INVESTIGATION OF THE MECHANISM OF ACTION OF PTEROCARPAN AND PTEROCARPANQUINONES NEW COMPOUNDS IN ACUTE LEUKEMIA CELL LINES SENSITIVE OR RESISTANT TO ETOPOSIDE

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INTRODUCTION

The multidrug resistance (MDR) is an obstacle for the treatment of acute leukemias and the main cause of refractoriness to treatment with topoisomerase inhibitors. Tumors overexpress topoisomerases, leading these enzymes as good targets for the development of new anticancer drugs. In this context, the development of new compounds effective on cells with MDR phenotype is necessary.

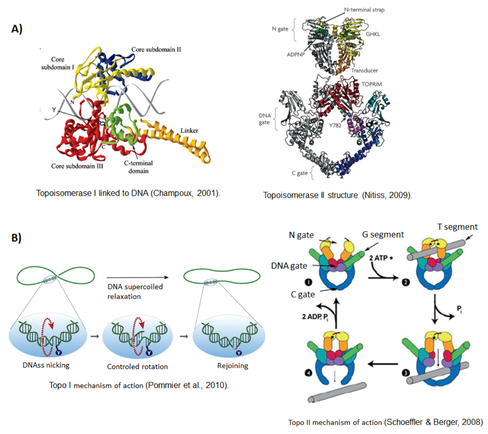


Figure 1: DNA topoisomerases scheme. A) Structure of topoisomerase I (Topo I) and topoisomerase II (Topo II). B) Topo I and Topo II mechanism of action.

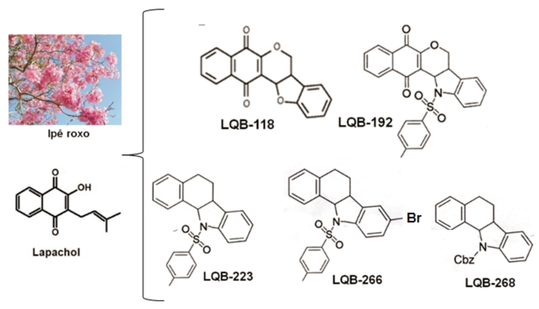


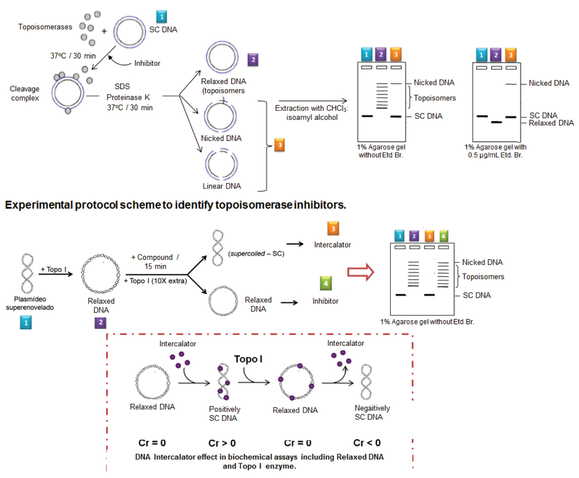
Figura 2: General structure of the synthetic compounds evaluated in this work: LQBs, initially structurally related to lapachol (LQB-118), an extracted natural *naphthoquinone of Tabebuia spp* plant. (Bignoniaceae), popularly known as "ipe".

OBJECTIVES

1)Evaluate the potential of LQBs compounds (LQB-118, -192, -223, -266, -268 and -326) as drug targeting human topoisomerases (hTopo I and IIα); 2)Develop acute leukemia cell lines resistant to etoposide (VP-16); 3)Investigate the effect of the most promising compounds in the induction of cell death and inhibition of Akt in cell lines sensitive and resistant to VP-16.

METHODS AND RESULTS

Molecular modeling docking, DNA relaxation and unwinding biochemical assays were used to investigate the interaction of LQBs to topoisomerases. Among 6 LQBs evaluated only LQB-118 and -223 were effective and also specific to hTopo IIα. They acted as catalytic inhibitors(with high affinity to bind to ATPase region of hTopo IIα), without intercalating into DNA. Acute lymphoid CEM-R and myeloid leukemia U937-R cell lines were exposed to increasing concentrations of VP-16 until they became, respectively, 40 and 8 times more resistant to VP-16 in comparison to parental cell lines. LQB-118 and -223 treatment reduced cell viability of both resistant and parental lineages. Flow cytometry was used to evaluate the level of MDR proteins, cell cycle profile and cell death after treatment with LQB-118 or LQB-223. All lineages presented functional BCRP protein and Pgp expression, however, resistant cell lines showed an overexpression of Pgp. Treatment with both LQBs altered the cell cycle (especially the LQB-223 that promoted stop at G2/M) and induced cell death. The level of miR-143 was analyzed by qRT-PCR and an upregulation of this miR was observed in both resistant cell lines. Western blot was used to access the expression of hTopo I and IIα, Akt, caspase-3 and PARP. Low levels of hTopo IIα protein was detected in resistant cells. LQB-118 treatment induced a decrease of Akt and it was not a consequence of apoptosis. Preliminary results suggested that LQB-118 is probably interfering with protein synthesis.



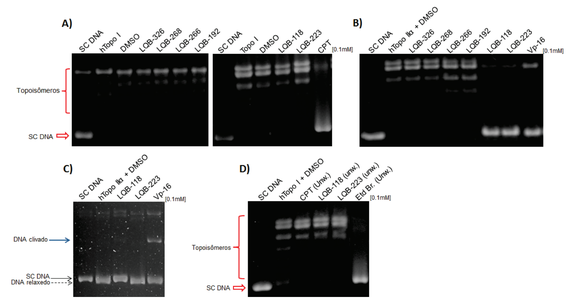


Figure 3: DNA topoisomerases inhibition evaluation. Relaxation DNA assay showing the effect of LQBs compounds on inhibition of (A) hTopo I and (B) hTopo IIα enzymes; C) Analysis of mechanism of hTopo IIα inhibition through differentiation of poison to catalytic inhibitors by DNA cleavage assay; D) Differentiation of topoisomerase inhibitors from DNA intercalators by unwinding assay.

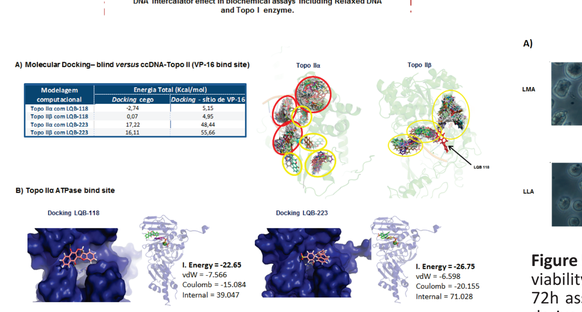


Figure 4: Theorical study of LQB-118 and LQB-223 biding in DNA topoisomerases. (A) Blind docking showing the areas with preferential interactions marked in yellow and red circles. (B) Molecular docking on Topo IIα ATPase bind site.

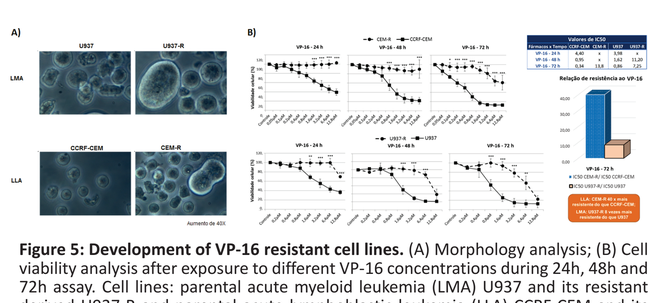


Figure 5: Development of VP-16 resistant cell lines. (A) Morphology analysis; (B) Cell viability analysis after exposure to different VP-16 concentrations during 24h, 48h and 72h assay. Cell lines: parental acute myeloid leukemia (LMA) U937 and its resistant derived U937-R and parental acute lymphoblastic leukemia (LLA) CCRF-CEM and its resistant derived CEM-R. Statistical analysis (ANOVA Followed by post-test Bonferroni) were performed comparing parental with resistant cell line (* p <0.05, ** p <0.01 and *** p <0.001).

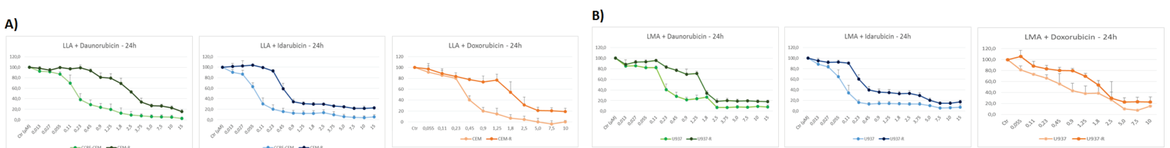


Figure 6: Characterization of resistance to different drugs. Cell viability assay after (A) LLA or (B) LMA exposure to daunorubicin, idarubicin or doxorubicin during 24h.

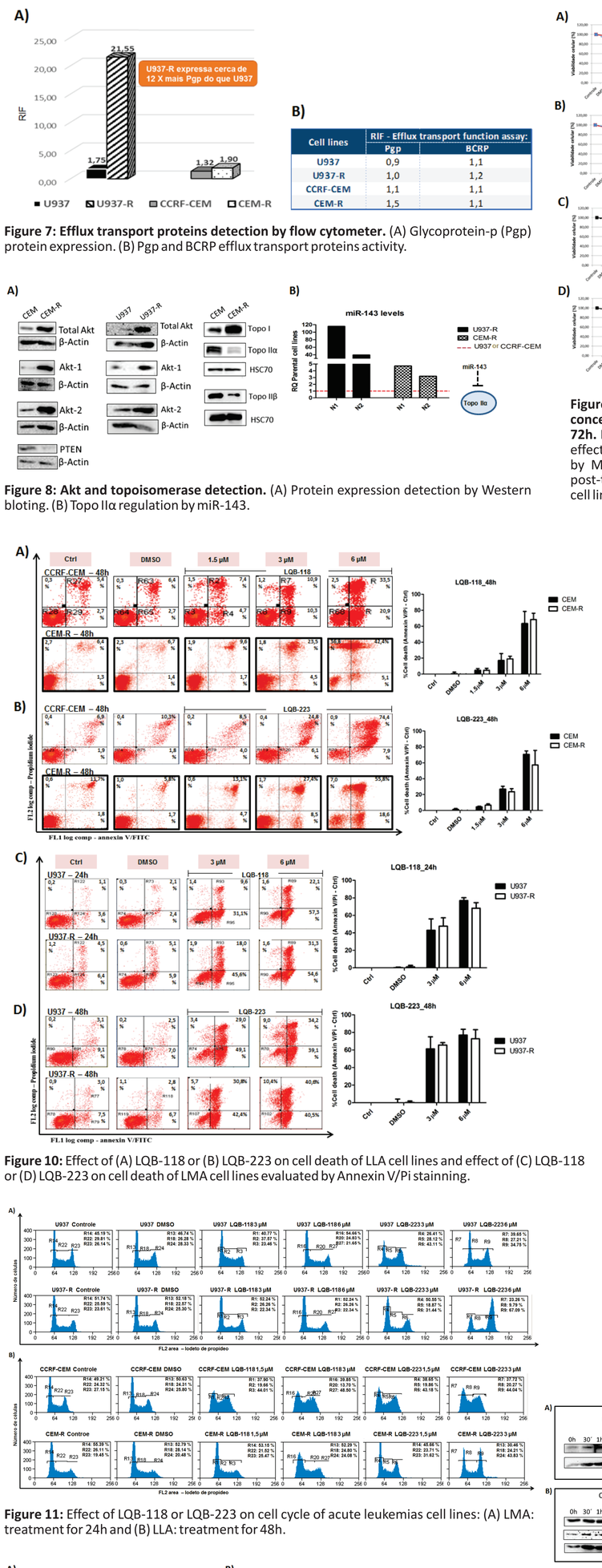


Figure 7: Efflux transport proteins detection by flow cytometer. (A) Glycoprotein-p (Pgp) protein expression. (B) Pgp and BCRP efflux transport proteins activity.

Figure 8: Akt and topoisomerase detection. (A) Protein expression detection by Western bloting. (B) Topo IIα regulation by miR-143.

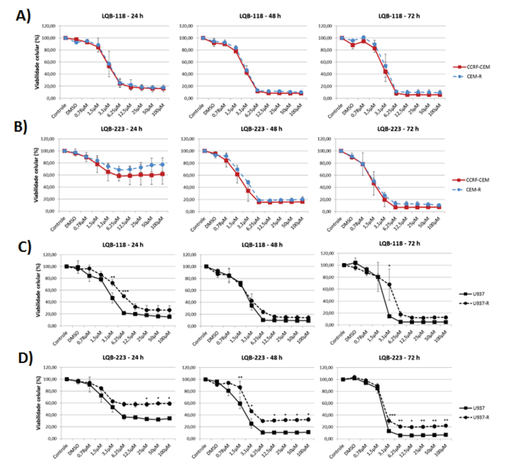


Figure 9: Cell viability analysis after exposure to different concentrations of LQB-118 or LQB-223 during 24h, 48h and 72h. Effect of (A) LQB-118 or (B) LQB-223 on LLA cell lines and effect of (C) LQB-118 or (D) LQB-223 on LMA cell lines evaluated by MTT. Statistical analysis (ANOVA followed by Bonferroni post-test) were performed comparing parental with resistant cell line (* p <0.05; ** p <0.01 and *** p <0.001).

Figure 10: Effect of (A) LQB-118 or (B) LQB-223 on cell death of LLA cell lines and effect of (C) LQB-118 or (D) LQB-223 on cell death of LMA cell lines evaluated by Annexin V/Pi stainning.

Figure 11: Effect of LQB-118 or LQB-223 on cell cycle of acute leukemias cell lines: (A) LMA: treatment for 24h and (B) LLA: treatment for 48h.

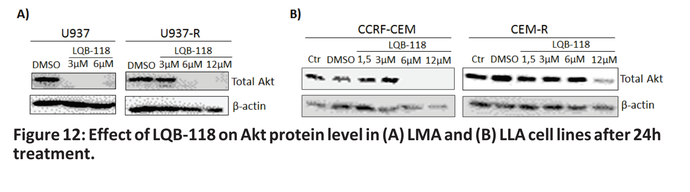


Figure 12: Effect of LQB-118 on Akt protein level in (A) LMA and (B) LLA cell lines after 24h treatment.

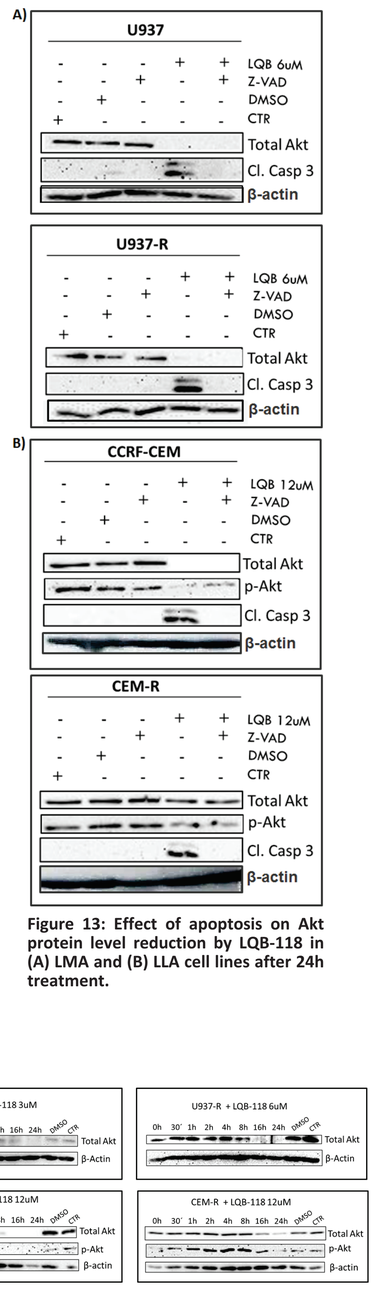


Figure 13: Effect of apoptosis on Akt protein level reduction by LQB-118 in (A) LMA and (B) LLA cell lines after 24h treatment.

Figure 14: Effect of time on Akt protein level reduction by LQB-118 in (A) LMA and (B) LLA cell lines after 24h treatment.

CONCLUSION

Our results suggest that LQB-118 and -223 are potential antitumor compounds for the treatment of acute leukemias including the MDR phenotype.

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