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Pathway toward catastrophe: cellular dynamics on hit-to-lead compound optimisation for current drug discovery pipelines

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Microtubules (MTs) are intracellular polymers that act as fundamental components of eukaryotic cells. Recent advances in protein purification techniques have begun to shed light on the importance of MTs intrinsic dynamic properties such as growth and shrinkage—both regulated by a cohort of molecular motors and microtubule-associated proteins. The dynamic instability of the MT cytoskeleton is crucial for cell division and motility, core processes that underpin abnormal behaviour in cancer development. (1) Within this framework, we have been carrying out in Brazil a PhD project entitled "Discovery of New Bioactive Ligands with Anticancer Properties" (FAPESP grant 2018/25289-7), working on MT-targeting agents for the treatment of triple-negative breast and hormone-refractory prostate cancers—both of which are metastatic. From a set of thirty-two initial compounds, ten passed the screening involving a series of assays: cell proliferation, migration, and invasion; experimental determination of pharmacokinetic parameters; tubulin polymerisation and site competition. The compounds' effect on tubulin polymerisation was found to be inhibitory, i.e., they promote destabilisation by favouring the switch from growing to shrinking ("catastrophes") at MT assembly in the colchicine binding site. (2) That said, here we describe the research proposal outcomes of the effects of these hit-compounds on MT dynamics, during a Research Internship Abroad programme under the supervision of Prof. Dr. Anna Akhmanova at Utrecht University. For those samples, using time-lapse microscopy, kymographs were generated to annotate and quantify MT dynamics both in cells and in *in vitro*.reconstitutions. At Prof. Akhmanova's laboratory, the conjunction of cellular expertise and advanced microscopy made possible to perform several experiments that were significant for our already screened compounds, in order to select those that will be forwarded for pre-clinical trials with animal models and thus, to elect the lead ones. Here, we used live-cell imaging and *in vitro*.reconstitution assays to show that the **cis-2e**.indolizine lactone and **para-3c**.quinazoline-chalcone hybrid indeed inhibit MT polymerisation and they are the most potent representative for each respective chemical series. Both induced catastrophes in cells stably expressing EB3-GFP and led to a decrease in the growth rate (Gr) [**Gr(cis-2e)**. 6.76 ± 0.44; **Gr(para-3c)**. 8.15 ± 0.44] and an increase in the catastrophe frequency (Cf) [**Cf(cis-2e)**. 8.92 ± 0.64; **Cf(para-3c)**. 7.38 ± 0.39] in comparison with the negative control (nc) and the vehicle DMSO 0.1% (v) [**Gr(nc)**. 20.59 ± 1.95; **Gr(v)**. 20.98 ± 1.10; **Cf(nc)**. 2.94 ± 0.25; **Cf(v)**. 2.87 ± 0.15]. Furthermore, in *in vitro*.reconstitution assays, they led to a mild decrease in the growth rate [**Gr(cis-2e)**. 1.46 ± 0.17; **Gr(para-3c)**. 1.97 ± 0.22] whilst resulted in more frequent catastrophes [**Cf(cis-2e)**. 1.75 ± 0.11; **Cf(para-3c)**. 0.82 ± 0.10] in comparison with the negative ones [**Gr(nc)**. 2.91 ± 0.45; **Gr(v)**. 2.66 ± 0.20; **Cf(nc)**. 0.49 ± 0.05; **Cf(v)**. 0.51 ± 0.07]. Our results suggest that the novel compounds

contribute to the non-maintenance of microtubule integrity, with values tending towards the positive control colchicine (col) in both assays [cells: **Gr(col)**. 6.07 ± 0.63; **Cf(col)**. 9.99 ± 1.04 / *in vitro*. **Gr(col)**. 1.59 ± 0.17; **Cf(col)**. 1.72 ± 0.11]. (3)

Palavras-chave: Cancer. Cellular dynamics. Microtubules.

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