing project.

Keywords: Cyclen; L-DOPA; Peptides; Solid-phase synthesis; *In vitro* cytotoxicity

Introduction

Macrocyclic polyamines are widely used in biology and medicine. The new methodologies for their selective functionalization are demanding due to their importance for a variety of diagnostic and therapeutic pharmaceuticals [1,2] and in the development of new MRI (Magnetic Resonance Imaging) contrast agents [3,4]. Recently, cyclen-based bifunctional chelators have attracted much interest in cancer therapy [5]. On the other hand, L-DOPA (3,4-dihydroxyphenylalanine) derivatives play a crucial role in the therapy of Parkinson disease (PD) as they increase the BBB penetration capacity of DOPA, which is well known medicine in the treatment of PD since 1960s. The DOPA peptidomimetics with amino acid cross-linked via oxygen atom were prepared and their antioxidant activities were studied [6].

In order to mimic biochemical processes, a number of artificial receptor molecules have been synthesized for many biologically vital molecules and enzymes. It is well known, that in such receptors, hydrogen bonding, hydrophobic or electrostatic interactions play significant role, as complementary features of the host-guest molecules. The Zinc- macrocyclic polyamine mode complexes with [12]aneN3 (cyclam) and [12]aneN4 (cyclen) showed the strong nucleophile $Zn^{II}-OH^-$ is forms at physiological pH from $Zn^{II}-H_2O$ species [7] and logk value (6.4) indicates that OH⁻ group is weakly bound to the metal ion and thus, can attack to CO₂ or carboesters. By complexation of Zn^{II} with [12]aneN3 (cyclam) and [12]aneN4 (cyclen) the pKa is lowering from 9.0 ($Zn^{II}-H_2O$) to 7.3 and 7.9, respectively, at 25°C.

The modification of macrocyclic polyamine receptor molecules with additional ligands (arms) enables to interact with nucleobase, sugar and other biomolecules moieties for a more efficient "multipoint" recognition, as well as for thermodynamic stabilization of the ternary complexes in aqueous solution [8]. Cyclen derivatives showed antibacterial [9], anti-HIV and anti-malarial activities [10,11], zinc(II)-cyclen-peptide hybrid compounds as potential inhibitor for Ras-Ras interactions [12], as well as, macrocyclic polyamines, their derivatives, and metal complexes as potential therapeutic agents in Alzheimer's disease treatment, were reported [13-16]. The most advantage of the macrocyclic polyamines is their unique capacity to bind some biologically important metals (Zn, Cu, Co, Fe) and their role

was dramatically increased as behavior models for enzymes and other metalloproteins. On the other hand, it is important, to inhibit protein-protein interactions by small molecules, which are considered to be bi- or multivalent ligands leading to increase binding affinities providing additional binding sites [12]. In addition, Zn^{2+} and Cu^{2+} chelators are proposed as potential therapeutic agents for AD where redox reactions of metal-A β aggregates can be inhibited by chelators [17]. Recently, derivatives of 1,1'-xylyl-bis-1,4,8,11-tetraazacyclododecane have been shown to be effective in reducing copper concentration and to maintain copper normal levels in rats [13]. On the other hand, macrocyclic polyamines such as cyclen and cyclam and their derivatives, as well as their metal complexes have wide applications in medicine [14,15]. It is important that toxicity of cyclen and cyclam is very low [18,19]. The detail investigation of these macrocyclic amines on the effect of metal-induced aggregation was carried out [20] and the chelators showed ability to increase solubility of large aggregates and prevented the formation of the β -sheet structure, as well as they inhibit hydrogen peroxide (H_2O_2) production by Cu-A β 40 and therefore cyclen and cyclam as proposed as good candidates to cross blood-brain barrier and with low toxicity as potential neuroprotective or neurorescue agents in the treatment of Alzheimer's disease.

Polyphenolic compounds, like coumarins and its derivatives are able to bind transition metal ions and inhibit hydroxyl radical and hydrogen peroxide formation produced by Fenton's reactions [21] and by this way, potent reactive oxygen species (ROS) scavengers and metal chelators [21]. Polyphenol derivatives (or metabolites) found numerous therapeutic applications, such as central nervous system stimulants [22],

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antitumor and anti-HIV therapy [23], antibacterial [24], anticoagulants [25] etc. By-phenolic compound, such as 6,7-hydroxycoumarin, with two hydroxyl moieties prevent formation and scavenging of ROS and influence radical-mediated oxidative damage, are considered as one of the most effective antioxidant compounds and used as an protectors against DNA damage, cancer and aging [26,27].

Materials and Methods

General

2-chlorotriylchloride resin, N,N' -diisopropylcarbodiimide (DIC), piperidine (PIP) and Fmoc-protected amino acids: Fmoc-His(trt)-OH, Fmoc-Asp(tBu)-OH, Fmoc-Glu(tBu)-OH were purchased from Iris Biotech GmbH (Marktredwitz, DE); triisopropylsilane (TIS) was from Novabiochem (Darmstadt, DE); N,N' -diisopropylethylamine (DIEA) was obtained from Merck (Darmstadt, DE); 1-hydroxybenzotriazole (HOBt), 1,4,7,10-tetraazacyclododecane (cyclen), 1,4,7-tri-Boc-10-(carboxymethyl)-tetraazacyclododecane (3N-Boc-cyclen), A β (1-40), zinc chloride (anhydrous, $ZnCl_2$) and copper sulphate pentahydrate ($CuSO_4$) were purchased from Sigma-Aldrich (St. Louis, MO, USA); A β (1-16), and Fmoc-DOPA(acetonide)-OH were obtained from Bachem (Bubendorf, Switzerland). High performance liquid chromatography (HPLC) grade acetonitrile (ACN), isopropyl alcohol (IPA) and dimethylformamide (DMF) were from Lachner (Neratovice, CZE); All other chemicals were of analytical or reagent grade.

Purification and identification

After the synthesis, the crude products (dipeptides, cyclen-dipeptides, DOPA-dipeptides, DOPA-dipeptide-DOPA and cyclen-DOPA) were purified by RP-HPLC using a 100 min (3 ml/min) gradient from 0 to 100% ACN. For identification of synthesized compounds were analyzed by MALDI-TOFMS spectrometer.

Experimental part

Solid-phase synthesis of cyclen/DOPA-peptides: All peptide conjugations were synthesized manually by a stepwise strategy. The general procedure for each synthetic cycle, based on initial resin, was as follows:

(1) attachment of Fmoc-His(Trt)-OH or Fmoc-DOPA(ac)-OH (Figure 1) on 2-chlorotriyl resin ($S=1.55$ mM/g), washings: DMF, *i*-PrOH, DMF, each 3x1 min; (2) Deprotection-20% PIP in DMF for ~30 min; washings: DMF, *i*-PrOH, DMF, each 3x1 min; (3) Coupling: HOBt 5 eq in DMF, coupling reagent (Fmoc-AA, 3N-Boc-Cyclen- CH_2COOH , Fmoc-DOPA(ac)-OH), 4 eq in DMF; 2M DIC/DMF, 7 eq;

Bromophenol blue (BB, 0,1% in DMF)-26-31 μ L; shaking the syringes at r.t. for at least 5 h, the reaction time was corrected depending upon the indicator (BB) color change in yellow; (4) washings: DMF, *i*-PrOH, DMF, each 3x1 min; (5) Cleavage of synthesized compounds from the resin and deprotection of side chains were performed in solution of 95% TFA, 2.5% TIS and 2.5% H_2O , the reaction time was 3.5 h followed by 3-4 TFA washing; TFA was removed with the stream of nitrogen. The products were precipitated with *tert*-BuOMe and $(Et)_2O$ ethers and collected by centrifugation (2000xg, 2 min).

Cyclen-HisHis-OH: 3N-Boc-cyclen-55 mg (102 μ M, 1.0 eq) in 200 μ L, His(Trt)-His(Trt)- * -382 mg (204 μ M, 1.0 eq), HOBt-29.0 mg (214 μ M, 2.1 eq) in 50 μ L DMF, BB-26 μ L, DIC-153 μ L (306 μ M, 3.0 eq) in 50 μ L DMF. Yield: crude-105.0 mg (87.2%), pure-54.1 mg (44.8%); MS ($M + H^+$) 501.

Cyclen-AspHis-OH: 3N-Boc-cyclen-55 mg (102 μ M, 1.0 eq) in 200 μ L, Asp(O-tBu)-His(Trt)- * -277 mg (208 μ M, 1.0 eq), HOBt-30.0 mg (218 μ M, 2.1 eq) in 50 μ L DMF, BB-27 μ L, DIC-156 μ L (312 μ M, 3.0 eq) in 50 μ L DMF. Yield: crude-88.0 mg (79.3%), pure-69.0 mg (62.2%); MS ($M + H^+$) 482.95.

Cyclen-GluHis-OH: 3N-Boc-cyclen-55 mg (102 μ M, 1.0 eq) in 200 μ L, Glu(O-tBu)-His(Trt)- * -387 mg (204 μ M, 1.0 eq), HOBt-29.0 mg (214 μ M, 2.1 eq) in 50 μ L DMF, BB-27 μ L, DIC-153 μ L (306 μ M, 3.0 eq) in 50 μ L DMF. Yield: crude-106.0 mg (87.4%), pure-59.4 mg (49.1%); MS ($M + H^+$) 497.

DOPA-HisHis-OH: His(Trt)-His(Trt)- * -470 mg (250 μ M, 1.0 eq), Fmoc-DOPA(ac)-OH-150 mg (326 μ M, 1.3 eq) in 450 μ L DMF, HOBt-51.0 mg (375 μ M, 1.5 eq) in 300 μ L DMF, BB-32 μ L, DIC-288 μ L (575 μ M, 2.3 eq). Yield: crude-117.0 mg (86.5%), pure-76.0 mg (56.2%); MS ($M + H^+$) 471.49.

DOPA-AspHis-OH: Asp(O-tBu)-His(Trt)- * -332 mg (250 μ M, 1.0 eq), Fmoc-DOPA(ac)-OH-150 mg (326 μ M, 1.3 eq) in 450 μ L DMF, HOBt-51.0 mg (375 μ M, 1.5 eq) in 250 μ L DMF, BB-32 μ L, DIC-288 μ L (575 μ M, 2.3 eq). Yield: crude-112.0 mg (84.1%), pure-80.6 mg (60.5%); MS ($M + H^+$) 449.49.

DOPA-GluHis-OH: Glu(O-tBu)-His(Trt)- * -474 mg (250 μ M, 1.0 eq), Fmoc-DOPA(ac)-OH-150 mg (326 μ M, 1.3 eq) in 450 μ L DMF, HOBt-51.0 mg (375 μ M, 1.5 eq) in 250 μ L DMF, BB-32 μ L, DIC-288 μ L (575 μ M, 2.3 eq). Yield: crude-107.0 mg (79.2%), pure-60.8 mg (45.0%); MS ($M + H^+$) 463.49.

Cyclen-DOPA: 3N-Boc-cyclen-335 mg (631 μ M, 1.1 eq) in 1.0 mL DMF, DOPA(ac)- * -800 mg (576 μ M, 1.0 eq), HOBt-156.0 mg (1152

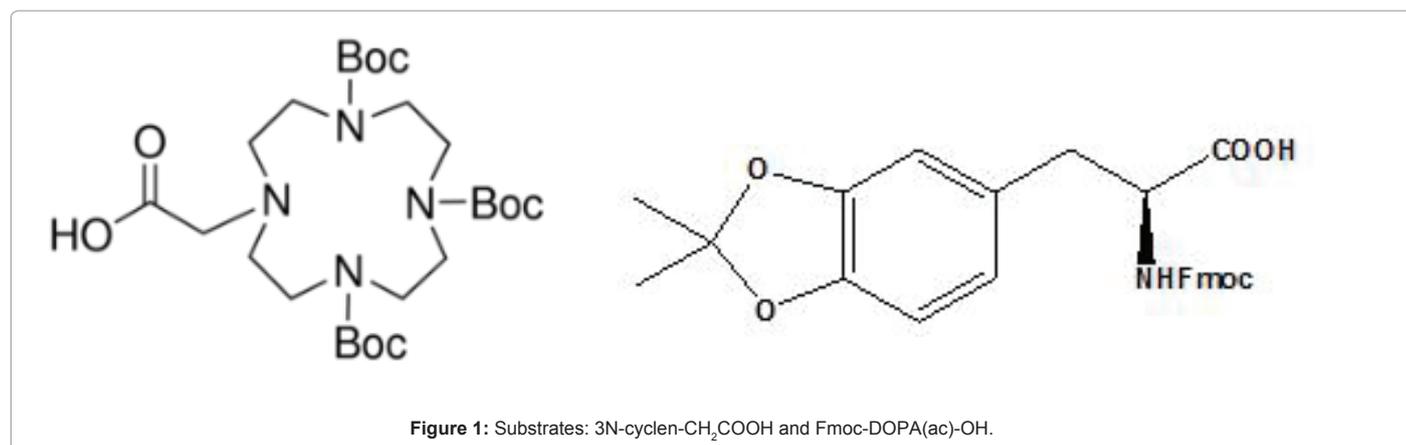


Figure 1: Substrates: 3N-cyclen- CH_2COOH and Fmoc-DOPA(ac)-OH.

μM , 2.1 eq) in 250 μL DMF, BB-74 μL , DIC-864 μL (1728 μM , 3.0 eq). Yield: crude-291.0 mg (90.1%), pure-201.0 mg (62.2%); MS ($\text{M} + \text{H}^+$) 409.49.

DOPA-HisHis-DOPA: Fmoc-His(Trt)-OH-303 mg (490 μM , 2.0 eq) in 650 μL DMF, DOPA(ac)- $^{\circ}$ -340 mg (245 μM , 1.0 eq), HOBt-83.0 mg (612.5 μM , 2.5 eq) in 120 μL DMF, BB-31 μL , DIC-429 μL (858 μM , 3.5 eq). Coupling with another His: Fmoc-His(Trt)-OH-380 mg (613 μM , 2.5 eq) in 800 μL DMF, HOBt-102.0 mg (760 μM , 3.1 eq) in 180 μL DMF, BB-31 μL , DIC-536 μL (1072 μM , 4.38 eq). Coupling with another DOPA: Fmoc-DOPA(ac)-OH-123 mg (270 μM , 1.1 eq) in 150 μL DMF, HOBt-66.0 mg (490 μM , 2.0 eq) in 300 μL DMF, BB-31 μL , DIC-370 μL (735 μM , 3.0 eq). Yield: crude-215.1 mg (80.3%), pure-190.2 mg (70.9%); MS ($\text{M} + \text{H}^+$) 650.38.

DOPA-AspHis-DOPA: Fmoc-His(Trt)-OH-303 mg (490 μM , 2.0 eq) in 650 μL DMF, DOPA(ac)- $^{\circ}$ -340 mg (245 μM , 1.0 eq), HOBt-83.0 mg (612.5 μM , 2.5 eq) in 120 μL DMF, BB-31 μL , DIC-429 μL (858 μM , 3.5 eq). Coupling with Asp: Fmoc-Asp(O-tBu)-OH-252 mg (613 μM , 2.5 eq) in 800 μL DMF, HOBt-102.0 mg (760 μM , 3.1 eq) in 180 μL DMF, BB-31 μL , DIC-536 μL (1072 μM , 4.38 eq). Coupling with another DOPA: Fmoc-DOPA(ac)-OH-123 mg (270 μM , 1.1 eq) in 150 μL DMF, HOBt-66.0 mg (490 μM , 2.0 eq) in 300 μL DMF, BB-31 μL , DIC-370 μL (735 μM , 3.0 eq). Yield: crude-247.0 mg (85.6%), pure-203.1 mg (70.4%); MS ($\text{M} + \text{H}^+$) 628.38.

DOPA-GluHis-DOPA: Fmoc-His(Trt)-OH-303 mg (490 μM , 2.0 eq) in 650 μL DMF, DOPA(ac)- $^{\circ}$ -340 mg (245 μM , 1.0 eq), HOBt-83.0 mg (612.5 μM , 2.5 eq) in 120 μL DMF, BB-31 μL , DIC-429 μL (858 μM , 3.5 eq). Coupling with Glu: Fmoc-Glu(O-tBu)-OH-261 mg (613 μM , 2.5 eq) in 800 μL DMF, HOBt-102.0 mg (760 μM , 3.1 eq) in 180 μL DMF, BB-31 μL , DIC-536 μL (1072 μM , 4.38 eq). Coupling with another DOPA: Fmoc-DOPA(ac)-OH-123 mg (270 μM , 1.1 eq) in 150 μL DMF, HOBt-66.0 mg (490 μM , 2.0 eq) in 300 μL DMF, BB-31 μL , DIC-370 μL (735 μM , 3.0 eq). Yield: crude-188.2 mg (77.2%), pure-102.1 mg (41.9%); MS ($\text{M} + \text{H}^+$) 642.38.

Cytotoxicity Assay

Monitoring of cell growth with the RTCA DP instrument: Experiments were carried out using the xCELLigence RTCA DP instrument (Roche Diagnostics GmbH, Mannheim, Germany) which was placed into a incubator (37°C and 5% CO₂). Cell proliferation and cytotoxicity experiments were performed using modified 16-well plates (E-plate, Roche Diagnostics GmbH, Mannheim, Germany).

Microelectrodes were attached at the bottom of the wells for impedance-based detection of attachment, spreading and proliferation of the cells. Initially, 100 μL of cell-free growth medium (10% FBS, 1% MEM) was added to the wells.

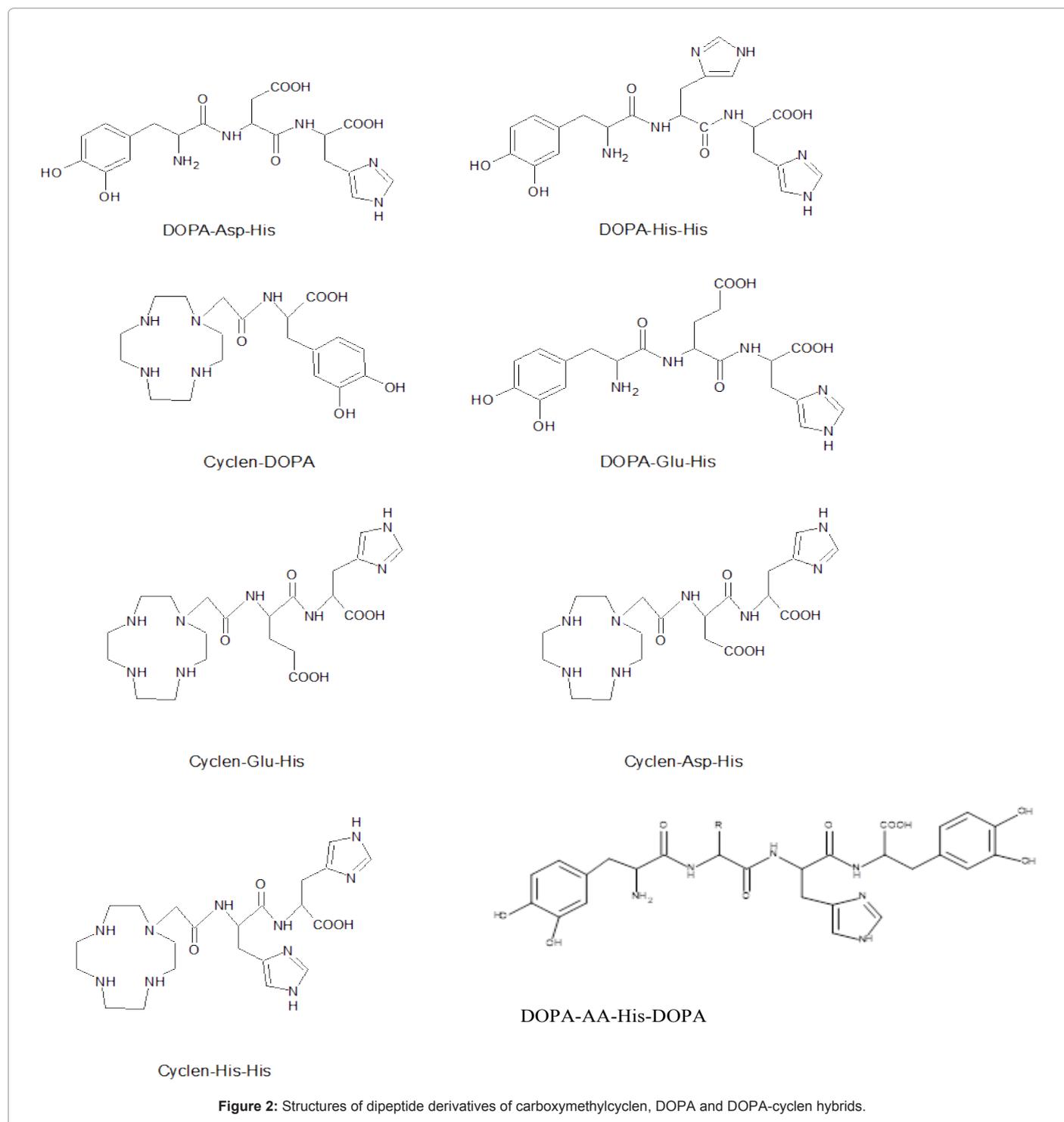
Cells were harvested from exponential phase cultures by a standardized detachment procedure using 0.25% Trypsin-EDTA and counted automatically using Roche's CASY Cell Counter and Analyzer. 100 μL of the cell suspension was seeded into the wells as 10⁶ HEK-293T cells/ml for cytotoxicity experiments. Twenty-four hours after cell seeding were added tested substances dissolved in water (concentration 50 $\mu\text{g}/\text{ml}$) and also during a period of 72 hours with. Water was added to control wells. CI (cell index) was monitored every 60 min during the experiment for 72 hours. This results into growth curves (dependence of the impedance expressed by the "cell index" value on time) of monitored cells in the presence of individual substances. HEK-293T ATCC[™] CRL-11268[™] are epithelial cells derived from kidney of human fetus. HEK-293T cells were cultivated in Dulbecco's modified

Eagle's medium (DMEM; with 4.5 g/L glucose and L-Glutamine), supplemented with 10% fetal bovine serum (FBS) and 1% of MEM (mix of vitamins-Gibco, GB. Cells were cultured at 37°C and 5% CO₂; cultivation medium was changed every 2 to 3 days. For experimental procedures, cells were seeded in 96-well plates at a concentration of 10⁵ cells/ml per well.

Results and Discussion

The new small peptide functionalized cyclen and DOPA derivatives were synthesized: cyclen-HisHis, cyclen-AspHis, cyclen-GluHis, DOPA-HisHis, DOPA-AspHis, DOPA-GluHis, DOPA-HisHis-DOPA, DOPA-AspHis-DOPA, DOPA-GluHis-DOPA and DOPA-cyclen (Figure 2). The solid-phase synthesis strategy was used for preparation of new compounds. The His-rich cyclen conjugations could be serve as DNA, ATP and other biomolecules recognition models, as bifunctional molecules (protein interaction and metal chelation) in metal chelation therapy approach and polyphenolic DOPA derivatives, as metal chelators and radical scavengers. The general synthetic procedure is given on Scheme 1 and 2. We prepared the cyclencarboxymethyl derivatives with tert Bu- and Trt-protected dipeptides, including HisHis, GluHis and AspHis, and Dopa-substituted carboxymethylcyclen. Compound 1 (Fmoc-His(Trt)-OH, 4.61 g, 7.44 mM, 1.2 eq.) was dissolved in DMF and 4.21 mL (24.8 mM, 4.0 eq) diisopropylethylamine (DIEA) was added to the syringe with 2-chlorotriyl chloride resin (S=1.55 mg/g, 4.0 g, 6.2 mM, 1 eq), which was preliminary washed with THF (3x) and swelled in DCM (CH₂Cl₂). The syringe was shaken for ~5 hrs at room temperature. As color turned from yellow to brown-orange, the reaction content was washed with DMF, i-PrOH, DMF (3x). The Fmoc-His(Trt)- $^{\circ}$ was dried and half of this His-attached resin was swelled for 20 min, 20% Piperidine (PIP) in DMF was added for Fmoc-deprotection for 30 min. The another portion of Fmoc-His(Trt)- $^{\circ}$ (2.73 g, 4.4 mM, 2 eq), HOBt (0.74 g, 5.5 mM, 2.5 eq), 2M DIC/DMF solution (3.85 mL, 7.7 mM, 3.5 eq) and 0.1% bromophenol blue (286 μL) were added. The reaction was shaken overnight until color turned from bluish yellow. The Fmoc group was deprotected again 20% PIP, washed and dried. Finally, the crude H-His(Trt)-His- $^{\circ}$ product was deprotected with 95% THF (2.5% TIS, 2.5% H₂O), then acids were removed under N₂ stream and product was extracted by MeOBu and Et₂O ethers. The crude products were purified by HPLC. Solvents were removed by rotovap and lyophilized to obtain colorless crystals.

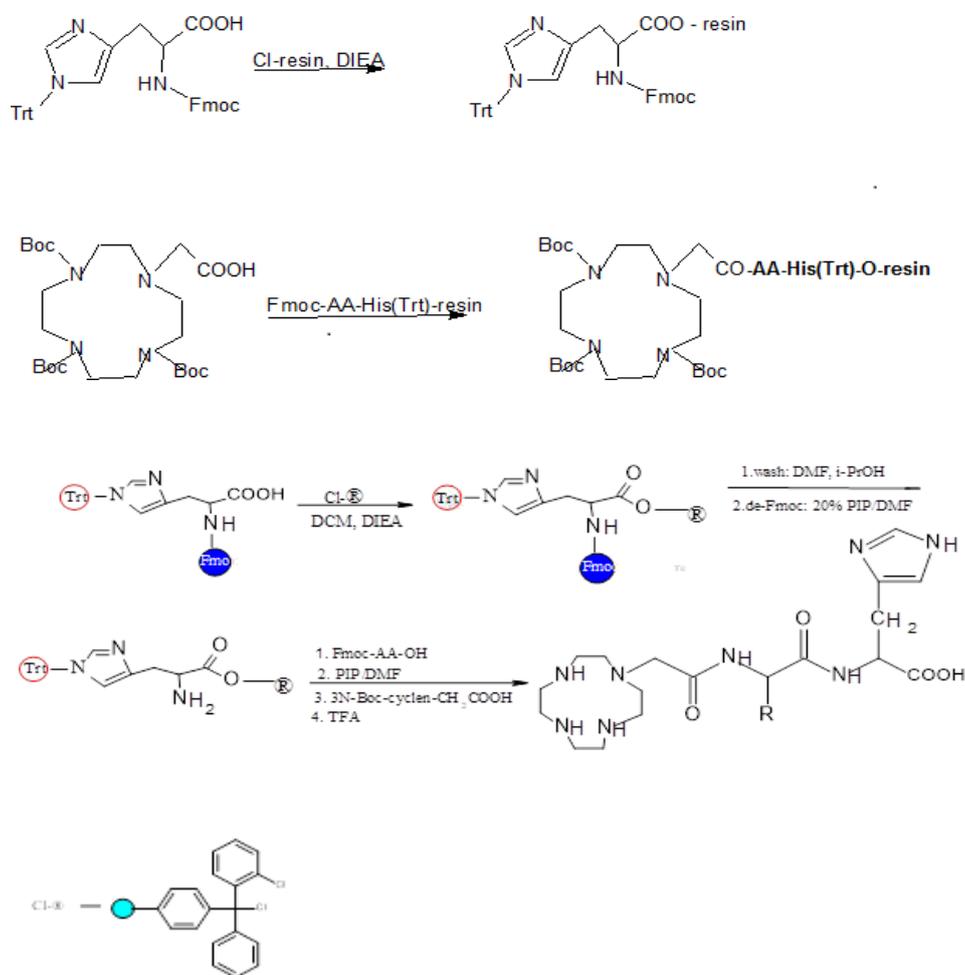
N-functionalization of tetraazamacrocycles and their derivatives has been reported [28-30]. Generally, modification of such compounds is occurring via n-derivatization by a) derivatization and b) protection-derivatization-deprotection [28-30]. Synthesis of tetraazamacrocyclic-KLVFF hybrids-the amide- or triazole linked derivatives and their metal complexes were reported [31] using Fmoc-strategy solid phase synthesis on Wang resin and subsequent coupling with tri-Boc-tetraazamacrocyclic-acetic acid. These hybrid compounds showed neuroprotective effects of Ab in Alzheimer's disease. The cyclen and cyclam were attached to the synthetic pentapeptide-KLVFF, a short Ab fragment (Ab16-20) which was chosen for amyloidic recognition and disruption of aggregates into fibrils [32, 33]. The glycine and triazole spacers were additionally incorporated in tetraazamacrocyclic hybrids. On the other hand, dopamine precursors, such as levodopa (L-Dopa) or dopaminergic compounds are generally used for the treatment of Parkinson's disease [34, 35]. Parkinson's disease treatment efforts are concerned to the improvement of BBB permeability of candidate molecules, and thus, brain delivery [36, 37]. With this aim, L-dopa dipeptide derivatives using non-natural amino acids were prepared and



their antiparkinsonic activity was studied [38] and amide derivatives of L-Dopa [39], from which the higher promising prodrug was diacetyl derivative of the simple L-Dopa amide.

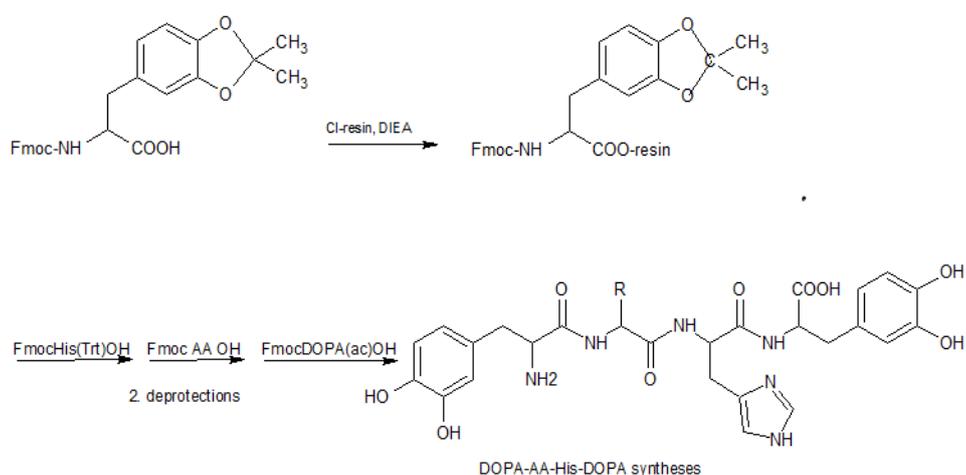
The new compounds were derived with good yield. Possible cytotoxicity of new biological active peptides to mammalian cells is another disadvantage in their potential clinical use. Testing of the biological properties of peptides includes the determination of cytotoxic activity to the basic test parameters. To measure cytotoxicity,

a system was used to assess the viability of adhering cells based on their degree of adhesion to the bottom of the microtiter plate, or a decrease in resistance between the surface of the electrodes and the culture medium. Next we investigated the toxic effect against mammalian cells human embryonic kidney cell line HEK293T ATCC®CLR-11268TM with each peptide. Toxic activity was determined in concentration 50 µg/ml. Figure 3 shows proliferation curves of cell line HEK 293T after adding group of cyclens of different dipeptides. The inhibition effect of

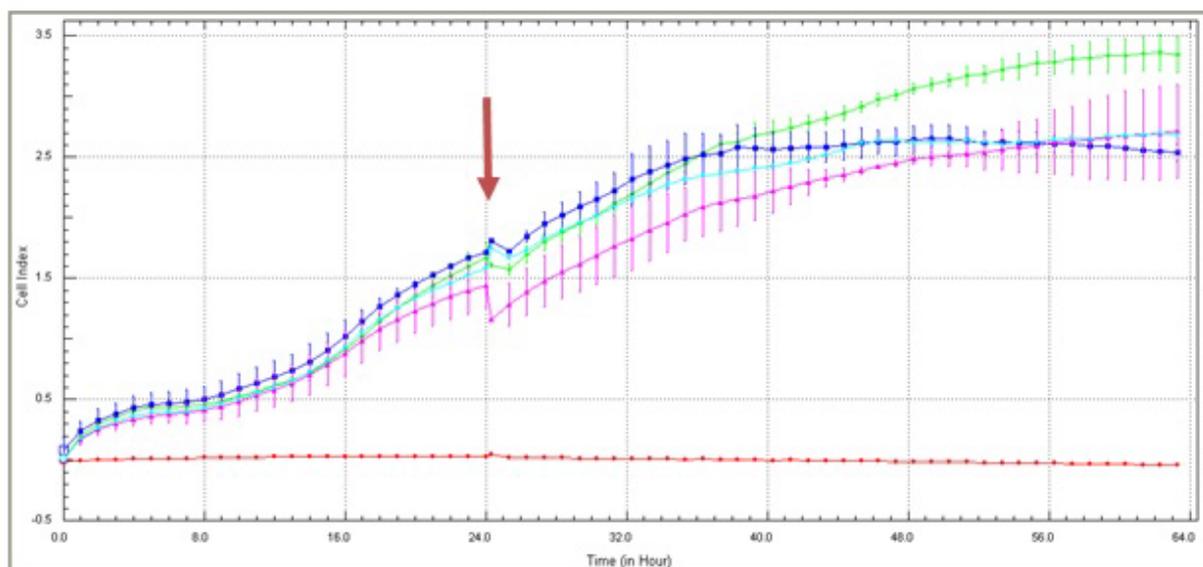


Cl-® - polystyrene-bound 2-chlorotryl resin

Scheme 1: Stepwise reaction of functional derivation of tri-N-Boc-Cyclen-CH₂COOH with dipeptide (HH, DH, EH).



Scheme 2: Stepwise reaction of modification of Fmoc-DOPA(ac)-OH with dipeptides.



Cell line HEK 293T (concentration of cell 10^6 cell/ml) Concentration of samples 50 μ g/ml

Figure 3: Proliferation curves of cell line HEK 293T (concentration of cell 10^6 /ml) after adding tested samples (green-control, blue-cyclen-HisHis, pink-cyclen-GluHis, turquoise- cyclen-AspHis; red arrow-sample added).

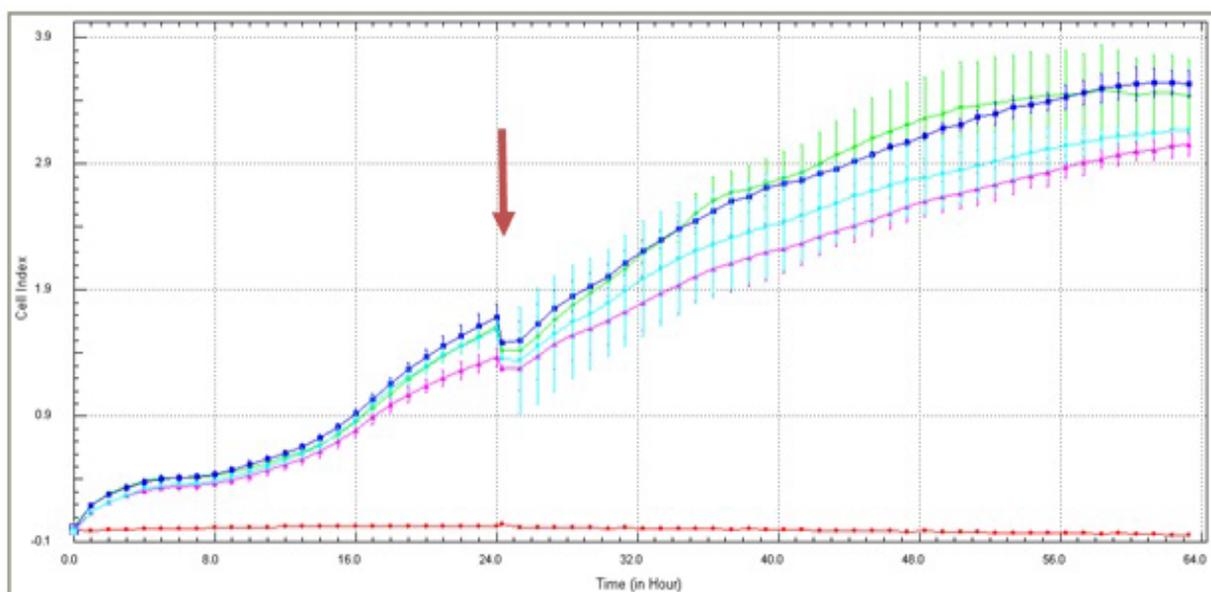


Figure 4: Proliferation curves of cell line HEK 293T (concentration of cell 10^6 /ml) after adding tested samples (green-control, blue-DOPA-HisHis, pink-DOPA-GluHis, turquoise-DOPA-AspHis; red arrow-sample added).

all tested cyclen-dipeptides on kidney cells was approximately about 30% after 24 hours. The minimal rate of toxicity against human liver cells showed all tested dipeptides with DOPA, their inhibition effect was maximal 10% (Figure 4). The acute inhibition effect of sample DOPA-GluHis-DOPA was 14% immediately after adding (Figure 5).

The obtained results showed that further analyses and testing will be followed, such as anticancer and antioxidant activities, interaction with aggregated amyloid beta peptide and toxicity on neuronal cells will be observed *in vitro*.

Conclusion

The new small peptide functionalized cyclen and DOPA derivatives - cyclen-HisHis, cyclen-AspHis, cyclen-GluHis, cyclen-DOPA, DOPA-HisHis, DOPA-AspHis, DOPA-Glu-His, DOPA-HisHis-DOPA, DOPA-AspHis-DOPA and DOPA-Glu-His-DOPA were prepared. The solid-phase synthesis strategy was used for preparation of new compounds. Synthesized cyclen- and DOPA-oligopeptide hybrid conjugations were purified by RP-HPLC and analyzed using MALDI TOF MS spectrometer. The toxic effect was determined

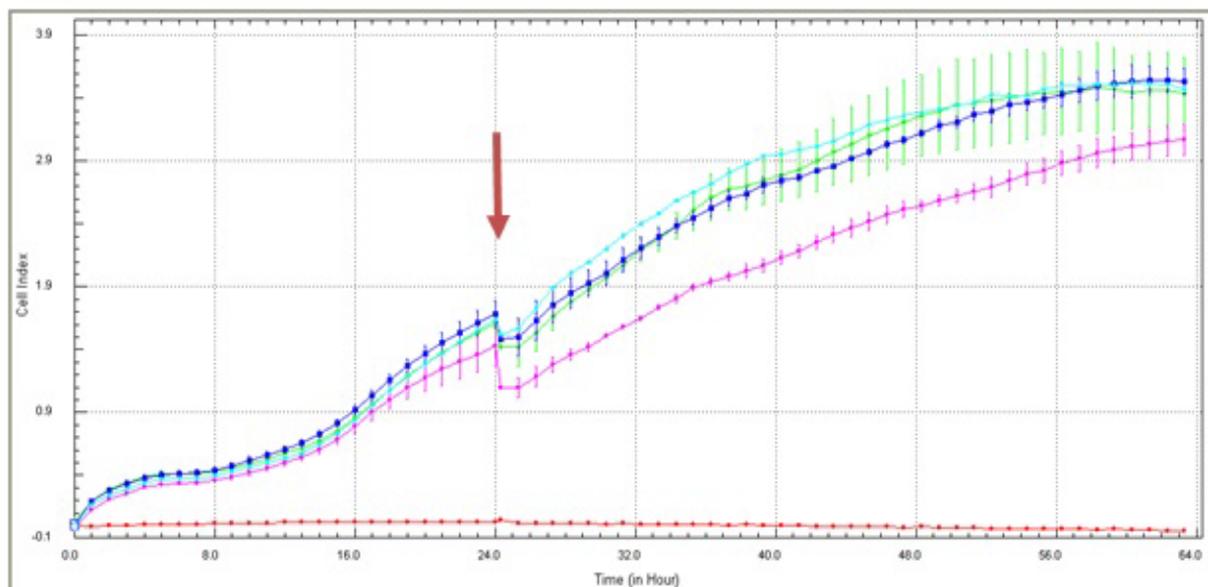


Figure 5: Proliferation curves of cell line HEK 293T (concentration of cell 10^6 /ml) after adding tested samples (green-control, blue-DOPA-HisHis-DOPA, pink-DOPA-GluHis-DOPA, turquoise-DOPA-AspHis-DOPA; red arrow-sample added).

against mammalian cells human embryonic kidney cell line HEK293T ATCC®CLR-11268™ for each new compound. Toxic activity was determined in concentration $50 \mu\text{g}/\text{ml}$. The inhibition effect of all tested cyclen-dipeptides on kidney cells was approximately about 30% after 24 hours. The minimal rate of toxicity against human liver cells showed all tested dipeptides with DOPA, their inhibition effect was maximal 10%. The acute inhibition effect of sample DOPA-GluHis-DOPA was 14% immediately after adding. Their antioxidant and anticancer activities will be studied based on the obtained toxicity results.

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