Establishing the Secondary Metabolite Profile of a Marine Fungus: *Tolypocladium geodes* MF458

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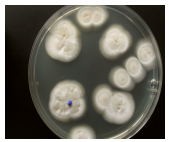
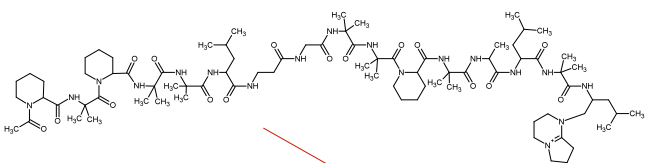
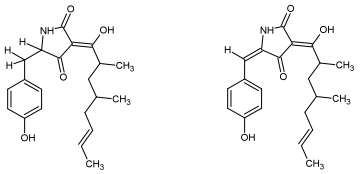
The aim of *Marine Fungi* is to evaluate the potential of secondary metabolites from fungi associated with marine macro-organisms to provide lead compounds for the development of cancer treatments. Extracts from fermentations of fungi isolated from Mediterranean sponges, Indonesian corals and Chilean macroalgae were screened against a preliminary cell line panel comprising the 786-0, M14 and MCF-7 tumour cell lines. Extracts from fermentations of the sponge-associated strain MF458, subsequently identified as *Tolypocladium geodes*, were found to have potent anti-tumour effects with predominantly anti-proliferative rather than overtly cytotoxic profiles. Assay-guided purification and structural characterisation has revealed the presence of compounds expressed by five different biosynthetic pathways. While some of these have been identified as compounds known to have effects on mammalian cells – pyridoxatin, cyclosporins and efrapeptins – other minor components are new compounds that have not previously been described, and their anti-tumour properties are being assessed.



Tolypocladium geodes MF458 was isolated from a Mediterranean sponge (*Tethya aurantium*) and identified by morphological and molecular characterisation. The strain was cultivated in a variety of media in order to characterise the metabolite spectrum of this strain. The best antitumour activity in extracts was found following cultivation on Wickerham medium containing 30 g/L tropic marine salt.

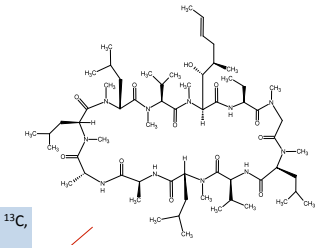


- Efrapeptin D or J. Efrapeptins have potent anti-tumour effects resulting from inhibition of mitochondrial F1F0-ATPase and down-regulation of HSP90 chaperone activity.[1-3]

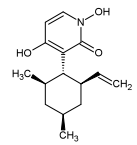


MF458-7 MF458-8

- Novel acyltetramates: structures elucidated by interpretation of ^{1}H, ^{13}C, COSY, HSQC, HMBC and NOESY NMR spectra
- MF458-7 showed more tumour growth inhibitory potency than MF458-8

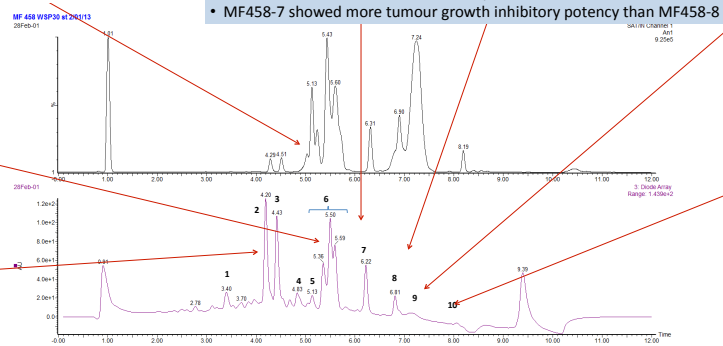


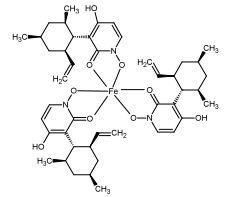
- Major metabolite present in these fermentation: cyclosporin A
- Known *Tolypocladium* spp. metabolite.[6]



- Pyridoxatin: first isolated from *Acremonium* sp. BX-86[4] and subsequently from *T. geodes*.[5] Known to be cytotoxic to HeLa cells

- MF458-2&3
- New molecules (MWs <300).
- Anti-tumour potential under evaluation

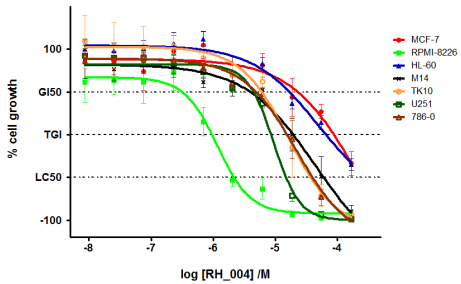




- Terricolin: (pyridoxatin)$_3$Fe complex known to be produced by *T. geodes*.[5]

HPLC-MS analysis of ethyl acetate extract of MF458 Fermentation: ELSD chromatogram (top), diode array UV-visual chromatogram (200-500 nm) bottom.

Pharmacological Relevance NCI60 panel



- Compound A is known selective kinase inhibitor against VEGFR2
- RPMI-8226 cells express VEGFR2
- NCI- panel wide screen gives provisional indication of underlying target

Non-Cancer cell line control (W138 fibroblast, lung epithelium) GI_{50} > 100uM

Growth Inhibitory Effects (GI_{50}s in µM) of MF458 Compounds on Selected Human Tumour Cell Lines

Cell Line	MF458-2	MF458-3	MF458-4 Efrapeptin D or J	MF458-6 Pyridoxatin	MF458-7 New acyl-tetramate	MF458-9 Cyclosporin A
786-0	22	17	-	4	75	-
DU-145	41	32	-	5	106	12
HL60	26	14	-	4	130	-
M14	14	17	1.4	4	150	12
MCF-7	16	14	0.05	0.9	56	12
UO-31	53	35	-	5	71	20
SF539	19	9	1.6	5	150	11
TK10	25	7	0.5	15	140	14
MDA-MB-468	18	6	-	0.8	110	4
OVCAR-3	15	7	-	0.7	56	-

For assay methods, see reference.[7]

Discusssion

Tolypocladium geodes MF458, isolated from the sponge *Tethya aurantium*, is a prolific producer of secondary metabolites and we have purified and structurally characterised the compounds with anti-tumour effects expressed by five different biosynthetic pathways under one particular set of fermentation conditions. Compounds produced by employing different fermentation conditions represent a future avenue of exploration. *Tolypocladium* spp. have attracted significant attention as producers of bioactive secondary metabolites. The efrapeptins,[1] pyridoxatin[5] and terricolin[5] have previously been reported as products of terrestrial isolates of *T. geodes*, while production of cyclosporins is usually associated with other *Tolypocladium* spp.[6] Metabolites from marine *Tolypocladium* spp., such as the new efrapeptin J, are also being reported.[2]

Extracts of MF458 fermentations had potent anti-tumour effects with a predominantly anti-proliferative activity profile. We used an assay-guided purification approach to characterise the active compounds. The most potent activities found were due to compounds known to have anti-tumour effects: efrapeptins and pyridoxatin.

We also found new molecules. The novel acyltetramates MF458-7 and MF458-8 only had very moderate anti-tumour potency. MF458-2 and MF458-3 have more potent effects and are being evaluated further. This work shows that new marine isolates of even previously well-researched species have the potential to produce new compounds with potentially useful biological activities.

References

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