In order to investigate in more details relationship between structure and activity of Tat cell penetrating peptide and also to clarify possible mechanism of its cell penetrating a series of 16 peptides homologues to the original GRKKRRQRRRPPQ sequence was synthesized. Each of those peptides represented either lipophylic, aromatic, neutral or unnatural amino acid residues located in different arrears of original sequence, mostly in between Arginine residues of original peptides. All peptides displayed same number of positive charges as native Tat peptide, but differed by their physico-chemical properties. All peptides were purified over 95% according HPLC and characterized by MS. After labelling with different fluorescence labels all peptides were tested for their cell penetrating ability on different cell lines in several conditions. The results of biological testing are presented and discussed.