

EVALUATION OF NAPHTHOQUINONES MANNICH BASES AGAINST ORAL SQUAMOUS CELLS CARCINOMA

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Introduction

Oral squamous cells carcinoma (OSCC) is the sixth most incident cancer in men worldwide. The survival rate for OSCC is around 5 years and the prognosis has exhibited little improvement in the last three decades. Among the drugs mostly used for the treatment are carboplatin and cisplatin. However, in addition to the high costs of the treatment for nephrotoxicity side effects, acquired resistance have been associated with these chemotherapeutics. Thus, the search for more effective antineoplastic agents is justified. The present study aims to show the synthesis of eighteen Mannich bases synthetic naphthoquinone compounds and testing them on OSCC models. A series of experiments were performed *in vitro* on OSCC lines and normal cells, and *in vivo* assays on mice (*Mus musculus*).

Method

Compounds synthesis occurred from a multicomponent reaction between lawsone, benzylamine and different aromatic aldehydes generating Mannich adducts in yields ranging from 83-98%. Then, treatment of the intermediates with methyl chloroformate in potassium carbonate and THF medium produced the methylcarbamates. Cytotoxicity and selectivity were evaluated by MTT assays in OSCC (SCC4, SCC9, SCC25) and fibroblast. The following assays were also carried out: hemolysis assays; clonogenic assay; *in vivo* acute toxicity (CEUA: 982); *in silico* prediction; molecular docking; pyknotic nucleus assay; caspase inhibitor assay; necroptosis inhibition; autophagy inhibition.

Results / Discussion

Among the eighteen 1,4-naphthoquinones tested, only the methyl benzyl (4-chlorophenyl) (3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl carbamate (MB10) demonstrated a selectivity index higher than 2 (2,36) (SI: IC₅₀ on normal cells / IC₅₀ on tumor cells) considering the 3 strains of OSCC (SCC9; SCC4 and SCC25) and normal mouth cells (human fibroblasts) this proved to be more selective than the treatment used in the clinic with carboplatin (IS: 0,847).

The most selective substance (MB10) reduced the formation of clones in SCC9 via clonogenic assay. The tests for reactive oxygen species (ROS) demonstrated that the substance MB10 did not present a good redox capacity in SCC9. The absence of cell death due to apoptosis was confirmed by tests of pyknotic nucleus and inhibition of caspases. *In vivo* tests showed that the LD₅₀ of MB10 was around 150 mg/kg and signs of toxicity were observed in the lungs and kidneys in higher concentrations. *In silico* predictions of MB10 indicated that its chemical and biological characteristics can provide a good drug profile and the computational target fishing strategy used indicated that the MB10 could exert its anticancer action by inhibiting topoisomerase I and II β and hPKM2. Finally, cell death assays showed that MB10 does not induce cell death by apoptosis and necroptosis, preliminary results indicate a possible cell death by autophagy.

Conclusion

The results indicated that 1 of the 18 Mannich bases synthetic naphthoquinone compounds has a good profile for the treatment of OSCC. Additionally tests are needed to elucidate the possible cell death by autophagy.

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