2,4-DIOXO-5-HEXENOIC ACID DERIVATIVES ARE NOVEL SELECTIVE NON-NUCLEOSIDE INHIBITORS OF MAMMALIAN TERMINAL DEOXYNUCLEOTIDYL TRANSFERASES, WITH POTENT CYTOTOXIC EFFECT AGAINST LEUKEMIC CELLS

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Mammalian terminal deoxynucleotidyl transferase (TDT) catalyzes the non-template-directed polymerization of deoxynucleoside triphosphates *in vitro*. It was initially isolated from thymus, but it is also expressed in bone marrow. Evidences continue to accumulate that TDT is a key player in influencing the outcome of V(D)J recombination during lymphocyte and repertoire development. Over 90% of leukemic cells in acute lymphocytic leukemia and approximately 30% of leukemic cells in the chronic myelogenous leukemia crisis exhibit elevated TDT activity, and the TDT activity of such leukemic cells is associated with a poor prognosis on chemotherapy and survival time.

TDT belongs to the family X of DNA polymerases (pols), whose other members are pol β, pol λ and pol μ in mammalian cells and pol IV in the yeast *S. cerevisiae*. Pol β and *Sc*pol IV do not display terminal transferase (tdt) activity, whereas pol λ has been shown to be endowed with strong *bona fid*e tdt activity. Biochemical studies suggested that pol λ and pol μ might be involved in the nonhomologous end joning (NHEJ) recombinational repair pathway of DNA double strand breaks (DSBs). The availability of specific inhibitors for these enzymes might help the investigation of their cellular functions. Moreover, the correlation between high TDT activity and malignancy of acute lymphocytic leukemia, further increases the interest in developing TDT-specific inhibitors.

In an effort to identify potent and selective inhibitors of the tdt activity of pol λ and TDT, we undertook a random screening of synthetic non-nucleoside analogues. Here, we report the characterization of the mechanism of action of three diketo hexenoic acid (DKHA) derivatives [1], which proved to be extremely selective for the tdt activity of pol λ and TDT. To the best of our knowledge, these are the first non-nucleoside specific inhibitors of mammalian terminal transferases reported so far. Moreover, these DOHA analogs were not toxic towards HeLa cells (CC_{50} > 100 μM), whereas they showed potent cytotoxicity against the TDT^+ acute leukemia cell line MOLT-4 (CC_{50} = 14 μM).

[1] R. Costi, R. Di Santo, M. Artico, A. Roux, *Bioorg. Med. Chem. Lett.* **2004,** *14*, 1745.