UNIVERSITI TEKNOLOGI MARA

BIOLOGICAL SCREENING AND MOLECULAR MECHANISM STUDIES OF SYNTHESIZED STILBENES AGAINST HUMAN CHRONIC MYELOID LEUKEMIC K562 CELLS

HASLAN ROSLIE

Thesis submitted in fulfillment of the requirements for the degree of
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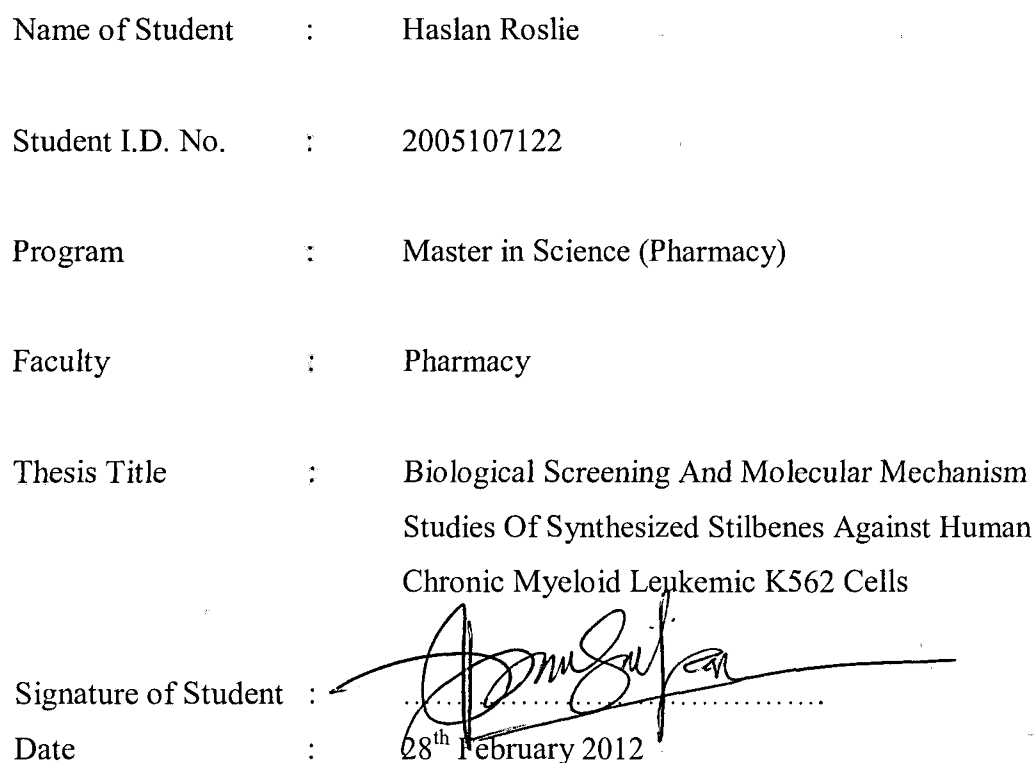
AUTHOR DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicate or acknowledge as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that i have been supplied with the Academic Rules and Regulation for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

Name of Student	:	Haslan Roslie
Student I.D. No.	:	2005107122
Program	:	Master in Science (Pharmacy)
Faculty	:	Pharmacy
Thesis Title	:	Biological Screening And Molecular Mechanism Studies Of Synthesized Stilbenes Against Human Chronic Myeloid Leukemic K562 Cells



Signature of Student : ....

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ABSTRACT

Stilbenes such as resveratrol, pterostilbene and picetannol were known to exhibit wide range biological activities including anticancer and anti-leukemic properties. In this study, a series of stilbene derivatives were synthesized incorporating acetoxy-, benzyloxy-, carboxy-, chloro-, hydroxy- and methoxy functional groups. The cytotoxicity of 23 stilbenes in human K562 chronic myelogenous leukemia cells were evaluated. Only four compounds were cytotoxic namely VS31, SY1/11B-25, VS30 and VS27 with IC_{50}s of 78μM, 38μM, 67μM and 19.5μM, respectively. By using Ferric Reducing "Antioxidant Power" (FRAP) assay, all compounds were investigated for their antioxidant activities and only compounds that possessed hydroxyl-group (VS27, VS30 and VS31) have antioxidant activities. However, the FRAP value was much lower compared to resveratrol which possessed 3 hydroxyl-groups. Genotoxicity assessment was carried out on two (2) most potent compounds. Compounds SY1/11B-25 and VS27 showed no DNA damage as assessed using Alkaline Comet assay in K562 cells which suggested that the cytotoxicity was independent of primary DNA damage. The apoptosis assessment using Acridine Orange/Propidium Iodide staining on VS27 and SY1/11B-25 were found to induce apoptosis at their IC_{50} concentration within 24 hours and the number of apoptotic cells increased after 48 hours. On the other hand, flow cytometric analysis of phosphatidylserine exposure confirmed that the cells underwent apoptosis. Since VS27 was found to be more potent and active compared to SY1/11B-25, further studies were carried out only on VS27. The loss of mitochondrial membrane potential was observed on K562 treated with VS27. Importantly, a concentration-dependent activation of caspase-9 as early as 2 hours with resultant caspase-3 cleavage in VS27-induced apoptosis was observed. Taken together, these data suggest that the pro-apoptotic effects of VS27 involve the intrinsic mitochondrial pathway characterized by an early activation of caspase-9.

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