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Search of new proteasome inhibitors in freshly prepared pre-fractionated Brazilian plant natural products libraries

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Abstract: Natural products continue representing the most important source of hits and leads in modern drug discovery [1]. Considering this potential, natural products have emerged in drug discovery, including efforts on high throughput screening (HTS). Pre-fractionated natural product libraries have been pointed as a valuable type of natural product libraries for HTS, regarding either time for their preparation, costs and efficiency for HTS and hit identification [2]. The proteasome complex is a validated target for anti-cancer chemotherapy [3]. The currently available proteasome inhibitors display several problems as high toxicity, low absorption and distribution, as well as development of resistance [4]. Therefore, better-designed proteasome inhibitors are needed. Natural products are the main sources of proteasome inhibitors, and have revealed new classes and enzyme inhibition mechanisms, which are useful for proteasome drug design [5]. Brazilian natural products however, have not been in deep explored as sources of proteasome inhibitors. In this context, this work aimed to discover new proteasome inhibitors from freshly prepared pre-fractionated Brazilian plant natural product libraries. Plants were collected in Brazilian biomes, processed in crude extracts and submitted to automated pre-fractionation using semi-preparative reverse phase LC-UV. Crude extracts and fractions were allocated in 384 plates using standardized concentrations and submitted to biological evaluation. We then carried out a target-based HTS assay using the human 20S proteasome and screened the first 1,000 samples. The hit rate was 1% (hit cutoff= 50% enzyme inhibition). These were further validated and characterized. Concentration-response curves were carried out allowing us to extract the IC_{50} values of each hit. Additionally, time-dependence inhibition experiments were conducted to check for reversible or irreversible h20S inhibitors. We detected 4 fractions exhibiting reversible 20S proteasome inhibition in the low µg/ml range. Hits were then analyzed by UPLC-PDA-MS and candidate chromatographic peaks were selected. MS information was used for searching public databases as an initial approach for finding candidate active substances. We found known and unknown proteasome inhibitors in this search. We then proceeded to bioguided isolation of one of the known inhibitors as a proof of concept of the methods applied for screening and indication of the active substance. The next steps will be to proceed to the isolation of the unknown proteasome inhibitors. For further perspectives, we are aiming to include MS/MS based molecular networking [6] as a dereplication strategy for hit prioritization – prior to isolation - focusing in the discovery of new natural products exhibiting biological activity.

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