A natural oxadiazine isolated from cyanobacteria kills cancer cells in multicellular culture systems by impairing cellular respiration

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State of Art

A more spread and low cost-effective genomic techniques and high-throughput screening allied to the crescent need of new drugs has triggered a new impulse to the discovery of natural resources

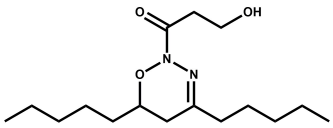
Cyanobacteria exhibit the presence on non-ribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) genes

Anti-cancer compounds isolated from Cyanobacteria

Antitubulin agent Brentuximab vedotin (SGN35) for Hodkin's lymphoma – FDA Approved, Depatuxizumab mafodotin(ABT-414) for Glioblastoma, Pediatric Brain Tumors - Clinical Trials phase III

Assessement of anti-cancer activity *in vitro* using 3D models are more representative of tumour environment, easier to sieve promising compounds and HTS is already possible.

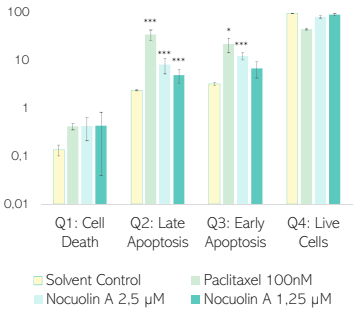
Cyanobacterial Oxadiazine: Nocuolin A



Isolated from *Nodularia sp.* LEGE 06071
Bioguided assay – MTT assay in 2D cultures
First time seen a natural azole ring with N-N-O linkage
IC50 of 1,35 µM was calculated for HCT116 (colon cancer cell line)

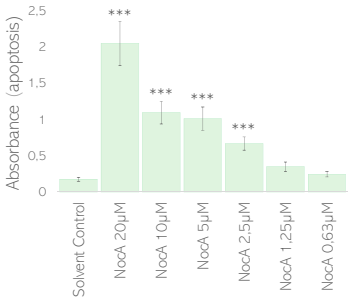
What makes a Nocuolin A a plausible antitumoral candidate?

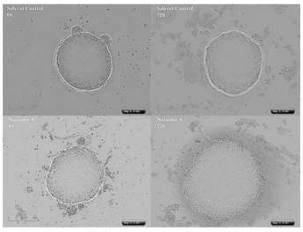
Flow cytometry with Annexin V/ PI on HCT116 2D culture
→ Apoptosis was verified



Effects against immortalized epithelial cell line hTERT RPE-1
→ Low cytotoxicity

Effects on 3D spheroids of HCT116 cell monitored over 72h
→ Apoptosis was verified





What is Nocuolin A mode of action?

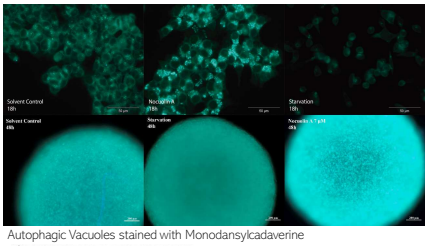
1 RNA next generation sequencing 2D

C-MAP analysis:
NocA molecular signature has similarities with a wide broad C-Map classes, revealing no similarity with known compounds regarding its molecular pathway

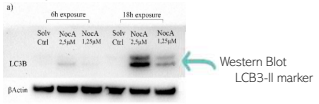
GSEA analysis reveal alterations in:
Cell regulation
Immune response
Stress response
Starvation

2 Follow GSEA results…

✗ Cell regulation:
No alterations on cell cycle using FACS
✗ Immune response:
No expression of Nrf2 in HCT116 reporter cells as oxidative stress marker
✗ ER stress:
No significant phosphorylation of translation initiation factor (elF2α)
✓ Starvation:
Autophagy

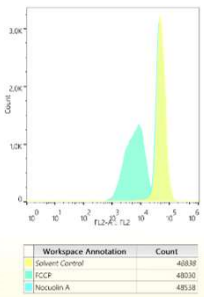


Autophagic Vacuoles stained with Monodansylcadaverine

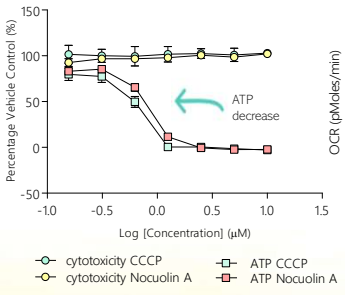


Western Blot LCB3-II marker

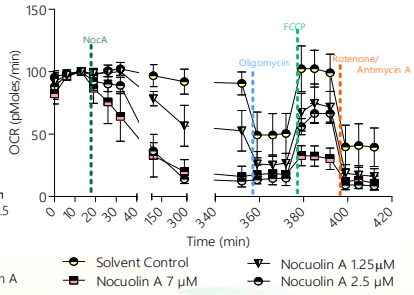
3 Mitochondrial membrane potential



4 ATP content



5 OXPHOS impairment



FINAL REMARKS

1. High cytotoxicity on carcinogenic cells grown as 2D and 3D
2. Cytotoxicity of NocA could be related to a starvation response, involving autophagy and metabolic inhibition on proliferating cells
3. Impairment of mitochondrial respiration and non-mitochondrial respiration rates were more catastrophic in carcinogenic vs non-carcinogenic cells, which may relate to a higher energy demand and different proliferation rates.

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