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Amphiphilic cationic calixarenes in HEK-293 transfection

R.V. Rodik[1], M.Yu. Kuznietsova[2], I.V. Bodenchuk[2], E.V. Zhuravel[2], V.I. Kalchenko[1], S.A. Zozulya[2]

[1] Institute of Organic Chemistry, NAS of Ukraine
5, Academician Kukhar Str., Kyiv, Ukraine, 02094
[2] Enamine Ltd.
78, Winston Churchill Str., Kyiv, Ukraine, 02094
dmso@ukr.net

Aim. Development of effective transfection agents remains an actual topic in biotechnology. The human embryonic kidney (HEK-293) cell line is commonly used for transfections, including the large-scale format. Previously [1, 2], we have found that cationic calixarenes with choline or *N*-methylimidazolium groups condense DNA in nano-sized particles and promote transfection of HeLa and Cos-7 cells. This study includes the toxicity and transfection experiments on HEK-293 cells with calixarenes modified with choline, *N*-methylimidazolium, 3-pyridinium, aminoammonium (CXN), and amidoammonium (CXN3FAc) groups at the upper macrocycle rim and long alkyl chains (C8, C12 and C16) at the lower rim. **Methods.** Synthesis of calixarenes was done by standard methods [1, 2]. The transfections were performed in a serum-free medium containing Calix/DNA complexes alone or mixed with 1,2-dioleoyl-sn-glycero-3-PE (DOPE) at 1:1 molar ratio of DOPE:Calix. For the transfections N/P=5 ratio of a calixarene protonable nitrogens (quaternized ammonium groups) to the DNA phosphate content (pPB-Puro-EF1A-EmGFP plasmid, 1 μg/ml) was used. The final concentrations of calixarenes were 3.7 μM. Commercial lipid-based transfection reagent (TransFast™) was used as a reference control. Green Fluorescent Protein (GFP) signal of transfected cells was quantitated by fluorescent confocal cell imaging (IN Cell Analyzer 6500, GE Healthcare). Cytotoxicity was assessed by resazurin fluorometric assay measuring the metabolic capability of cells. Fluorescence was measured with EX 555 nm, EM 585 nm on SpectraMax i3x plate reader. **Results.** TransFast™ control treatment resulted in 7% transfection efficiency (GFP-positive cells). All the studied calixarenes did not reveal any effect in the absence of DOPE. CX8N3Fac, CX8N and CX16N3FAc demonstrated good transfection efficiency in combination with DOPE at the levels of 1.7%, 2.7% and 3.7% respectively. Addition of chloroquine at 100 uM did not improve transfection for CX8N and CX16N3Fac and provided a small increase for CX8N3Fac. In addition, the preliminary results of dodecyl analog of CX8N showed a significant improvement in the transfection efficiency. Cytotoxicity tests show IC_{50} values within the 8–77 μM range. The most promising macrocycles CX8N, CX8N3FAc and CX16N3FAc have cytotoxicity IC_{50}s of 18, 28 and 11 μM. Cytotoxicity decreases with increasing alkyl chain length. **Conclusions.** The transfection efficiencies of calix[4]arenes CX8N and CX16N3FAc for the commonly used HEK293 cells were comparable to the commercial transfection reagent control. This contrasts with the low results for CX8N obtained previously on HeLa and Cos-7 cells. At the used N/P=5 ratio of calixarenes to DNA, their working concentrations were 3.7 μM, which is well below their cytotoxicity IC_{50}s. Therefore, these compounds are promising agents for the transfection formulations.

Keywords: Calixarenes, transfection, HEK-293, cytotoxicity.

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