SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL STUDIES OF *P*-NITROANILIDE DERIVATIVES AS CHROMOGENIC SUBSTRATES FOR ENDOTOXIN SCREENING

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MASTER OF SCIENCE
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Thesis Submitted in Fulfillment of the Requirement for the Degree of Master of Science in the School of Fundamental Science Universiti Malaysia Terengganu
July 2016

Dedicated this thesis to:

My super awesome and hardworking supervisor, Dr Maisara Abdul Kadir

My chemistry teacher at SMKP, cikgu Harun

My beloved parents (umie and bak) and siblings

Thanks for the endless love

Indeed We have granted you a clear victory (1)

And grant you a might victory (3)

[Surah Al- Fath]

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfillment of the requirement for the degree of Master of Science

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Main Supervisor : **Maisara Abdul Kadir, Ph.D**

Co-Supervisor : **Associate Professor Noraznawati Ismail, Ph.D**

School : **School of Fundamental Science**

The approach of using *p*-nitroanilide derivatives as chromogenic substrates has become a subject of interest due to their outstanding application in detecting endotoxin chromogenically. In this present study, five *p*-nitroanilide derivatives (two reported and three new compounds) namely *tert*-butyl (2-((4-nitrophenyl)amino)-2-oxoethyl)carbamate (**1**), *tert*-butyl (1-((4-nitrophenyl)amino)-1-oxopropan-2-yl)carbamate (**2**), *tert*-butyl (4-methyl-1-((4-nitrophenyl)amino)-1-oxopentan-2-yl)carbamate (**3**), *tert*-butyl (1-((2-((4-nitrophenyl)amino)-2-oxoethyl)amino)-1-oxopropan-2-yl)carbamate (**4**) and *tert*-butyl (1-((4-(methylthio)-1-((4-nitrophenyl)amino)-1-oxobutan-2-yl)amino)-1-oxopropan-2-yl)carbamate (**5**) have been successfully synthesized from the reaction of protected amino acids and protected peptides with *p*-nitroaniline. The structures of these compounds were characterized using combination of common spectroscopic techniques such as Fourier Transform Infrared (FTIR), Ultraviolet-Visible (UV-vis), ^{1}H and ^{13}C Nuclear Magnetic Resonances (NMR) and elemental analysis. Compounds **1-5** were subjected for biological assay analysis using Enzyme-linked immunosorbent Assay (ELISA) techniques to investigate the potential of these compounds to act as chromogenic substrates for endotoxin. Compounds **1-4** are able to detect endotoxin

until concentration at 0.03 EU/mL by forming a cloudy solution. However, compound **5** has limited solubility and unable to cleavage the peptide in the hydrolysis process. Among these compound, compound **3** gave the best endotoxin activity. From this research study, compound **3** is a suitable chromogenic substrate for endotoxin derived from simple amino acid and *p*-nitroaniline has been discovered.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

SINTESIS, PENCIRIAN DAN KAJIAN BIOLOGIKAL TERHADAP TERBITAN *P*-NITROANILIDA SEBAGAI KROMOGEN UNTUK PENYARINGAN ENDOTOKSIN

RUMAIZAH BINTI CHE ZULKIFLI

Julai 2016

Penyelia Utama : **Maisara Abdul Kadir, Ph.D**

Penyelia Bersama : **Profesor Madya Noraznawati Ismail, Ph.D**

Pusat pengajian : **Pusat Pengajian Sains Asas**

Pendekatan penggunaan terbitan *p*-nitroanilida sebagai substrat kromogen telah menjadi subjek yang mendapat perhatian disebabkan oleh aplikasinya yang tiada tandingan dalam mengesan kehadiran endotoksin secara kromogenik. Di dalam kajian ini, lima sebatian *p*-nitroanalida (dua telah dilaporkan, tiga sebatian baru) iaitu *tert*-butil (2-((4-nitrofenil)amino)-2-oksoetil)karbamat (**1**), *tert*-butil (1-((4-nitrofenil)amino)-1-oksopropana-2-il)karbamat (**2**), *tert*-butil (4-metil-1-((4-nitrofenil)amino)-1-oksopentana-2-il)karbamat (**3**), *tert*-butil (1-((2-((4-nitrofenil)amino)-2-oksoetil)amino)-1-oksopropana-2-il)karbamat (**4**) dan *tert*-butil (1-((4-(metiltio)-1-((4-nitrofenil)amino)-1-oksobutana-2-il)amino)-1-oksopropana-2-il)karbamat (**5**) telah berjaya dihasilkan daripada tindak balas antara beberapa jenis asid amino dan peptide terlindung, dan *p*-nitroanilina. Kesemua sebatian yang diperolehi telah dicirikan melalui teknik spektroskopi seperti spektroskopi Penukaran Fourier Inframerah (FTIR), Ultralembayung boleh nampak (UV-vis), ^{1}H dan ^{13}C Resonan Magnet Nukleus (RMN) dan analisis unsur. Sebatian **1-5** telah dihantar untuk analisis biologi dengan menggunakan teknik Asai Imunoserapan Terangkai Enzim (ELISA) untuk mengkaji potensi sebatian-sebatian ini sebagai

substrat kromogen untuk endotoksin. Di dalam pendekatan ini, satu Limulus Amebosit Lisat (LAL) yang diekstrak dari darah belangkas telah digunakan sebagai enzim proteolisis. Keputusan telah menunjukkan bahawa sebatian **1-4** berkeupayaan untuk mengesan endotoksin sehingga kepekatan pada 0.03 EU/mL dengan menghasilkan larutan keruh. Namun begitu, sebatian **5** mempunyai keupayaan keterlarutan yang terhad dan tidak berkeupayaan untuk memutuskan ikatan peptida di dalam proses hidrolisis. Di antara sebatian-sebatian ini, sebatian **3** telah memberi keupayaan yang terbaik untuk aktiviti endotoksin. Daripada penyelidikan kajian ini, satu kromogen substrat yang telah diterbitkan daripada amino asid ringkas dan *p*-nitroanilina untuk endotoksin telah ditemui iaitu sebatian **3**.