UNIVERSITI SAINS MALAYSIA

Pejabat Pengurusan Dan Kreativiti Penyelidikan
Research Creativity and Management Office

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No. Fail : F0396
Tarikh : 2 Disember 2011

Prof. Madya Amirin Sadikun
Pusat Pengajian Sains Farmasi
Universiti Sains Malaysia

Tuan,

LAPORAN AKHIR SKIM GERAN PENYELIDIKAN FUNDAMENTAL (FRGS)
Tajuk Projek : Synthesis of Liphophilic Isonicotinic Acid Hydrizide Derivatives and Mechanism Study of Its Action on Mycobacterium Tuberculosis
No. Akaun : 203/PFARMASI/671157

Dengan hormatnya perkara di atas dirujuk.

2. Terlebih dahulu saya ucapkan ribuan terima kasih di atas satu salinan laporan akhir untuk projek penyelidikan seperti tajuk di atas.

3. Adalah dimaklumkan walaupun projek ini telah selesai, kerjasama Jabatan Bendahari dipohon untuk menguruskan penutupan akaun projek pada selewat-lewatnya **31 Disember 2011**. Tempoh ini bertujuan untuk menyelesaikan semua urusan tuntutan dan bayaran yang telah dibelanjakan di dalam tempoh projek. Walau bagaimanapun, tuan dinasihatkan supaya tidak mengeluarkan borang-borang pesanan baru di dalam tempoh ini.

4. Selanjutnya sila ambil perhatian terhadap perkara-perkara berikut sekiranya berkaitan:

(i) Semua penerbitan harus merakamkan penghargaan kepada **Skim Geran Penyelidikan Fundamental (FRGS)** dan tuan dipohon mengemukakan satu salinan ke Pejabat ini.

(ii) Bahagian Penyelidikan & Inovasi boleh/akan mengagihkan semula peralatan yang telah dibeli menggunakan peruntukan geran ini seandainya terdapat penyelidik lain yang memerlukan peralatan tersebut.

5. Akhir sekali, tahniah di atas usaha dan kejayaan pihak tuan dapat menyelesaikan projek ini dengan jayanya.

Sekian, terima kasih.

"BERKHIDMAT UNTUK NEGARA"
'Memastikan Kelestarian Hari Esok'

Yang menjalankan tugas,

HAH, HAR, SM

(AMRA OTHMAN)
Penolong Pendaftar
Unit Pengurusan Geran & Kontrak

LAPORAN AKHIR SKIM GERAN PENYELIDIKAN FUNDAMENTAL (FRGS)

Tajuk Projek : Synthesis of Liphophilic Isonicotinic Acid Hydrizide Derivatives and Mechanism Study of Its Action on Mycobacterium Tuberculosis

No. Akaun : 203/PFARMASI/671157

s.k. Dekan Penyelidikan
Pelantar Sains Fundamental
Pejabat Pelantar Penyelidikan
Universiti Sains Malaysia

Dekan
Pusat Pengajian Sains Farmasi
Universiti Sains Malaysia

Timbalan Dekan
(Ijazah Tinggi & Penyelidikan)
Pusat Pengajian Sains Farmasi
Universiti Sains Malaysia

Ketua Pustakawan
Perpustakaan Hamzah Sendut
Universiti Sains Malaysia

} Disampaikan satu salinan laporan akhir projek untuk simpanan Perpustakaan

Penolong Bendahari Kanan
Unit Kumpulan Wang Penyelidikan
Jabatan Bendahari
Universiti Sains Malaysia

Pegawai Sains
Pelantar Sains Fundamental
Pejabat Pelantar Penyelidikan
Universiti Sains Malaysia

} Mohon kerjasama pihak puan untuk menguruskan penutupan akaun projek selewat-lewatnya pada **31 Disember 2011** dan mohon kemukakan satu salinan penyata kewangan terakhir ke Pejabat ini untuk tujuan rekod

FINAL REPORT
FUNDAMENTAL RESEARCH GRANT SCHEME (FRGS)

Laporan Akhir Skim Geran Penyelidikan Asas (FRGS) IPT
Pindaan 1/2010

A

RESEARCH TITLE : Synthesis of lipophilic isonicotinic acid hydrazide derivatives and mechanism study of its action on *Mycobacterium tuberculosis*
Tajuk Penyelidikan

PROJECT LEADER : Assoc. Prof. Dr. Amirin Sadikun
Ketua Projek

PROJECT MEMBERS (including GRA) : 1. Assoc. Prof. Dr. Pazilah Ibrahim
2. Assoc. Prof. Dr. Nornisah Mohamed
Ahli Projek

PROJECT ACHIEVEMENT *(Prestasi Projek)*

B

ACHIEVEMENT PERCENTAGE			
Project progress according to milestones achieved up to this period	**0 - 50%**	**51 - 75%**	**76 - 100%**
Percentage			85

RESEARCH OUTPUT		
Number of articles/ manuscripts/ books *(Please attach the First Page of Publication)*	**Indexed Journal**	***Non-Indexed Journal***
		1
Conference Proceeding *(Please attach the First Page of Publication)*	**International**	***National***
	2	1
Intellectual Property *(Please specify)*	1 (poster presented at Majlis Sambutan Hari TB Sedunia 2009, Peringkat Negeri Pulau Pinang)	

HUMAN CAPITAL DEVELOPMENT

Human Capital	**Number**				**Others** (please specify)
	On-going		**Graduated**		
Citizen	Malaysian	Non Malaysian	Malaysian	Non Malaysian	
PhD Student					
Master Student	1				
Undergraduate Student					
Total	1				

EXPENDITURE *(Perbelanjaan)*

C

Budget Approved *(Peruntukan diluluskan)*	:	**RM** 105 000.00
Amount Spent *(Jumlah Perbelanjaan)*	:	**RM** 86 660.87
Balance *(Baki)*	:	**RM** 18 339.13
Percentage of Amount Spent *(Peratusan Belanja)*	:	82.5 %

ADDITIONAL RESEARCH ACTIVITIES THAT CONTRIBUTE TOWARDS DEVELOPING SOFT AND HARD SKILLS
(Aktiviti Penyelidikan Sampingan yang menyumbang kepada pembangunan kemahiran insaniah)

D

International

Activity	Date (Month, Year)	Organizer
(e.g : Course/ Seminar/ Symposium/ Conference/ Workshop/ Site Visit)	1. International Biohealth Science Conference (IBSC) 2010, 29th Nov – 1st Dec 2010, Bayview Beach Resort, Penang. 2. AASP-MPS Conference 2009, 10-13 June 2009, Vistana Hotel, Penang.	Advance Medical & Dental Institute, Universiti Sains Malaysia School of Pharmaceutical Sciences, USM & Malaysian Pharmaceutical Society, Malaysia

National

Activity	Date (Month, Year)	Organizer
(e.g : Course/ Seminar/ Symposium/ Conference/ Workshop/ Site Visit)	3rd National Conference Infectious Diseases, 26 – 27th October 2009, Renaissance Kota Bharu Hotel, Kota Bharu, Kelantan.	Department of Medical Microbiology & Parasitology, School of Medical Sciences, Universiti Sains Malaysia

PROBLEMS / CONSTRAINTS IF ANY *(Masalah/ Kekangan sekiranya ada)*

E

This project involves handling of *M. tuberculosis* cultures which is a very slow growing microorganism that requires around 4 – 6 weeks of incubation time to observe visible colonies. Indeed, *M. tuberculosis* is a class 3 pathogen. Hence, proper training and guidance of students in handling the cultures and other proper lab techniques is vital to avoid lab acquired infection. The characterization of the compounds through NMR and Mass spectroscopy also delays the project progress due to lack of facilities and skillful staff in the lab. This project also faced difficulty in getting interested staff to perform TB research which eventually drags the project.

RECOMMENDATION (*Cadangan Penambahbaikan*)

F

1. The current *in vitro* studies of hydrophobic derivatives should be further evaluated with *in vivo* models to undoubtedly declare them as prospective anti-TB drug in future. The *in vivo* studies indeed will lead to a better understanding of the pharmacokinetic and pharmacodynamic properties of the derivatives and ultimately, this will lead to infer the action mechanism of INH derivatives.

2. Molecular and biochemical studies can also be done to elucidate the action mechanism of the INH derivative, especially INH-C16. The studies can be focused on the mycolic acid composition in the cell wall since, the present study indicated that the target of INH-C16 is the cell wall. Besides, studies can also be conducted using fluorescence probe to detect the pathway of action mechanism of INH-C16. However, such method is a cost and time consuming but highly reliable.

RESEARCH ABSTRACT – Not More Than 200 Words *(Abstrak Penyelidikan – Tidak Melebihi 200 patah perkataan)*

G

Studies have indicated that the antimycobacterial activity of hydrophilic drug especially isoniazid (INH) can be improved if it is augmented into hydrophobic drug. Therefore, 13 hydrophobic INH derivatives with different hydrophilicity were synthesized by attaching the INH to a different hydrophobic moiety. The minimum inhibitory (MIC) concentration of INH derivatives was then determined via tetrazolium microplate assay. 1-isonicotinoyl-2-hexadecanoyl hydrazine (INH-C16), 1-isonicotinoyl-2-heptadecanoyl hydrazine (INH-C17), and 1-isonicotinoyl-2-octadecanoyl hydrazine (INH-C18) were shown to have lower MIC values compared to INH. The interaction between these derivatives and first-line anti-TB drugs were studied using fixed-ratio isobologram method. INH-C16 showed the most synergistic activity, so it was selected as a potential INH derivative to study the action mechanism. *M. tuberculosis* was susceptible to INH-C16 at the lag, log, and death phase of the growth cycle. This was shown by the significant reduction in the colony counts of the exposed cells compared to the control culture. When the cells were observed under light microscopy, the INH-C16 and INH treated cells tend to form clumps as well as lost their acid-fastness properties. The INH-C16 treated cells were further viewed under scanning electron microscope and we found that INH-C16 caused the cells to lose their cellular integrity which ultimately lead to cell death. In conclusion, INH-C16 has the potential to be a drug leads worthy of further investigations.

Date : 10/02/11
Tarikh

Project Leader's Signature:
Tandatangan Ketua Projek

COMMENTS, IF ANY/ ENDORSEMENT BY RESEARCH MANAGEMENT CENTER (RMC)
(Komen, sekiranya ada/ Pengesahan oleh Pusat Pengurusan Penyelidikan)

H

.....

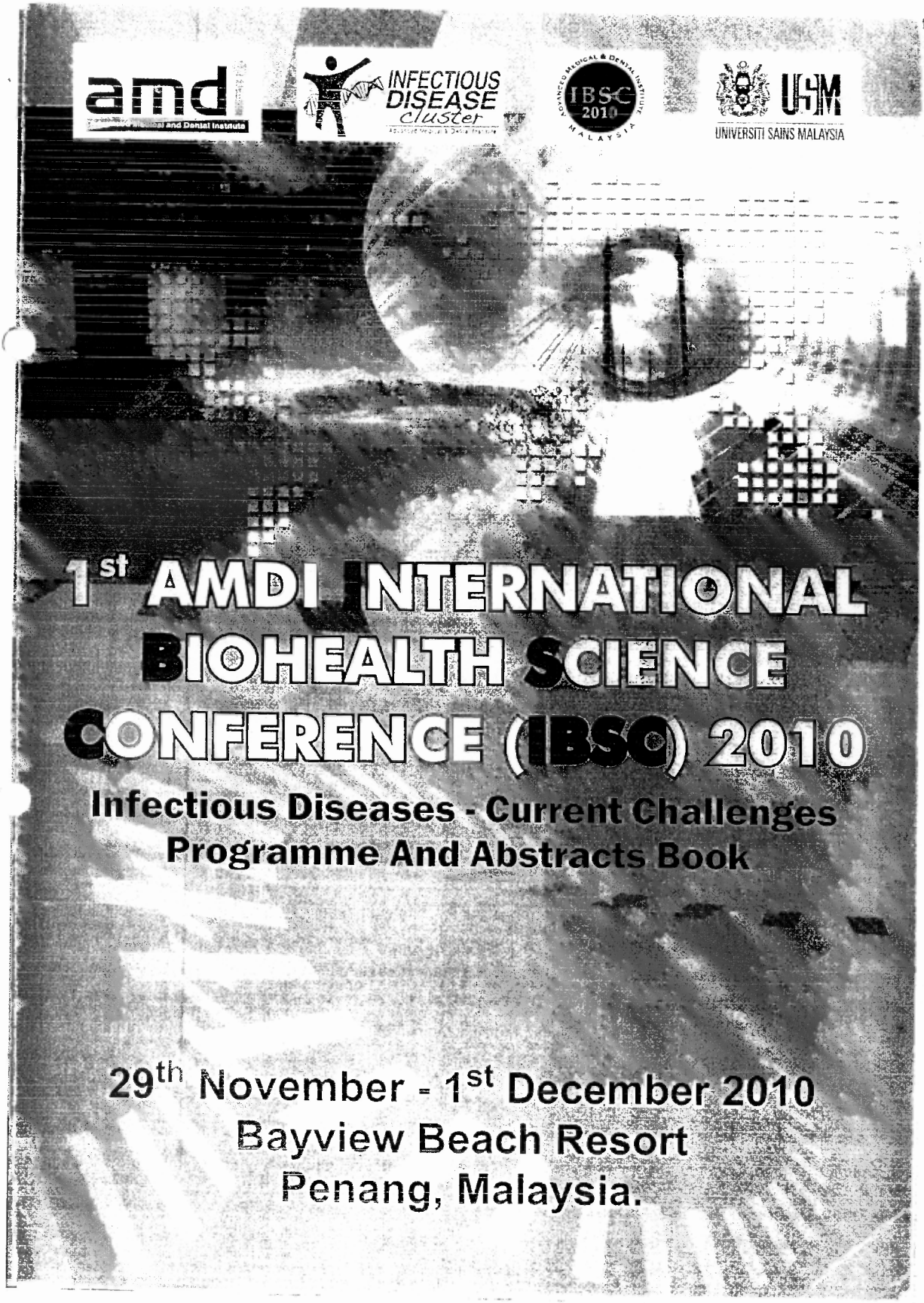
.....

.....

Name:
Nama:

Signature:
Tandatangan:

Date:
Tarikh:


amd
INFECTIOUS DISEASE cluster
IBSC 2010
UNIVERSITI SAINS MALAYSIA
1st AMDI INTERNATIONAL BIOHEALTH SCIENCE CONFERENCE (IBSC) 2010
Infectious Diseases - Current Challenges
Programme And Abstracts Book
29th November - 1st December 2010
Bayview Beach Resort
Penang, Malaysia.

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The study of *in vitro* interaction of 1-isonicotinoyl-2-octadecanoyl hydrazine with first line anti-TB drugs against *Mycobacterium tuberculosis* H37Rv

Thaigarajan Parumasivam[1], Naveen Kumar H. S.[1], Pazilah Ibrahim[1], Amirin Sadikun[1], Suriyati Mohamad[2]*

[1]School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia, [2]School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia

***Corresponding Author**: Pazilah Ibrahim
E-mail: pazilah@usm.my

Background/Aim: Isoniazid (INH) is the first choice drug in tuberculosis (TB) treatment. It is a bactericidal drug which has high activity against *Mycobacterium tuberculosis*. However, the emergence of multi-drug resistant strains had caused complication in TB treatment. This phenomenon has led to an urgent need for a newer and more powerful drug as well as the re-examination and re-evaluation of older drugs. One of the factors contributing to resistance in *M. tuberculosis* is the architecture of the bacterial cell envelope where the outer layer acts as an exclusion barrier towards hydrophilic drugs such as INH. According to Rastogi *et. al.* (1990), the anti-mycobacterial activity of hydrophilic drug can be improved if it is augmented into the hydrophobic compound. Therefore, an INH derivative was synthesized by attaching the INH to a hydrophobic moiety. The aim of this research is to study the interaction between the INH derivative, 1-isonicotinoyl-2-octadecanoyl hydrazine (INH-C18) with INH, streptomycin (STR), rifampicin (RIF) and ethambutol (EMB) respectively

Methodology: The INH-C18 was synthesized chemically and confirmation of the structure of the compound was achieved by standard procedures involving IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectroscopy. The drug interaction study was carried out using fixed-ratio isobologram method with slight modification

Results: INH-C18 showed additive or indifferent to synergistic interaction with INH, while synergistic interaction was observed with STR and RIF. Additive or indifferent interaction was observed when INH-C18 combined with EMB.

Conclusion: Thus, it can be concluded that INH-C18 has the potential to be used as a TB drug in the future.

PP12684/9/2009
Vol. 1 Issue 7. June 2009

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PPC – 05 (000019)

Synthesis and Pharmacological Evaluation of 5-((4,5-Diphenyl-2-Substituted-1h-Imidazol-1-Yl)Methyl)-1H-Tetrazoles

K.Anandarajagopal, J. Anbu Jeba Sunilson, M.Muthappan, M.Vignesh, V.Venu, Neelopar S.M.
School of Pharmacy, Masterskill University College of Health Sciences, Batu 9, Cheras, Selangor, Malaysia

Tetrazole and imidazole nucleus are versatile lead molecules and possess wide range of biological activities. Various substituted imidazoles were synthesized using benzil, ammonium acetate and various aldehyde which being a secondary amine was cyanoethylated with acetonitrile and Tritan - B yield 2-(4,5-diphenyl-2-substituted-1*H*-imidazol-1-yl)acetonitriles which is readily converted to 1,2,3,4-tetrazole derivative, 5-((4,5-diphenyl-2-substituted-1*H*-imidazol-1-yl)methyl)-1*H*-tetrazoles by cycloaddition with sodium azide and ammonium chloride in dimethylformamide. The compounds were differentiated by mixed melting point and thin layer chromatography and characterized by IR and NMR spectral data. The synthesized compounds were tested for their analgesic activity using pentazocine as a standard drug. All compounds possess ($P < 0.001$) significant analgesic activity.

PPC – 06 (000261)

Synthesis And Characterization Of Novel Analgesic And Anti-Inflammatory Agents

Amitava Das[1], Jay Jyoti Roy[2], J. Saravanan[2], S. Mohan[2], J. Anbu Jeba Sunilson[1], M.Muthappan[1]
[1] School of Pharmacy, Masterskill University College of Health Sciences, Taman Kemacahaya 11, Jalan Kemacahaya, Cheras, Selangor, Malaysia
[2] PES College of Pharmacy, Department of Pharmaceutical Chemistry, 50 Feet Road, Hanumanthanagar, Bangalore-560050

Pyrimidine derivatives have continued to attract the interest of medicinal chemists due to a wide variety of biological activities such as analgesic, anti-inflammatory, anticancer, antiviral, antimicrobial, and antifungal. Pyrazoles on the other hand are also reported to possess a wide array of pharmacological activities. Various marketed NSAIDs like phenylbutazone, and oxyphenylbutazone contain the pyrazole nucleus. So it was thought of interest to synthesize pyrimidines and pyrazoles in one framework and screen for analgesic and anti-inflammatory activity. In the present work, a mixture of ethylcyanoacetate, acetic anhydride and triethyl orthoformate under reflux yielded ethylethoxyethyene cyanocarboxylate(1) which on refluxing with phenyl hydrazine in ethanol produced 5-amino-4-carbethoxy-1- phenyl pyrazole(2). The parent compound 2-methyl 3-amino -8-phenyl pyrazolo[3,4-d] pyrimidine-4[3H]-one (3) was synthesized by acetylating (2) with acetic anhydride followed by cyclization using hydrazine hydrate. Later, the parent compound (3) was derivatized by using different acid chlorides to obtain a series of amides (a-d). Treating (3) with chloroacetyl chloride yielded compound (e) in which the labile chlorine was replaced with morpholine and piperazine to form compounds (f,g). The representative compounds were characterized by spectral data. All the derivatives (a-d, f,g) were screened for analgesic activity. The compounds which showed comparable analgesic activity were further screened for anti-inflammatory activity using diclofenac sodium as the standard. From the analgesic and anti-inflammatory activity results, it can be inferred that the compounds having electron-withdrawing groups as substitution showed significant analgesic activity but moderate anti-inflammatory activity.

PPC – 07 (000329)

Synthesis of Isonicotinic Acid Hydrazide Derivatives and Its Action on *Mycobacterium tuberculosis*

HS Naveenkumar[1], P Thaigarajan[2], A Sadikun[1] and P Ibrahim[2]
[1] Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia
[2] Department of Microbiology, School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia

Tuberculosis (TB) remains the leading cause of mortality worldwide even in 21st century and the situation is getting worse due to the increase in prevalence of HIV/AIDS and emergence of multidrug-resistant strains of TB. One of the contributing factors to the resistance of *M.tuberculosis* is the architecture of the cell wall, which is a triple layered structure. Isoniazid (INH) is one of the most potent drug available for tuberculosis.

The antibacterial action of hydrophilic drug like INH may be enhanced if it contained a lipophillic side chain. In this study, a series of lipophillic isoniazid derivatives have been synthesized and evaluated for their invitro antibacterial activity against *M.Tuberculosis* H37Rv using tetrazolium micro plate assay and the activity expressed as the minimum inhibitory concentration (MIC) in µm/mL. Among the synthesized INH derivatives, compounds INHC10 and INHC12 were exhibited excellent antibacterial activity when compared with isoniazid, making them suitable for further exploration.

PPC – 08 (000006)

New Alpha-glucosidase And Tyrosinase inhibitors From Microbial Transformation of Tibolone And Hydroxytibolones And SAR Studies Of Their active Metabolites

S A A Shah[1], M I Choudhary[2], Atta-ur-Rahman[2]
[1]Faculty of Pharmacy, University Technologi MARA, Shah Alam, Selangor D. E Malaysia
[2]H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan

Sixteen new and one known metabolites **4-20** were obtained by incubation of tibolone (**1**) and hydroxytibolones (**2** and **3**) with various fungal cultures. Their structures were elucidated by means of a homo and heteronuclear 2D NMR and by HREI-MS techniques. The relative stereochemistry was deduced by 2D NOESY experiment. These metabolites have shown promising inhibitory activities against α-glucosidase and tyrosinase enzymes. Metabolites **4**, **7**, and **8** exhibited a potent inhibitory activity against the α-glucosidase enzyme. All the analogs also exhibited mild to potent inhibition against the enzyme, tyrosinase, except compounds **7**, **11**, **13** and **14**, while compound **9** was the most potent in the series.

PPC – 09 (000256)

Total Flavonoid: Standardization Parameter of *Stelechocarpus burahol* [(Bl.) Hook. F. & Th.] Extract For Hyperuricaemic Therapy

I Purwantini[1], Purwantiningsih[2]
[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Gadjah Mada University, Indonesia
[2]Department of Pharmaceutical Biology, Faculty of Pharmacy, Gadjah Mada University, Indonesia

Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases. Currently, the major pharmaceutical companies have demonstrated renewed interest in investigating the development of standardized phytotherapeutic agents to ensure its efficacy, safety and quality. Because of this, it is important to select the extract-standardization parameter. The aim of this research was to find out correlation between total flavonoid content and its activity. Ethanolic extract of *S. burahol* and its n-hexane fraction have been reported to have potent anti hyperuricaemic activity in rat. The n-hexane fraction is more potent than the ethanolic extract. Moreover, both extracts also showed xanthine oxidase inhibitor activity, a key enzyme associated with the incidence of hyperuricemia-related disorders. The total flavonoid content in ethanolic extract was 6.84% whereas n-hexane fraction was 9.35%. The higher amount of flavonoid content leads to a more potent anti hyperuricaemi effect and the total flavonoid content can be used to standardize *S. burahol* extract for the development as hyperuricaemic phytopharmaceutical.

PPC – 10 (000239)

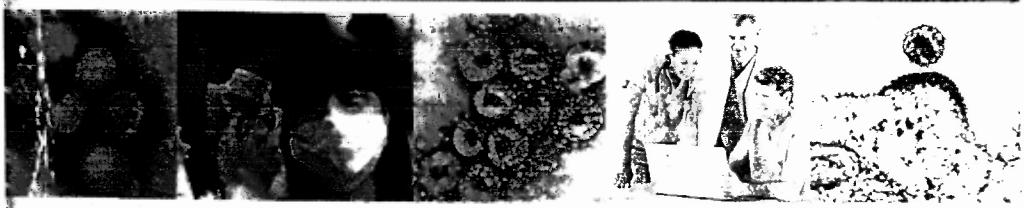
Anticancer Activity Screening Of Eleven Thai Indigenous Plants

Natthida Weerapreeyakul[1], Sasipawan Machana[2], Thaweesak Thitimetharoch[1], Bungorn Sripanidkulchai[1]
[1] Center for Research and Development of Herbal Health Product, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand
[2] Graduate School, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand

Cytotoxicity screening provides an important preliminary data for selection of plant extracts with potential anticancer properties for future work. This study was aimed to investigate the cytotoxicity of 50% ethanolic-water crude extracts of 11 Thai indigenous plants in leukemia MOLT-4 cell compared to the normal Vero cell

3rd NATIONAL CONFERENCE ON INFECTIOUS DISEASES

Innovative Approaches in Infectious Diseases: Towards Sustainable Healthcare



26-27 October 2009
Renaissance Kota Bharu Hotel

Department of Medical Microbiology & Parasitology
School of Medical Sciences
Universiti Sains Malaysia

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ORAL PRESENTATION

O6: DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION OF ISONIAZID AND ITS DERIVATIVES AGAINST *Mycobacterium tuberculosis*

Thaigarajan Parumasivam, Pazilah Ibrahim, Naveen Kumar HS, Amirin Sadikun

School of Pharmaceutical Sciences, Universiti Sains Malaysia, Malaysia

Background and aims
Isoniazid (INH) is one of the first-line drugs for tuberculosis. It is a bactericidal drug which has high activity against *M. tuberculosis*. Studies have indicated that the antibacterial action of a hydrophilic drug like INH can be enhanced if it contained a lipophilic side chain. This clarification was also supported by molecular modelling studies that INH derivatives have higher favorable binding to InhA enzyme than INH. InhA enzyme is a crucial enzyme in synthesis of mycolic acid; important component in mycobacterium cell wall. Therefore, the objective of this research is to study the susceptibility of *M. tuberculosis* towards the INH derivatives.

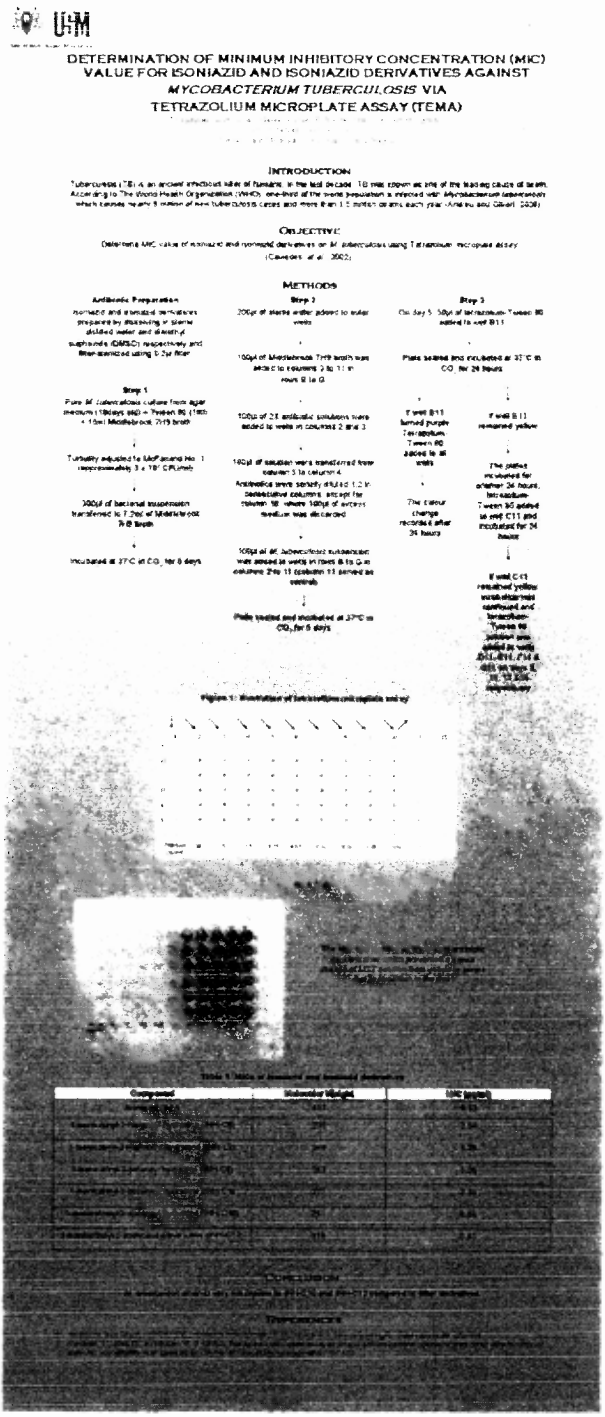
Methods
The INH derivatives were synthesized chemically. Confirmation of the structure of the product was achieved by standard procedures involving IR, 1H-NMR, 13C-NMR, and mass spectroscopy. The MIC of the derivatives against *M. tuberculosis* was determined using tetrazolium microplate assay (TEMA).

Results
The MIC value of INH was 2.26μM, while the MIC of the derivatives were as follows: 1-isonicotinyl-2-hexanoyl hydrazine (INH-C6) 5.32μM, 1-isonicotinyl-2-heptanoyl hydrazine (INH-C7) 2.53μM, 1-isonicotinyl-2-octanoyl hydrazine (INH-C8) 2.39μM, 1-isonicotinyl-2-nonanoyl hydrazine (INH-C9) 4.51μM, 1-isonicotinoyl-2-decanoyl hydrazine (INH-C10) 1.07μM, 1-isonicotinoyl-2-dodecanoyl hydrazine (INH-C12) 0.97μM, 1-isonicotinoyl-2-tetradecanoyl hydrazine (INH-C14) 1.81μM, 1-isonicotinyl-2-hexadecanoyl hydrazine (INH-C16) 3.33μM and 1-isonicotinoyl-2-octadecanoyl hydrazine (INH-C18) 3.10μM.

Conclusion
Thus, it can be concluded that INH-C10 and INH-C12 have the potential to be used as a TB drug in future.

Poster presented at Majlis Sambutan Hari TB Sedunia 2009,

Peringkat Negeri Pulau Pinang



DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) VALUE FOR ISONIAZID AND ISONIAZID DERIVATIVES AGAINST *MYCOBACTERIUM TUBERCULOSIS* VIA TETRAZOLIUM MICROPLATE ASSAY (TEMA)

INTRODUCTION

OBJECTIVE

METHODS