CONCLUSIONS

Considering human telomerase as a promising target of anti-cancer therapy, the thesis deals with the study of inhibitory potency of selected ANP diphosphates towards telomerase, and the capability of nucleoside-type DNA methylation inhibitors to inhibit *hTERT* expression, knowing that *hTERT* expression closely correlates with telomerase activity *in vitro* and *in vivo*. The results can be summarized as follows:

• All the purine ANP diphosphates except for (*S*)-PMPApp and 6-Me$_2$PMEDAPpp show dose-dependent inhibition of human telomerase in cell-free assay, the adenine derivatives are less effective inhibitors than the guanine derivatives. The only two pyrimidine ANP diphosphates tested (PMECpp and PMETpp) do not show any significant inhibitory potency towards telomerase.

• Activity of tested ANPs on telomerase is limited to their diphosphates (ANPpp) only.

• (*R*)-enantiomers are more inhibitory compared to (*S*)-enantiomers. This indicates that absolute configuration plays an important role in the telomerase inhibition and that the enzyme distinguishes between the (*R*)- and (*S*)-enantiomers.

• PMEGpp is the most potent human telomerase inhibitor among all ANPs studied with the IC_{50} value of 12.7 ± 0.5 mol.l^{-1} at 125 M dNTPs. Its inhibitory potency towards telomerase is comparable to that of ddGTP (IC_{50} value of 8.1 ± 0.4 mol.l^{-1} at 125 M dNTPs), which is known to be one of the most effective nucleotide analogue based telomerase inhibitors.

• (*S*)-PMPApp and 6-Me$_2$PMEDAPpp do not inhibit telomerase, on the contrary, they increase the repeat addition processivity of telomerase in a dose-dependent manner in cell-free assay.

• Although PMEGpp is a much more potent human telomerase inhibitor than any of the other ANPs tested, only a moderate and reversible telomere shortening can be achieved

by exposure to 0.75 M PMEG over a period of 9 weeks of treatment – CCRF-CEM cells lost about 20% of their initial mean telomere length. In contrast, treatment with 20 M PMEDAP caused a progressive and irreversible telomere shortening in CCRF-CEM cell line. Cells lost more than 60% of their initial mean telomere length until the removal of PMEDAP from the growth media in the end of 11_{th} week.

• The increase of telomerase processivity *in vitro* caused by (*S*)-PMPApp has not been shown to be manifested in telomere elongation in the growing cells - (*S*)-PMPA does not cause any significant changes in telomere length in CCRF-CEM cells when supplied in the growth medium for 11 weeks at concentration of 100 $mol.l^{-1}$.

• Both -5-azadCyd and -5-azadCyd down-regulate *hTERT* expression, however, treatment with these compounds induces a distinct pattern of *hTERT* expression in HL-60 cells. -5-AzadCyd inhibited *hTERT* expression in the whole range of tested concentrations whereas the beta anomer (decitabine) causes a transient elevation of *hTERT* mRNA at low micromolar concentrations followed by subsequent *hTERT* down-regulation at higher concentrations of -5-azadCyd. The increase of *hTERT* expression correlates with up-regulation of c-*myc*, however, the subsequent decrease in *hTERT* expression seems to be independent of c-*myc* expression.

• The reversible SAH-hydrolase inhibitor (*S*)-DHPA causes up-regulation of *hTERT* within a broad range of concentrations up to 1000 M. c-*Myc* expression is significantly elevated at all tested concentrations.

• The irreversible SAH-hydrolase inhibitor (*R,S*)-AHPA-*ibu* exhibites stronger potency to inhibit *hTERT* expression when compared to (*S*)-DHPA. In contrast to (*S*)-DHPA, we observed a significant decrease in *hTERT* mRNA levels from concentration corresponding to its GIC_{50} value (174 M). Again, there is an up-regulation of *hTERT* expression at lower concentrations of (*R,S*)-AHPA-*ibu* and c-*myc* remains overexpressed within the whole range of tested concentrations. Similar to -5-azadCyd and (*S*)-DHPA, the down-regulation of *hTERT* seems to be independent of c-*myc* expression.

• Treatment with (*S*)-HPMPazaC results in the decrease of *hTERT* mRNA levels. The effect of (*S*)-HPMPazaC on expression of *hTERT* and c-*myc* is similar to that of -5-azadCyd, however, at considerably higher concentrations. No transient elevation of *hTERT* mRNA levels and no c-*myc* overexpression is observed.

• From the studied compounds, F-PymRf was shown to have the highest potency to increase c-*myc* and *hTERT* mRNA levels.