Discovery of Protease Inhibitors from Marine Cyanobacteria Targeting Breast Cancer

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Introduction

Proteases are involved in the regulation of many physiological processes and their overexpression and dysregulated activity are linked to many diseases including, but not limited to, cancer. Marine cyanobacteria are known to produce modified peptides with propensity to inhibit various proteases with different selectivity profiles. A group of cyanobacterial protease inhibitors contain a characteristic statine (γ-amino-β-hydroxy acid) unit and was found to confer activity towards aspartic proteases. Another group of cyanobacterial secondary metabolites characterized by a 16-membered ring cyclic depsipeptide scaffold bearing the 3-amino-6-hydroxy-2-piperidone (Ahp) moiety is well recognized for its serine protease inhibitory activity.

Experimental

Samples of marine cyanobacteria were collected from Cetti Bay, Guam and Kemp Channel, Florida Keys. The freeze dried samples were subjected to extraction, solvent partitioning, and silica chromatography. Pure compounds were purified by reversed-phase high performance liquid chromatography (HPLC). Structures were elucidated using NMR spectroscopy and mass spectrometry (MS). The absolute configuration was established by enantioselective HPLC-MS. In terms of biological assays, protease inhibition assays, in vitro cleavage of recombinant proteins, ELISA, and migration assays were carried out.

Results and Discussion

Our efforts exploring the cyanobacteria collected from Cetti Bay and Kemp Channel have led to the discovery of grassystatins D−F (**1**−**3**) and kempopeptin C (**4**), respectively (Fig. 1). The antiproteolytic activity of (**1**−**3**) was evaluated against cathepsins D and E and **3** was the most potent analogue with IC_{50} values of 50 and 0.5 nM, respectively. Compound **4** was tested against trypsin, plasmin and matriptase and found to exhibit IC_{50} values of 0.19, 0.36 and 0.28 μM, respectively. Because cathepsin D is a biomarker in aggressive forms of breast cancer and linked to poor prognosis, the effects of cathepsin D inhibition by **1** and **3** on the downstream cellular substrates cystatin C and PAI-1 were investigated. Grassystatin F (**3**) inhibited the cleavage of cystatin C and PAI-1, the activities of their downstream targets cysteine cathepsins and tPA, and the migration of the highly aggressive triple negative breast cancer cells. Due to the significance of matriptase in cancer progression and metastasis, the effects of **4** on the downstream cellular substrates of matriptase: CDCP1 and desmoglein-2 (Dsg-2) were investigated. Kempopeptin C (**4**) was shown to inhibit the cleavage of both substrates in vitro and reduced the cleavage of CDCP1 in MDA-MB-231 cells up to 10 μM. Additionally, **4** inhibited the migration of the invasive MDA-MB-231 cells by 37 and 60% at 10 and 20 μM, respectively.

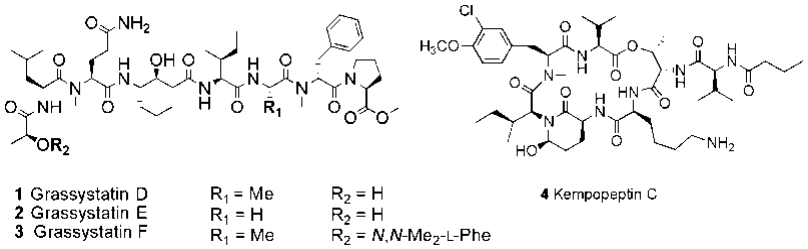

Fig.1 Structures of grassystatins D–F (**1**–**3**) and kempopeptin C (**4**)

Conclusions

The implication of dysregulated protease activity in cancer progression has been extensively studied and highlights the importance of proteases as therapeutic targets. The discovery of grassystatins D−F (**1**−**3**) and kempopeptin C (**4**) add to the growing family of cyanobacterial protease inhibitors. These classes of compounds might be developed into probes to further investigate the biology of cathepsin D/E and matriptase mediated processes and serve as a starting point for the design and development of more potent and selective leads with therapeutic potential.

Acknowledgements

The research was supported by the National Institutes of Health grant R01CA172310.

References

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[2] Al-Awadhi, F. H., *et al*., J. Nat. Prod., in press (2017).