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Anti-proliferative Activity of the Combination of Salan Ti(IV) Complexes with Other Organic and Inorganic Anticancer Drugs Against HT-29 and NCI-H1229 Cells: Synergism with Cisplatin

Supplementary Information

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Experimental Section

The ligands L^1H_2 and L^2H_2 and their complexes $L^1Ti(OiPr)_2$ and $L^2Ti(OiPr)_2$ were synthesized as previously described.[1] Azatoxin was synthesized as previously described.[2] Camptothecin and cis-dichlorodiammine platinum (II) 99% were purchased from Acros.

Cytotoxicity was measured on a HT-29 colon and NCI-H1229 non-small cell lung cancer cells obtained from ATCC Inc. using the MTT assay as previously described.[3] Approximately 0.9×10^6 cells in medium (contains: 1% penicillin/streptomycin antibiotics; 1% L-glutamine; 10% fetal bovine serum (FBS) and 88% medium RPMI-1640, all purchased from Biological Industries Inc.) were seeded into a 96-well plate (approximately 13640 cells per well) and allowed to attach for a day. The cells were consequently treated with the reagent or combination of reagents tested at 10 different concentrations. Salan titanium(IV) complexes and azatoxin were administered in THF, cisplatin and CPT in DMSO, and combinations accordingly in the corresponding mixtures of solvents. After a standard of 3 days incubation at 37 °C in 5% CO_2 atmosphere, MTT (0.1 mg in 20 µL) was added and the cells were incubated for additional 3 hours. For experiments where one compound was inserted after the other, the incubation time was measured starting from the first administration. After the incubation period, the MTT solution was then removed, and the cells were dissolved in isopropanol (200 µL). The absorbance at 550 nm was measured by a Bio-Tek EL-800 microplate reader spectrophotometer. Relative IC_{50} values were determined by a nonlinear regression of a variable slope (four parameters) model by Graph Pad Prism 5.0 program, with error values based on the STD of the repetitions.

The interactions between the combined reagents were evaluated with the isobolographic method,[4] using OriginPro 8 program.

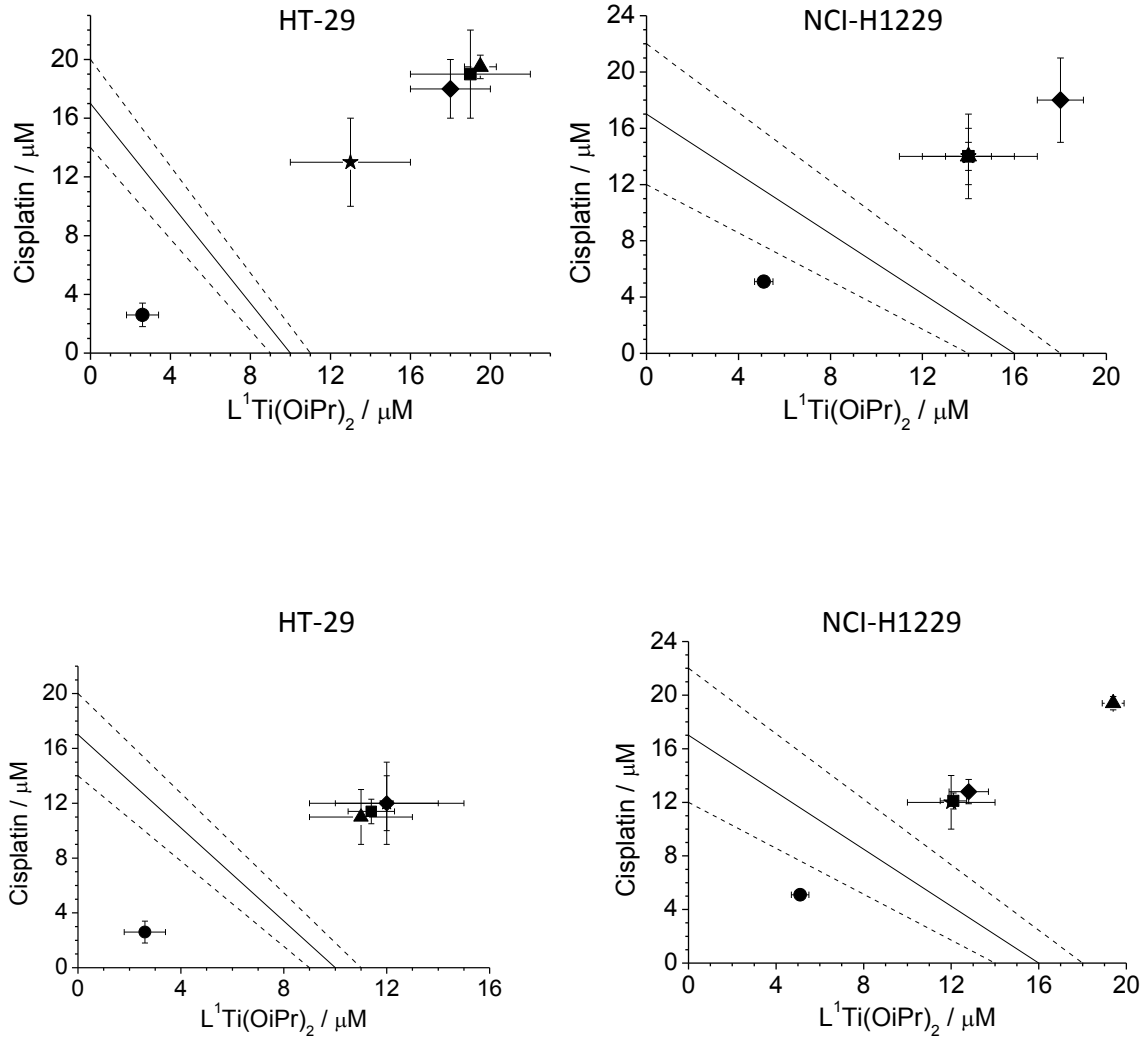

Fig. 1: Isobolographic analysis of the anti-proliferative activity of the combination of $L^1Ti(OiPr)_2$ and cisplatin against human colon HT-29 and non-small cell lung NCI-H1229 cells; top: where the cells were treated first with $L^1Ti(OiPr)_2$ and then with cisplatin; bottom: where the cells were treated first with cisplatin and then with $L^1Ti(OiPr)_2$; with varying time intervals in between: ●-0h, ■-1h, ▲-2h, ◆-5h, ★-24h; with replacement of medium prior to addition of the second drug.

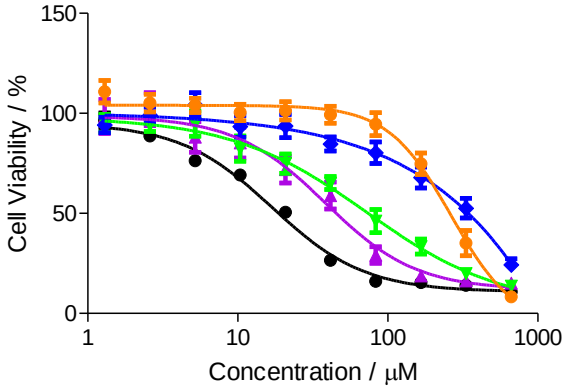

Fig 2.: Dose-response curves of cisplatin administered following ●-0, ▲- 1, ▼- 2, ◆- 5, and ●- 24 hours in DMSO, against human colon HT-29 cancer cell line (3 day incubation period; based on 3 times 3 repetitions)

references

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