SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF HYSTATIN 2 DERIVATIVES

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January 2016

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Marine sponge is a marine life that enrich with natural resources which is significant in medical development. Most of these are secondary metabolites produced by sponge used as defend mechanism against pathogenic bacteria, algae, fungi and other potential predator. The rapid evolution of virus has strongly withstand the equipped current pharmaceutical that cause continuous and consistently development of new anti-virus compounds from the marine sponge.Marine sponge contains a unique and potentially useful source of substitute nitrogen heterocyclic including a series of 1*H*-benzo[*de*][1,6]-naphthyridine skeleton. The parent naphthyridine known as 'aaptamines' was first isolated by Nakamura and co-worker in 1982. Aaptamine and their derivatives have contributed significantly toward biological activities.Hystatin is a group of compounds which formed from the derivatization of aaptamines. This group of compounds have been synthesized by Pettit (2004). Hystatin 1 was designed to stabilize isoaaptamine from oxidation. Hystatin 2 and hystatin 3 were prepared from aaptamine *via* alkylation reaction with benzyl bromide and 4-methoxybenzyl bromide, respectively.A series of hystatin 2 derivatives (**14, 88, 90** and**130-138**) have been synthesized *via* alkylation reaction at N-1 and/or N-4 position. The synthesized compounds were characterized using spectroscopic techniques, namely FT-IR, UV-Vis, ^{1}H and ^{13}C NMR and mass spectrometer. All of the compounds were evaluated for their antibacterial activities against selected bacterial strains of both Gram positive (*Bacillus cereus, Staphylococcus aureus, Micrococcus* sp) and Gram negative group (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*), using standard microbiological procedure of disc-diffusion and broth microdilution methods. The antibacterial activities of the compounds were assessed by the presence or absence of inhibition zone and minimum inhibition concentration (MIC) values, respectively.In general, from disc-diffusion test showed that hystatin 2 derivatives (**14,90** and**131-138**) have significant activity against Gram positive bacteria strain. However, compound **88, 130** and **136** did not have any activity against both Gram positive and Gram negative bacterial strains. Interestingly, compound **134** exhibited greatly significant activity against all tested bacteria. Further evaluation by broth

microdilution showed the same pattern of antibacterial activity as disc-diffusion where most of the compounds exhibited activity toward Gram positive. In addition, all of the compounds were active against *micrococcus* sp. and the minimum inhibition concentration (MIC) were observed at range 16.0-1.0 μg/ml. Meanwhile, no activity observed toward *Pseudomonasaeruginosa* except compound **14** and **137** at 500 μg/ml.

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SINTESIS, PENCIRIAN DAN AKTIVITI ANTIBAKTERIA TERBITAN HYSTATIN 2

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Span laut merupakan hidupan laut yang kaya dengan sumber semulajadi yang amat penting dalam pembangunan perubatan. Kebanyakan sumber semulajadi tersebut adalah metabolik sekunder yang dihasilkan sebagai mekanisma pertahanan terhadap bakteria patogenik, alga, kulat dan pemangsa lain yang berpotensi. Evolusi virus yang pesat masa kini mendorong pembangunan yang berterusan dan konsisten bagi menghasilkan sebatian anti-virus baru daripada span laut.Span laut mengandungi sumber yang berguna, unik dan berpotensi terdiri daripada gantian nitrogen heterosiklik termasuk struktur rangka 1*H*-benzo[*de*][1,6]-naftridina. Struktur induk naftridina dikenali sebagai 'aaptamine' yang pertama kali diasingkan daripada span laut oleh Nakamura dan rakan-rakan pada tahun 1982. Aaptamine dan terbitannya didapati telah memberikan sumbangan yang berguna terhadap aktiviti-aktiviti biologi. Hystatin merupakan suatu kumpulan sebatian yang terhasil daripada terbitan aaptamine. Terbitan hystatin pertama telah dihasilkan oleh Pettit et al. pada tahun 2004. Hystatin 1 telah direka bagi menstabilkan *iso*aaptamine daripada teroksida. Hystatin 2 dan hystatin 3 masing-masingnya telah dihasilkan daripada aaptamine melalui tindakbalas pengalkilan dengan benzil bromida dan 4-metoksibenzil bromida. Satu siri terbitan hystatin 2 (**14**, **88**, **90** dan**130-138**) telah dihasilkan melalui tindakbalas pengalkilan pada posisi N-1 dan/atau N-4. Sebatian yang telah disintesis dicirikan menggunakan kaedah spektroskopi iaitu FT-IR, UV-Vis, ^{1}H dan ^{13}C NMR dan Mass spektrometer. Sebatian yang telah disintesis dinilai untuk aktiviti anti-bakteria terhadap strain bakteria terpilih daripada kumpulan Gram positif (*Bacillus cereus, Staphylococcus aureus, Micrococcus* sp) dan Gram negatif (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*), menggunakan kaedah cakera resapan dan pencairan mikro mengikut protokol mikrobiologipiawai. Aktiviti antibakteria terbitan masing-masingnya dinilai dengan kehadiran atau ketiadaan zon perencatan dan nilai kepekatan perencatan minima (MIC). Secara umumnya, ujikaji daripada resapan cakera menunjukkan terbitan hystatin 2 (**14**, **90** dan **131-138**) mempunyai aktiviti antibakteria terhadap Gram positif. Walau bagaimanapun, sebatian **88**, **130** dan **136** tidak menunjukkan sebarang aktiviti terhadap kedua-dua

bakteria Gram positif dan Gram negatif. Menariknya, sebatian **134** mempamerkan aktiviti yang sangat berkesan terhadap kesemua bakteria yang diuji. Penilaian selanjutnya melalui kaedah pencairan mikro menunjukkan polar aktiviti antibakteria yang sama seperti ujian resapan cakera dimana kebanyakkan terbitan hystatin 2 menunjukkan aktiviti terhadap bakteria Gram positif. Tambahan pula, kesemua sebatian adalah aktif melawan bakteria *micrococcus* sp. dan menunjukkan kepekatan perencatan minima (MIC) pada julat 16.0-1.0 µg/ml.Sementara itu, tiada aktiviti yang dapat diperhatikan terhadap bakteria *Pseudomonas aureus* kecuali sebatian **14** dan **137** pada MIC 500 µg/ml.