Discovery, Synthesis, Pharmacological Profiling and Biological Characterization of Brintonamides A–E, Novel Dual Protease and GPCR Modulators from a Marine Cyanobacterium

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Introduction

Cancer metastasis is considered the major cause of death among cancer patients. Therefore, understanding the mechanisms involved in metastasis is critical for the development of treatment strategies to limit tumor progression. In addition to proteases, which have been extensively studied, recent studies report the involvement GPCRs in regulating the metastatic process of various tumors. Marine cyanobacteria are a rich source of bioactive molecules with diverse activities. In addition to being prolific producers of protease inhibitors, they often produce structurally diverse compounds that resemble endogenous ligands of GPCRs. Herein we describe the isolation, structure determination, and synthesis of five novel modified peptides and other structurally related analogues. Also, we report the biological evaluation of their dual protease and GPCR targeting activities and subsequently their effects on breast cancer migration.

Experimental

Samples of intertidal cyanobacterial mats (collected from Brinton Channel, Florida) were freeze dried, extracted and fractionated. Pure compounds were purified by HPLC. Structures were elucidated using NMR spectroscopy and mass spectrometry. The absolute configuration was established by enantioselective HPLC-MS. Biological evaluation involved protease and GPCR profiling, cell viability and migration assays.

Results and Discussion

Five novel modified linear peptides named brintonamides A–E (**1**–**5**) were discovered (Fig.1). The total synthesis of **1**–**5** in addition to two other structurally related analogues was achieved. The cancer related serine protease kallikrein 7 (KLK7) was inhibited to similar extents with an IC_{50} near 20 μM by both representative members **1** and **4**. In contrast to the biochemical protease profiling study, clear SAR was observed in the functional GPCR screens, where five GPCRs in antagonist mode (CCR10, OXTR, SSTR3, TACR2) and agonist mode (CXCR7) were modulated by brintonamides to varying extents. Chemokine receptor 10 (CCR10) was potently modulated by brintonamide D (**4**) with an IC_{50} of 0.44 μM. Due to the significance of CCR10 in cancer progression we demonstrated the ability of **4** at 10 μM to inhibit CCL27-induced CCR10-mediated proliferation and the migration of highly invasive breast cancer cells.

Conclusions

We demonstrated for the first time the discovery of cyanobacterial compounds with dual protease and GPCR modulatory activities, which may have a therapeutic potential in targeting invasive breast cancer. Given the significance of CCR10 in cancer progression and metastasis, future studies could be directed towards the design of improved small molecule antagonists of CCR10, which could be utilized as valuable probes to understand the downstream cellular pathways mediating the antimetastatic effects in breast cancer.

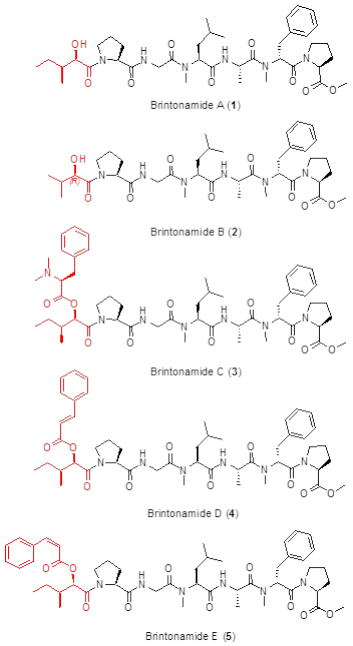

Fig.1 Structures of brintonamides A−E (**1**–**5**)

Acknowledgements

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References

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