Synthesis of New Cyclen-Peptide Conjugations

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Macrocyclic polyamines have wide biological and medicinal applications. The new methodologies for their selective functionalization are of high interest due to their importance for a variety of diagnostic and therapeutic pharmaceuticals (Aoki S., Kimura E.,1999; Bradshaw J.S. et al, 1993) and in the development of new MRI (Magnetic Resonance Imaging) contrast agents (Caravan P., 1999). Recently, cyclen-based bifunctional chelators have attracted much interest in cancer therapy (Liu S., Edwards D.S., 2001). On the other hand, L-DOPA (3,4-dihydroxyphanylalanine) 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 kross-linked via oxygen atom were prepared and their antioxidant activities were studied (Bazzarri B. M. et al., 2015). After the synthesis, the crude products (dipeptides, cyclen-dipeptides, DOPA-dipeptides, DOPA-dipeptide-DOPA and cyclen-DOPA) were purified by RP-HPLC using an 100 min (3 ml/min) gradient from 0 to 100% CAN. For identification of synthesized compounds were analyzed by MALDI-TOF-MS spectrometer.In our current works, the new small peptide functionalized cyclen and DOPA derivatives were synthesized: cyclen-HisHis, cyclen-AspHis, cyclen-GluHis, DOPA-HisHis, as well as their Cu(II) and/or Zn(II) coordination compounds 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 MS-ES spectrometer. 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. Cytotoxicity testing on mammalian cells *in vitro* showed that they are non-toxic compounds, now their anticancer and antioxidant activity testing is under experiment. The new small peptide functionalized cyclen and DOPA derivatives - cyclen-HisHis, cyclen-AspHis, cyclen-GluHis, DOPA-HisHis, as well as their Cu(II) and/or Zn(II) coordination compounds were prepared. 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% CO_2). 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. 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 MS-ES spectrometer. The *in vitro* testing of cytotoxicity showed that cyclen-dipeptide and Dopa hybrids are non-toxic compounds for cell line Hep G2 - ATCC® HB-8065™ (cells are derived from human liver) and HEK-293T - ATCC® CRL-11268™ (epithelial cells derived from kidney of human fetus), and their antioxidanr and anticancer activities will studied based on the obtained toxicity results.

Abbreviations: *Cyclen - 1,4,7,10-tetraazacyclododecane; L-DOPA - 3,4-dihydroxyphenylalanine; Cyclen-HisHis-OH- 10-(carboxymethyl-histidyllhistidine)-1,4,7,10-tetraazacyclododecane; Cyclen-AspHis-OH- 10-(carboxymethyl-aspartylhistidine)-1,4,7,10-tetraazacyclododecane; Cyclen-GluHis-OH- 10-(carboxymethyl-glutamylhistidine)-1,4,7,10-tetraazacyclododecane; DOPA-HisHis-OH- 3,4-dihydroxyphenylalanine-6-histidylhistidine.*

Key words: *peptide, cyclen, L-DOPA, macrocyclic, cytotoxicity, synthesize, derivative, anticancer, chromatogram.*