11A-N-TOSYL-5-DEOXI-PTEROCARPAN, LQB-223: CYTOTOXIC ANTITUMOR AGENT IN MULTIDRUG RESISTANT ACUTE LEUKEMIAS CELL LINES ACTS THROUGH INHIBITION OF DNA TOPOISOMERASE II

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Introduction

The multidrug resistance (MDR) is one of the causes for the onset of refractoriness to treatment with topoisomerase inhibitors. Therefore, the development of effective new compounds on MDR cells is necessary.

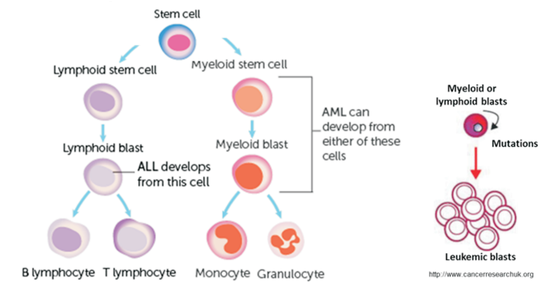


Figure 1: Acute leukemias arising scheme

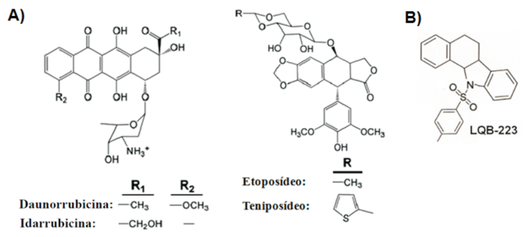


Figure 2: A) Topoisomerase inhibitors used in the treatment of acute leukemias. B) Structure of the synthetic compound evaluated in this work: 11A-N-TOSYL-5-DEOXI-PTEROCARPAN, LQB-223.

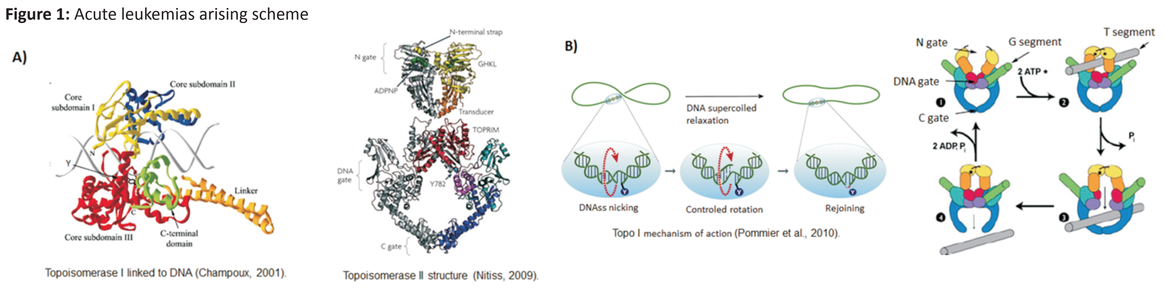


Figure 3: DNA topoisomerases scheme. A) Structure of topoisomerase I (Topo I) and topoisomerase II (Topo II). B) Topo I and Topo II mechanism of action.

Objectives

The aim of this work was: To develop acute lymphoid and myeloid leukemia cells resistant to etoposide (VP-16) and evaluate the MDR phenotype of these lineages; To investigate the effect of new compound LQB-223 in the induction of cell death and also in the inhibition of human DNA topoisomerases (hTopo I and IIα) as its mechanism of action.

Methods and Results

The developed VP-16 resistant cells also showed to be more resistant to idarubicin, daunorubicin and doxorubicin treatment than parental cells. Resistant cells presented an increase in P-glycoprotein expression, alterations on microRNAs expression and reduction of hTopo IIα/β proteins. LQB-223 treatment reduced the viability of all cell lines, altered the cell cycle and induced cell death. The biochemical and docking assays showed that LQB-223 act as catalytic inhibitor of hTopo IIα and that its bind may occur at hTopo IIα ATPase region.

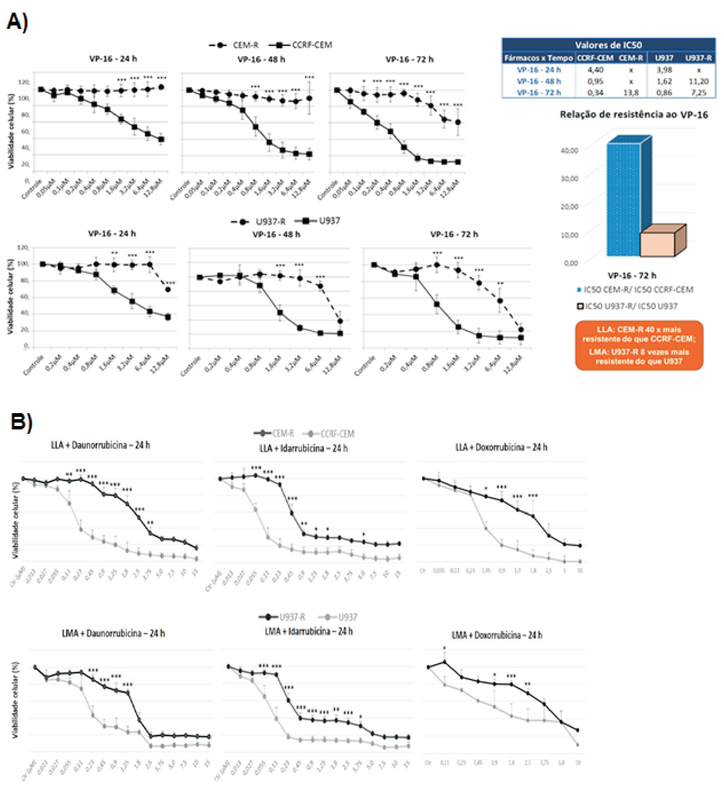


Figure 4: Development and characterization of acute leukemias resistant cells. Acquired resistance to: A) etoposide (V-16); B) anthracyclines: daunorubicin, idarubicin and doxorubicin.

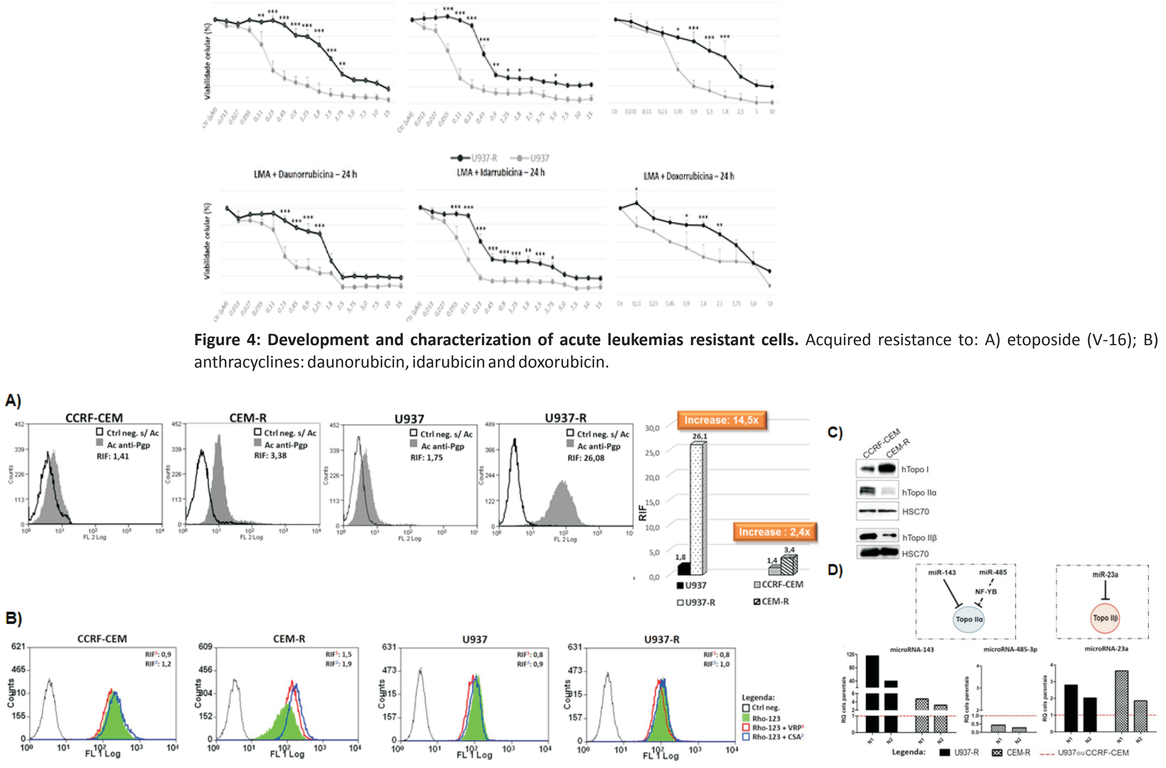
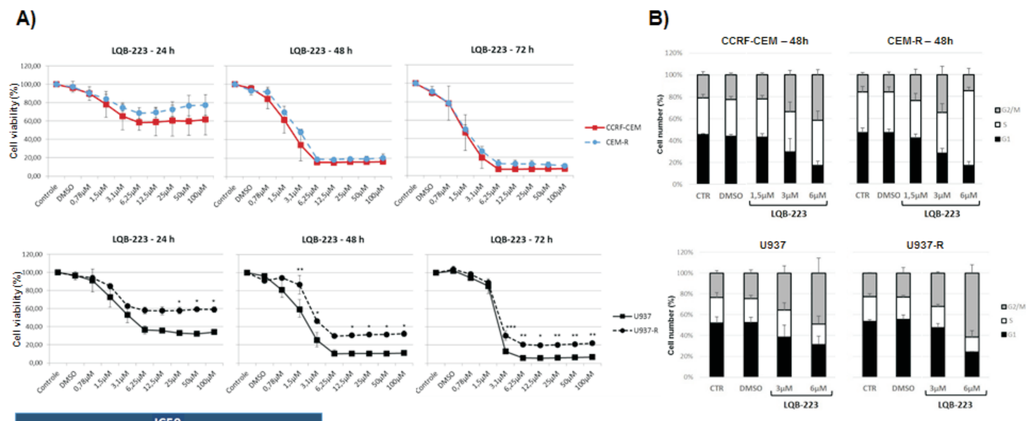


Figure 5: Evaluation of the multidrug resistance (MDR) phenotype of acute leukemias cells by: A) Pgp expression levels; B) Pgp drug efflux transport activity; C) Topoisomerase I and II proteins expression levels; D) Topoisomerases-targeted microRNAs expression levels.



IC50				
	CCRF-CEM	CEM-R	U937	U937-R
LQB-223 - 24 h	x	x	3,23	x
LQB-223 - 48 h	1,94	2,57	1,81	2,88
LQB-223 - 72 h	1,42	1,51	2,10	2,21

Figure 6: Analysis of the acute leukemias cells treatment with LQB-223 by: A) cell viability analysis; B) cell cycle analysis.

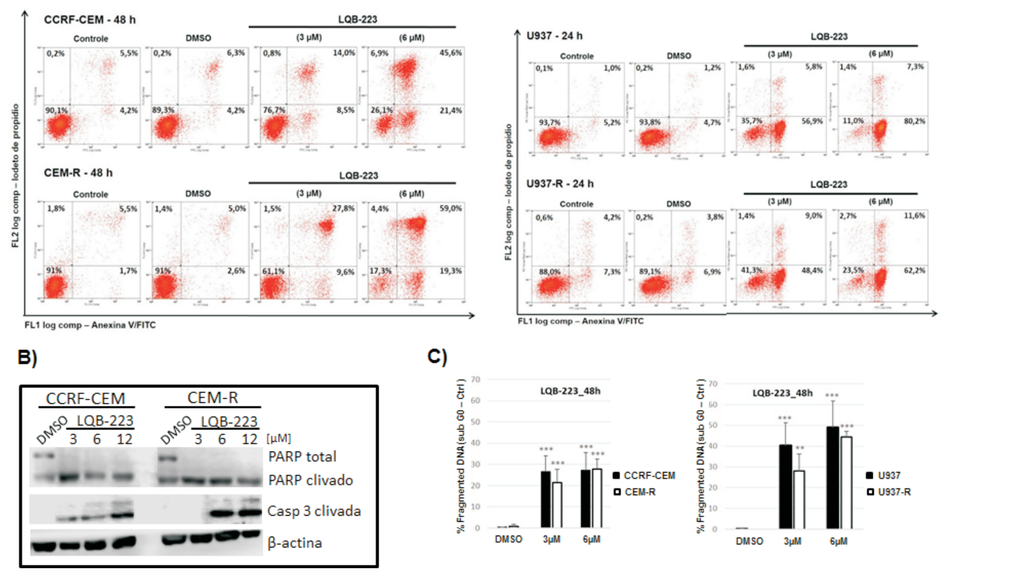


Figure 7: Detection of cell death induction after LQB-223 treatment of acute leukemias cells by: A) Anexin V/Pi detection; B) Apoptotic proteins detection; C) Fragmented DNA detection.

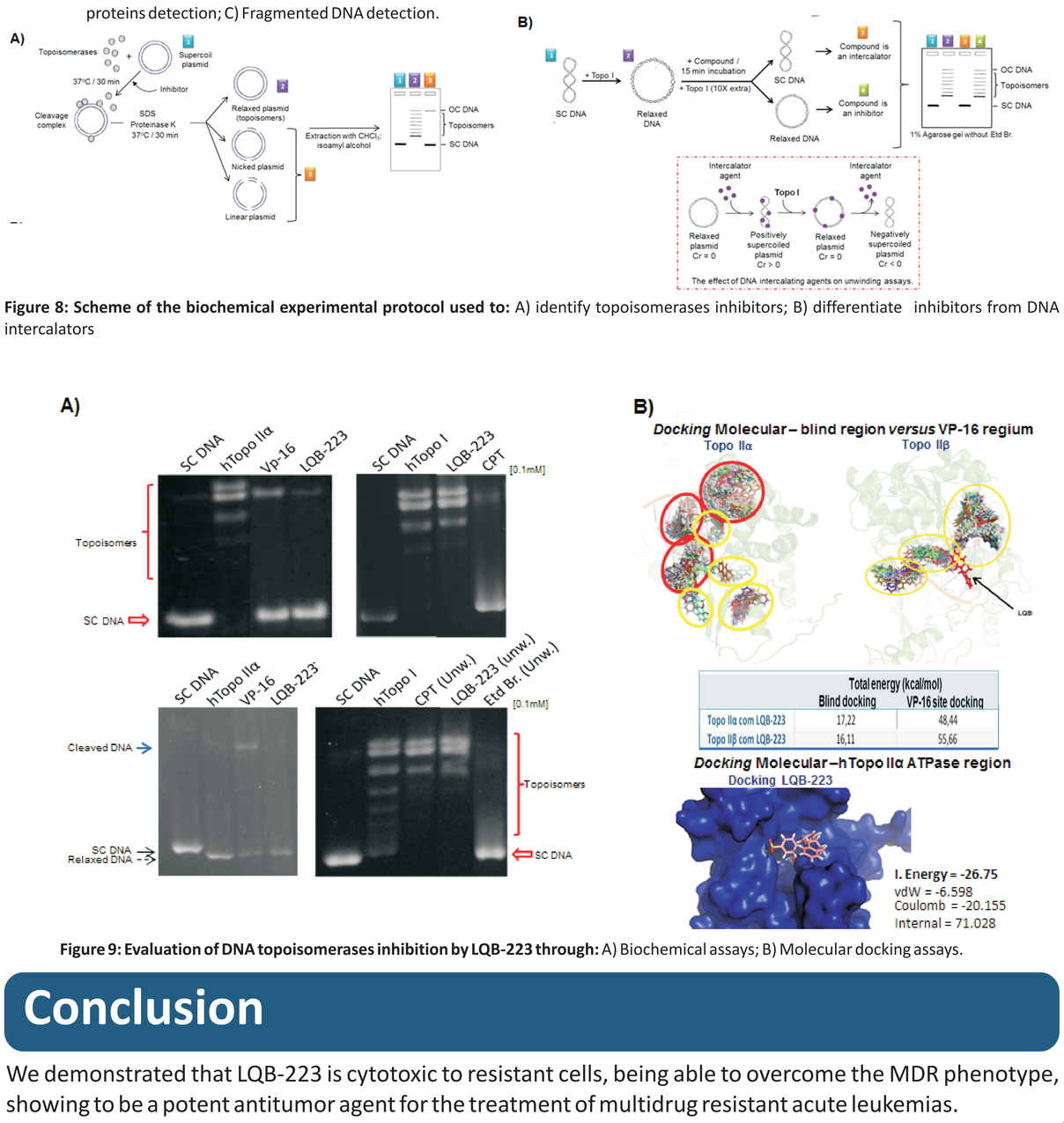


Figure 8: Scheme of the biochemical experimental protocol used to: A) identify topoisomerases inhibitors; B) differentiate inhibitors from DNA intercalators

Figure 9: Evaluation of DNA topoisomerases inhibition by LQB-223 through: A) Biochemical assays; B) Molecular docking assays.

Conclusion

We demonstrated that LQB-223 is cytotoxic to resistant cells, being able to overcome the MDR phenotype, showing to be a potent antitumor agent for the treatment of multidrug resistant acute leukemias.

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos/ Seitec / INCA