PHYTOCHEMICAL STUDY ON THE SHOOTS OF *DENDROCALAMUS ASPER*, AND SYNTHESIS OF PYRAZOLINE DERIVATIVES AND THEIR BIOLOGICAL ACTIVITIES

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by

WONG KOK TONG

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Dedicated to my beloved family

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LIST OF ABBREVIATIONS

ADC	Albumin, dextrose and catalase
ATR	Attenuated total reflectance
[bmim]PF_6	1-Butyl-3-methylimidazolium hexafluorophosphate
BSD	Dichloromethane extract of bamboo shoot
BSH	n-Hexane extract of bamboo shoot
BSM	Methanol extract of bamboo shoot
^{13}C NMR	Carbon-13 nuclear magnetic resonance
°C	Degree celsius
Calcd	Calculated
CC	Column chromatography
$CDCl_3$	Deuterated chloroform
CD_3COCD_3	Deuterated acetone
CD_3OD	Deuterated methanol
CHN	Carbon, hydrogen and nitrogen
CFU	Colony forming unit
cm^{-1}	Per centimeter
CO_2	Carbon dioxide
COSY	Correlation spectroscopy
d	doublet
dd	doublet of doublet
1D-NMR	One-dimensional nuclear magnetic resonance
2D-NMR	Two-dimensional nuclear magnetic resonance
D. asper	*Dendrocalamus asper*
DABCO	1,4-Diazabicyclo[2.2.2]octane

DCM	Dichloromethane
DEPT	Distortionless enhancement by polarization transfer
DMAP	4-dimethylaminopyridine
DMSO-d_6	Deuterated dimethyl sulfoxide
(E)	Trans configuration
equiv	Equivalent
EtOH	Ethanol
Fr	Fraction
FT-IR	Fourier-transform infrared
g	Gram
g/L	Gram per litre
h	hour
hrs	hours
^{1}H-NMR	Proton nuclear magnetic resonance
H_2O	Water
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
HIV	Human immunodeficiency virus
HMBC	Heteronuclear multiple bond correlation
HPLC	High-performance liquid chromatography
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear single quantum correlation
Hz	Hertz
IC_{50}	Inhibition concentration at 50%
INH	Isoniazid

IR	Infrared
J	Coupling constant
LDH	Lactate dehydrogenase
lit	Literature
m	Multiplet
mg	Miligram
min	Minutes
mL	Mililitre
mmol	Milimole
mol	mole
m.p	Melting point
m/z	Mass to charge ratio
M. tuberculosis	*Mycobacterium tuberculosis*
MHz	Mega hertz
MABA	Microplate alamar blue assay
MAO	Monoamine oxidase
MBC	Minimum bactericidal concentration
CH_3CN	Acetonitrile
CH_3OH	Methanol
MIC	Minimum inhibition concentration
MPLC	Medium-pressure liquid chromatography
MTB	*Mycobacterium tuberculosis*
MTBD	7-Methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
NaOH	Sodium hydroxide

NC	No cidal effect
OADC	Oleic, albumin, dextrose and catalase
OMP	Orotidine 5'-monophoste
ppm	Parts per million
P. falciparum	*Plasmodium falciparum*
P-HPLC	Preparative high-performance liquid chromatography
PMA	Phosphomolybdic acid reagent
q	Quartet
r.t	Room temperature
REMA	Resazurin microtiter assay
s	Singlet
t	Triplet
t_R	Retention time
TB	*Tuberculosis*
TBD	1,5,7-Triazabicyclo[4.4.0]dec-5-one
TEMA	Tetrazolium micro plate assay
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMG	N,N,N'N'-Tetramethylguanidine
TMS	Tetramethylsilane
TTL	2.3,5-Triphenyltetrazolium chloride
µg/mL	Microgram per mililitre
µL	Microlitre
µM	Micromolar
UPLC/MS	Ultra-performance liquid chromatography/ mass spectrometry

WHO	World Health Organisation
XTT	[2,3-bis-(2-Methoxy-4-nitro-5-sulpho phenyl)-2H-tetrazolium-5-carboxanilide]
$Zn(OTf)_2$	Zinc Triflate
%	Percentage
α	Alpha
β	Beta
γ	Gamma
δ	chemical shift

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KAJIAN FITOKIMIA KE ATAS REBUNG *DENDROCALAMUS ASPER*, DAN SINTESIS TERBITAN PIRAZOLINA DAN AKTIVITI BIOLOGI

ABSTRAK

Tesis ini terdiri daripada dua bahagian. Bahagian pertama adalah kajian fitokimia ke atas rebung *Dendrocalamus asper.* Sehingga kini, tiada banyak kajian dijalankan ke atas rebung ditemui. *Dendrocalamus asper* ialah tumbuhan yang gumpal dan padat daripada genus *Dendrocalamus* dan tergolong dalam keluarga Poaceae. Di Malaysia, tumbuhan ini dikenali sebagai buluh betung atau betung hitam dan telah lama digunakan sebagai makanan dan perubatan tradisional. Dalam kajian ini, rebung *Dendrocalamus asper* telah diekstrak dengan menggunakan tiga pelarut yang berlainan iaitu *n*-heksana, diklorometana dan metanol. Ekstrak *n*-heksana dan diklorometana difraksinasi dan dipencilkan dengan menggunakan pelbagai jenis teknik kromatografi termasuk MPLC, analitikal dan preparatif-HPLC. Sebatian tersebut telah dikenalpastikan dengan kaedah spektroskopik (IR, 1D dan 2D NMR), analisis spektrometri jisim (APCI/MS, UPLC/MS), dan juga melalui perbandingan dengan data literatur yang berkaitan. Berdasarkan data spektroskopik, satu sebatian baru **BSH F042** dan dua sebatian yang sudah dikenalpasti (**BSH F039**, **BSH F041**) telah diasingkan daripada ekstrak *n*-heksana manakala dua sebatian yang sudah dikenalpasti (**BSD F011**, **BSD F033**) telah diasingkan daripada ekstrak diklorometana. Empat sebatian semula jadi telah diuji untuk aktiviti antimalaria terhadap *Plasmodium falciparum* 3D7. Di antara semua sebatian semula jadi, **BSH F042** menunjukkan perencatan yang bagus dengan IC_{50} 3 μM. Bahagian kedua, pirazolina yang terdapat dalam tumbuhan ini, tetapi tidak banyak kajian aktiviti biologi dijalankan ke atas terbitan tersebut. Terbitan pirazolina juga dapat disintesis melalui tindak balas

siklokondensasi. Sejumlah dua puluh lima terbitan pirazolina daripada empat siri telah disintesis melalui tindak balas siklokondensasi terbitan kalkon dengan pelbagai jenis semikarbazida. Sebatian terbitan tersebut dicirikan dengan menggunakan IR, 1D NMR (^{1}H, ^{13}C and DEPT-135 NMR) dan 2D NMR (COSY, HMBC, HSQC) dan HRMS. Akhirnya, sebatian sintesis telah dinilai untuk aktiviti anti-tuberkulosis terhadap *Mycobacterium tuberculosis* H37Ra secara *in vitro*. Sebatian **94a** menunjukkan aktiviti perencatan yang paling kuat dengan nilai MIC 17 μM. Enam sebatian sintesis, **100a**, **100c**, **100d**, **100e**, **100f** dan **100g** mempamerkan aktiviti yang sederhana dengan nilai MIC masing-masing ialah 70 μM, 66 μM, 65 μM, 61 μM, 66 μM dan 134 μM. Bagi ujian MBC, sebatian **94a** menunjukkan aktiviti bakterisidal yang bagus dengan nilai MBC 34 μM terhadap *Mycobacterium tuberculosis* H37Ra.

PHYTOCHEMICAL STUDY ON THE SHOOTS OF *DENDROCALAMUS ASPER*, AND SYNTHESIS OF PYRAZOLINE DERIVATIVES AND THEIR BIOLOGICAL ACTIVITIES

ABSTRACT

This thesis consists of two parts. The first part is phytochemical study on the shoots of *Dendrocalamus asper*. To date, not many studies have been done on the shoots of *Dendrocalamus asper*. *Dendrocalamus asper*, a dense clumping plant from *Dendrocalamus* genus belongs to the family of Poaceae. In Malaysia, it commonly known as "buluh betung" or "betung hitam", which has been long used as traditional cuisine and medicine. In this study, the shoots of *Dendrocalamus asper* was extracted using three different types of solvents namely, *n*-hexane, dichloromethane and methanol. The *n*-hexane and dichloromethane extracts undergone fractionation and isolation by varies types of chromatography techniques including MPLC, analytical and preparative-HPLC. Then, these compounds were elucidated using spectroscopic methods (IR, 1D and 2D NMR), mass spectrometry analysis (APCI/MS and UPLC/MS) and also by referring with the related literature. Based on the spectroscopic data, one new compound **BSH F042** and two known compounds (**BSH F039**, **BSH F041**) were isolated from *n*-hexane extract while, two known compounds (**BSD F011**, **BSD F033**) were isolated from dichloromethane extract. Four isolated compounds were tested for antimalarial activity against *Plasmodium falciparum* 3D7. Among the isolated compounds, **BSH F042** showed good inhibition with the IC_{50} of 3 μM. The second part, pyrazoline had been found in this plant, however, not many biological studies have been done on its derivatives. Pyrazoline derivatives also can be synthesised through cyclocondensation reaction. A total of twenty-five pyrazoline

derivatives from the four series were synthesized through cyclocondensation reaction of chalcone derivatives with different type of semicarbazides. Then, these compounds were characterized with IR, 1D NMR (^{1}H, ^{13}C and DEPT-135 NMR) and 2D NMR (COSY, HMBC, HSQC) as well as HRMS. Finally, the synthesized compounds were tested for their anti-tuberculosis activity against *Mycobacterium tuberculosis* H37Ra in *in vitro* study. Compound **94a** showed the most potent inhibitory activity with MIC value of 17 μM. Six other synthesized compounds **100a**, **100c**, **100d**, **100e**, **100f** and **100g** exhibited moderate activity with the MIC values of 70 μM, 66 μM, 65 μM, 61 μM, 66 μM and 134 μM, respectively. For the MBC assay, compound **94a** showed good bactericidal activity with MBC value of 34 μM against *Mycobacterium tuberculosis* H37Ra.

CHAPTER 1

INTRODUCTION

This thesis consists of two main parts. Part 1 is phytochemical study and antimalarial activity on the shoots of *Dendrocalamus asper*. Part 2 is synthesis of pyrazoline derivatives through cyclocondensation reaction and the derivatives were tested for anti-tuberculosis activity.

1.1 Background of Study

Malaysia is one of the twelve knowns 'mega-diversity' countries in the world (Forestry Department of Peninsular Malaysia, 2020). The tropical rain forest in Malaysia is rich with flora and fauna, which nearly 16000 plants from about 4600 species as an important source of traditional medicine (Othman et al., 2011). The tropical rain forest is rich in biological and chemical resources which have a huge potential as defense agents against pest, diseases and predators (Jantan, 2004).

The phytochemistry has been developed in recent years as a rapid distinct discipline, in connection with natural product chemistry and plant biochemistry. This science is in touch with the myriad organic substances elaborated and accumulated from the plants and their chemical structures as well as their natural distribution, biosynthesis, metabolism and biological functions (Harborne, 1998). These chemical substances are broadly categorized into primary metabolites and secondary metabolites. Usually, the secondary metabolites are functioning as drugs for medical use (Kaur, Kapoor, & Kaur, 2011). Many of the natural occurring compounds including heterocyclic rings are an important part of coumarin, pyrazole, alkaloids, flavonoids and terpenoid structures. These compounds displayed considerable roles in medicine and human health (Arshad et al., 2011).

Poaceae plants, particularly the Bambusoideae subfamily, have been extensively investigated for their phytochemical and pharmacological potentials. Several types of bioactive compounds have been discovered from this family. They are steroids, terpenoids, flavonoids, phenolic acids, fatty acids, hydroxamic acids and alkaloids (Abdel Kader, Orabi, Sharma, & Assaf, 2016). Bamboo belongs to the family of Poaceae and one of the most valued medicinal plants (Wani, Prasad, & Prakash, 2019). Previous studies reported biological activities including anti-inflammatory, antimicrobial, immune-modulating effect, anti-stress, antioxidant and antimalarial activity of bamboo (Anigboro, 2018; Wani et al., 2019). In traditional Chinese medicine, bamboo leaves were used as a component to reduce the energy of "fire", and were usually used to treat hypertension, arteriosclerosis, and cardiovascular disease (Sun, Yue, Tang, & Guo, 2010).

Malaria, a tropical blood-borne protozoan disease caused by parasites of the genus *Plasmodium* is spread by female *Anopheles* mosquitoes. There are five types of *Plasmodium* causing malaria *viz*; *P. malariae, P. ovale, P. knowlesi, P. vivax,* and *P. falciparum* (Al-Adhroey, Nor, Al-Mekhlafi, Amran, & Mahmud, 2011). According to WHO (2019), in 2018, there were 228 million cases of malaria occurred worldwide. Fortunately, malaria disease in Malaysia was combated successfully, from 4164 cases in 2017 to zero cases last year (Malaysia Health Ministry, 2019).

On the other hand, tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (MTB) that usually affect the lungs when the airborne droplets nuclei inhaled in the lungs (WHO, 2015). TB is one of the leading causes of death in the world. From the WHO 2019 Global Tuberculosis Report, it was estimated that 1.5 million from the total of 10 million people with TB died in 2018. According to WHO, South-East Asian region are the most affected with TB cases (44 %),

followed by Africa (24 %), Western Pacific (18 %), Eastern Mediterranean (8 %), Americas (3 %) and Europe (3 %). In Malaysia, Health Ministry (2019) has reported that about 1500 to 2000 death by TB per year, with average of 6 deaths a day in 2018. Head of TB/ Leprosy sector, Dr Mohamed Naim Abdul Kadir also said that about 20000 to 25000 new TB cases are recorded in Malaysia each year. The increasing of cases mainly due to the delay in seeking medical treatment and low TB awareness among the public.

1.2 The Family of Poaceae

Member of family of Poaceae are classified as grasses, which are the fifth largest family of flowering plants and second most diverse family among the monocotyledons, also the most economically important and larger group of useful plants (Balcerek, Rak, Majtkowska, & Majtkowski, 2009; Campbell, 1985; Clark, 1990; Finot, Barrera, Marticorena, & Rojas, 2011). There are about 750-770 genera and 11000-12000 species of grasses (Kellogg, 2015; Soreng et al., 2017; Ullah, Khan, Ahmad, Zafar, & Ullah, 2011). They are mostly found in Southeast Asia, Africa, Australia, and North America (Campbell, 2016). Species from this family contain bioactive secondary metabolites including flavonoids, phenolic acids, triterpenes, saponins and sterols. Some of the species from Poaceae have been proved to show therapeutic effect including antioxidant, anti-ulcerogenic (Awaad, Mohamed, Maitland, & Soliman, 2008), antimicrobial (Singariya, Kumar, & Mourya, 2012), antifungal (Kanife, Odesanmi, & Doherty, 2012), anticancer (Hussein, Mostafa, Ghoneim, & El-Hela, 2015), antiflammatory (Adom & Liu, 2002), antiparasitic, antimalarial (Clarkson et al., 2004), anthelmintic (Kozan, Kupeli, & Yeslada, 2006), hepatoprotective (Shakya, Sharma, Saxena, & Shrivastava, 2012) and

antihyperglycemic activity (Balcerek et al., 2009; Karan, Pal, Mishra, & Mondal, 2013).

1.3 *Dendrocalamus* Genus and Medicinal Uses

Dendrocalamus genus is a large genus belongs to the subfamily Bambusoideae of Poaceae, which is a giant clumping bamboo with large branches and leaves, consisting of about 57 species (Nguyen & Xia, 2013). This genus is widely distributed in subtropical and tropical regions of Asia, especially in China, India, Burma, Thailand, Malaysia and Papua New Guinea (Schröder, S., 2010). Some of the species from genera of *Dendocalamus* have been made into healthy foods or pharmaceutical materials. For examples, the combination *D. strictus* leaf with *Curcuma longa* powder was cooked as decoction to treat cold, cough and fever (Kamble et al., 2010). Source of natural antioxidant can be found in leaf powder of *D. strictus* (Goyal, Middha, & Sen, 2011). In addition, it also can heal cut and wound (Mohapatra, Prusty, & Sahoo, 2008). Meanwhile, fermented succulent shoots of *D. strictus* are very useful in increase appetite, lowering the blood pressure and cholesterol (Sarangthem & Singh, 2003).

1.4 *Dendrocalamus asper*

Dendrocalamus asper (bamboo) (**Table 1.1**) is one of the *Dendrocalamus* genus belongs to the family of Poaceae and usually grown throughout the tropics and subtropics regions, especially in South China, North-eastern part of India, Thailand, Vietnam, Malaysia, Indonesia and Philippines (Banik, 2016). This species is usually found from lowlands to highlands in rich and heavy soils of humid regions (Chandramouli, Jagadish, & Viswanath, 2015). It commonly known as “buluh betung” or “Betung Hitam” in Malaysia (Ashaari & Mamat, 2000).

D. asper is a tall arborescent grass, which the colour of culm is dark green and the height is up to about 20-30 m, and the lower part was covered with a circle of rootlets. The culm internodes have a diameter between 8 to 20 cm, length of 20 to 45 cm and a thickness culm walls of 11-20 mm and covered with short brown hairs (**Figure 1.1**). Generally, the leaf length is between 15-30 cm, wide is between 10-25 mm and leaf-blades are lance-shaped (Rao, Willams, & Ramanatha Rao, 1998). The bamboo trees plays an important role in construction, making good-quality furniture, musical instruments, reinforcing fibers in paper, medicines and food sources (Banik, 2016; Singh, Dalal, Singh, Dhawan, & Kalia, 2012). The bamboo trees also possess various biological activities due to their antioxidant, antifungal (Fatriasari, Syafii, Wistara, Syamsu, & Prasetya, 2014), remedy of diarrhea in poultry, and antibacterial (Mulyono, Lay, Rahayu, & Yaprianti, 2012).

Table 1.1. Taxonomy classification of *Dendrocalamus asper*.

Taxonomy Classification	
Kingdom	Plantae
Phylum	Tracheophyta
Class	Liliopsida
Order	Poales
Family	Poaceae
Genus	Dendrocalamus
Scientific name	*Dendrocalamus asper*
Species authority	(Schult.) Backer
Common names	Giant bamboo, dragon bamboo, sweet bamboo

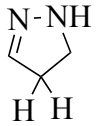
Figure 1.1: *Dendrocalamus asper* found near Cameron Highlands, Malaysia (Schröder, 2010)

Figure 1.2. The shoots of *Dendrocalamus asper* (Kendall, 2016)

Shoots of *D. asper* are young, new canes that are harvested for food when just emerged from the ground, generally 23-30 cm long (Choudhury, Sahu, & Sharma, 2012) (**Figure 1.2**). Bamboo shoots are crisp, tender and sweet which are used frequently in Asian Cuisine as an ethnic traditional food (Nirmala, David, & Sharma, 2017; Singh et al., 2012). It also having some health benefits including healthy weight

loss, antibacterial, cytotoxicity and anti-carcinogenic properties (Iwansyah, Kumalasari, Darmajana, & Ratnawati, 2019; Nirmala, Bisht, & Sheena, 2011).

1.5 Pyrazoline



Pyrazoline is a five-membered heterocyclic ring having an endocyclic double bond with two adjacent nitrogen atoms at position 1- and 2-. It can be found naturally and also can be produced through organic synthetic reactions. The first pyrazoline (4,5-dihydro-*1H*-pyrazole) was synthesized by Knorr in 1883. Pyrazolines are prominent for the stability of their ring system and the reactivity of several sites that permit a series of substitution reactions to take place. Structural modifications of pyrazolines can be achieved by decorating the stable fragments with different functional groups and aromatic scaffolds to afford benzylhydrazine moieties for the development of new potent compounds possessing biological activities. Previous studies reported that considerable biological activities are discovered when substitution is occurred at positions 1-, 3- and 5- of the pyrazoline such as anticonvulsant, antimalarial (G. Kumar et al., 2018), anticardiovascular (Malhotra, Pathak, Nath, Mukerjee, & Shanker, 2002), anticancer (Lv et al., 2011), antiamoebic (Abid, Bhat, Athar, & Azam, 2009), antimicrobial (Abdel-Wahad, Abdel-Aziz, & Ahmed, 2009) and antitumour activities (Ismaeil, Soliman, & Abd-El Monem, 2011). Meanwhile, carbothioamide derivatives were found to have significant pharmacological activities such as monoamine oxidase (MAO) inhibitors (Nayak et al., 2013; Upadhyay, Tripathi, Paliwal, & Saraf, 2017), antitubercular (Ahsan et al., 2011; Palleapati, Kancharlapalli, & Shaik, 2019) and anticonvulsant activities (Beyhan,

Kocyigit-Kaymakcioglu, Gümrü, & Aricioglu, 2017; Bhandari, Tripathi, & Saraf, 2013; Ozdemir, Kandilci, Gumusel, Calis, & Bilgin, 2007).

1.6 Problem Statement

Bamboo shoots are considered one of the widely acclaimed nutrient rich food items and medicine. It contains high content of protein, amino acids, minerals, fiber, carbohydrates and less cholesterol. Besides, the new phytochemical and pharmacological studies on different type species of bamboo shoot showed that the shoots have several biological activities such as antibacterial, antioxidant, antimalarial and anticancer (Awol, 2015; Fujimura, Ideguchi, Minami, Watanabe, & Tadera, 2005). Previously, there are many phytochemical and biological studies on different species of bamboo but limited research studies particularly on *Dendrocalamus asper* shoots (Kumalasari, Iwansyah, Ratnawati, Fitrianti, & Darmajana, 2019).

Drug-resistant malarial cases have increased dramatically over the past few years especially in the South East Asia which is a serious health concern (WHO, 2013). Despite the fact that Malaysia do not have any domestic malaria (indigenous) cases recorded since 2018, malaria continue to be one of the greatest health challenges to other south east Asian countries. The main drawbacks of the current treatment of malaria are the development of multiple drug resistance and the non-specific targeting to intracellular parasites. This is turn results in the requirement of high dose anti-parasitic drugs and subsequent intolerable toxicity. Hence, there is a need for novel chemotherapeutic agents.

TB is also another major health problems in the world, which the microbial infections potentially could cause death of human beings. It is estimated that 1.3 million lives among human immunodeficiency virus (HIV)-negative people died of

TB in 2016, which surpass the number of deaths caused by HIV. In addition, about 374 000 death cases of TB coinfected with HIV (WHO, 2017). Corbett, E. L. et al. 2006, reported that emergence anti-TB resistant and HIV are the vital contributors to death by TB. Recently, several drugs in the market used for TB treatment such as isoniazid (INH), rifampicin, pyrazinamide and delamanid, which were possess a nitrogen atom in their ring structure (Dixit et al., 2017). Electron-rich nitrogen heterocycles play vital role in various pharmacological activities (Cai et al., 2015). Previous study reported a new set of pyrazoline derivatives was synthesized and found significant antimycobacterial activity (Ahmad, Husain, Khan, Mujeed, & Bhandari, 2016). This information has prompted us to synthesize some new pyrazoline derivatives with possible anti-tuberculosis activity. We hope the derivatives could reduce the duration of the tuberculosis therapy and consequently reduce the adverse effects of the medication.

1.7 Research Objectives

This thesis consists of two parts. The research objectives are as below:

Part 1:

i) To extract, isolate and characterise the chemical constituents of bamboo shoots of *Dendrocalamus asper.*

ii) To study the anti-malarial activity found in the isolated compounds

Part 2:

i) To synthesise and characterise new pyrazoline derivatives (*N*-heterocyclic derivatives).

ii) To evaluate the *in vitro* anti-tuberculosis activity of the synthesised pyrazoline derivatives against *Mycobacterium tuberculosis* (MTB) H37Ra

1.8 Scope of Study

Part 1: This study focuses on the extraction, separation, fractionation, isolation and inhibitory studies of secondary metabolites isolated from *Dendrocalamus asper* shoots against *Plasmodium falciparum* 3D7. The *n*-hexane and dichloromethane extracts were subjected for fractionation using Medium Pressure Liquid Chromatography (MPLC), analysed by Analytical High-Pressure Liquid Chromatography (HPLC) and isolated by preparative High-Pressure Liquid Chromatography (Prep-HPLC) in Chemical Biology Research Group, Institute of Physical and Chemical Research (RIKEN), Japan. The isolated compounds were characterised by 1D and 2D NMR, FT-IR and UPLC/MS. All the isolated compounds were tested for antimalarial activity at Antibiotics Laboratory, RIKEN.

Part 2: This study focuses on the design, synthesis, characterisation and anti-TB studies of pyrazoline derivatives against *Mycobacterium tuberculosis* H37Ra. Pyrazoline derivatives were synthesised through cyclocondensation reaction and characterised using various spectroscopy techniques such as nuclear magnetic resonance (NMR) (1D and 2D) and Fourier transform infrared spectroscopic (FT-IR). Meanwhile, the high-resolution mass spectrometry (HRMS) analysis of all synthesised pyrazoline derivatives were conducted at Laboratorium Sentral, Universitas Padjadjaran, Sumedang, Indonesia. All the synthesised compounds were assayed *in vitro* for their anti-tuberculosis activities against *Mycobacterium tuberculosis* (MTB) strain at Biological Research Laboratory, School of Biological Sciences, Universiti Sains Malaysia.

CHAPTER 2

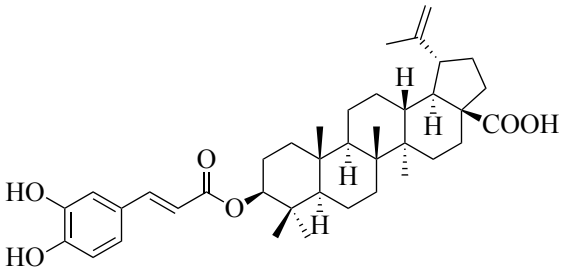
LITERATURE REVIEW

2.1 Previous Studies on Antimalarial Activity of Medicinal Plants

Natural products, in particular, plants play a prominent role in the discovery of leads for the development of drugs for the treatment of human diseases (Balunas & Kinghorn, 2005). It has been estimated that almost 50 % of present drugs are derived from plants of cultural importance (Tulp, Bruhn, & Bohlin, 2006). In the past decades, the exploration of natural products had been the main focus of scientists for the treatment of malaria (Anigboro, 2018).

In vitro antimalarial activity of the leaves extracts of *Phyllanthus emblica* and flower bud extracts of *Syzygium aromaticum* were evaluated against *Plasmodium falciparum* strain 3D7 (Bagavan, Rahuman, Kaushik, & Sahal, 2011). The results revealed that the leaves extract of *P. emblica* exhibited antimalarial activity against *P. falciparum* with 50 % inhibitory concentration (IC_{50}) values of 7.25 µg/mL (ethyl acetate extract) and 3.13 µg/mL (methanol extract). While the flower bud extract of *S. aromaticum* exhibited promising antimalarial activity against *P. falciparum* were ethyl acetate (IC_{50}: 13 µg/mL) and methanol (IC_{50}: 6.25 µg/mL).

Ma *et al*. (2008) reported that two compounds, betulinic acid 3-caffeate (**1**) and diospyrosin (**2**) were isolated from the up parts of *Disospyros quaesita*, which this plant in Laos known as “Muang Kout) and screened by in vitro antimalarial activity against *P. falciparum* clones D_6 (chloroquine-sensitive) and W_2 (chloroquine-resistant). According to the tested result, compound **1** demonstrated antimalarial activity against D_6 and W_2 clones with IC_{50} values of 1.40 and 0.98 µM, respectively (Ma et al., 2008).



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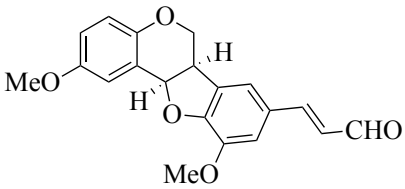

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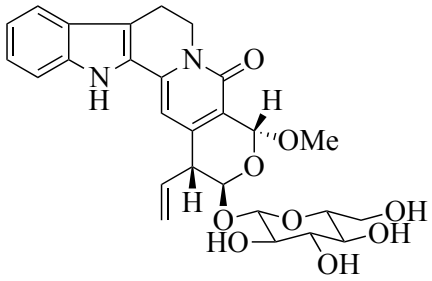
In addition, *in vitro* antiplasmodial activity of the leaves and fruits extracts of *Tamarindus indica* evaluated against *P. falciparum* (Koudouvo et al., 2011). The results indicated that the aqueous extract of fruit of *T. indica* was the most active (IC_{50} of 4.786 µg/mL) on the strains of *P. falciparum*. Meanwhile, methanol extract of fruit of *T. indica* was moderately active with IC_{50} value of 55.544 µg/mL. But both extracts of leave of *T. indica* (IC_{50} > 100 µg/mL) were devoid of intrinsic antiplasmodial activity.

Two novel compounds, naucleaorine (**3**) and epimethoxynaucleorine (**4**) with two known compounds: 3α,23-dihydroxyurs-12-en-28-oic acid (**5**) and oleanolic acid (**6**) were isolated from chloroform extract of the stems of *Nauclea orientalis* was estimated against *P. falciparum* clones D_6 (chloroquine-sensitive) and W_2 (chloroquine-resistant) strains (He et al., 2005). Compound **3**-**6** showed moderate *in vitro* activities against *P. falciparum* with IC_{50} values of 6.9/ 6.0 µM, 12.4/ 13.2 µM, 9.7/ 12.7 µM and 4.6/ 5.1 µM, respectively.

Two known alkaloids, 5-hydroxynoracronycine (**7**) and 1,5-dihydroxy-2,3-dimethoxy-10-methyl-9-aridone (**8**) were isolated from the methanol extract of the root barks of *Citropsis articulata* and tested for antimalarial activity against *P. falciparum*, which were identified as the best growth inhibitors of *P. falciparum* with

IC_{50} values of 2.8 μM and 10.0 μM, respectively (Lacroix, Prado, Kamoga, Kasenene, & Bodo, 2011).



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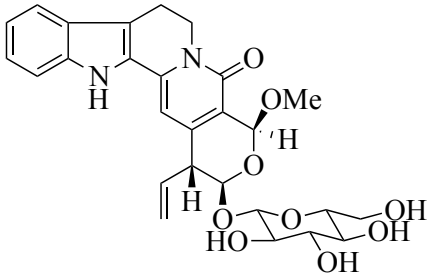

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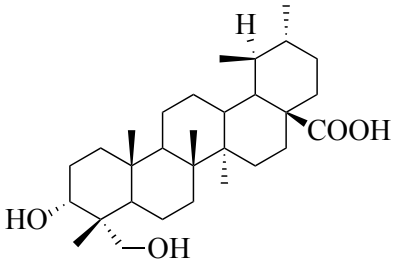

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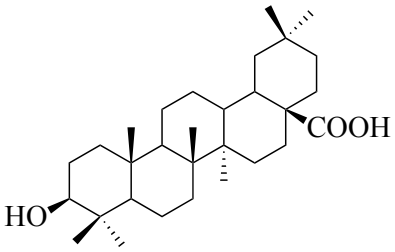

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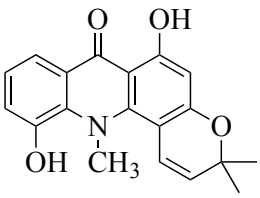

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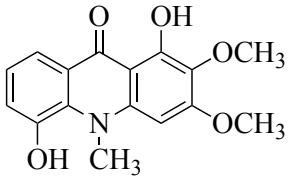

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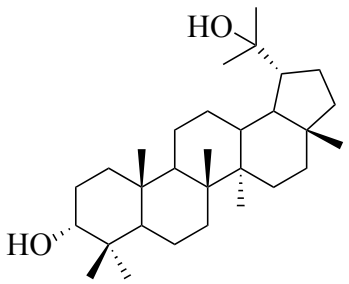
Besides that, ethanol extracts of *Dysoxylum caulostachyum* and *Garcinia celebica* leaves were tested against *P. falciparum* strain 3D7 (MRA-102, MR4, ATCC, Manassas, Va, USA) (Sofian et al., 2018). The tested result shows that the ethanol extract of *D. caulostachyum* leaf exhibited moderate to high antiplasmodial activity with IC_{50} value of 5.10 μg/mL, while the ethanol extract of *G. celebica* leaf was showed weak inhibition against *P. falciparum* (IC_{50} of 11.34 μg/mL).

Furthermore, *in vitro* antimalarial activity of five compounds: 3α,20-lupandiol (**9**), grewin (**10**), nitidanin (**11**), 2α,3β-dihydroxyolean-12-en-28-oic acid (**12**) and 2,6-dimethoxy-1-acetonylquinol (**13**) were isolate from the leaves, stems and twigs of *Grewia bilamellata* was estimated against *P. falciparum* clones D_6 (chloroquine-sensitive) and W_2 (chloroquine-resistant) strains which exhibited significant IC_{50} value ranging from 5.5-42.2 μM (Ma et al., 2006).



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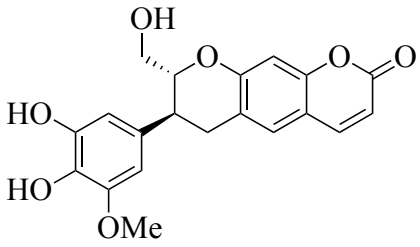

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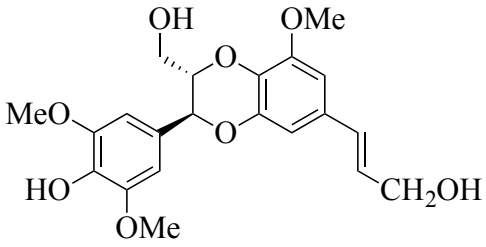

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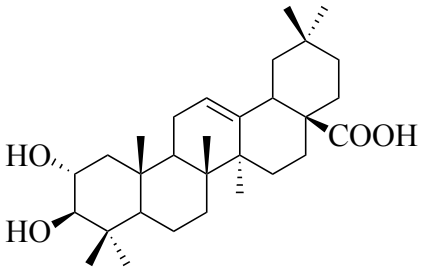

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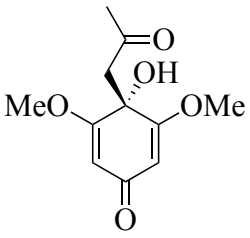
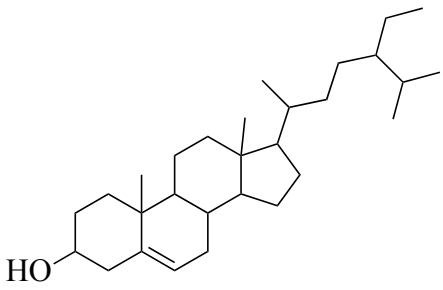


13

2.2 Previous Studies on Some *Dendrocalamus* Species

Several species from the genus *Dendrocalamus* have been investigated phytochemically. Ingudam et al., (2018) reported that the crude extract contained of three phytosterol compounds, β-sitosterol (**14**), campesterol (**15**) and stigmasterol (**16**) from the fermented products of bamboo shoot slices of *D. hamiltonii*. The crude and standard samples were subjected to thin layer chromatography and developed with Lieberman-Burchard reagent to confirm the presence of compounds **14-16**. Furthermore, compound **14** was isolated from crude extract by using TLC and HPLC techniques.



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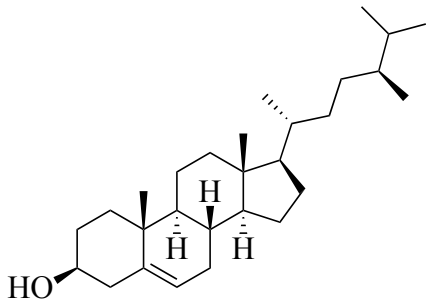

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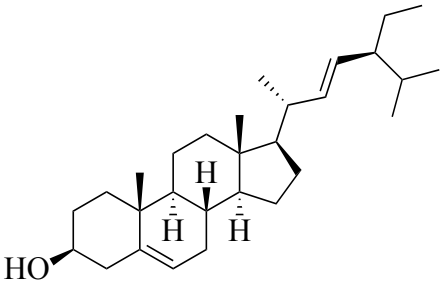

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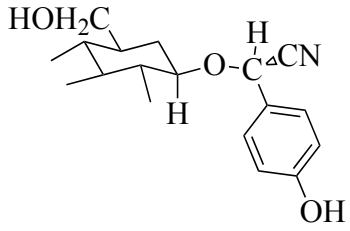
Phytochemical studies of the shoots of *D. giganteus* and *D. hamiltonii* by Schwarzmaier, (1977) had identified four compounds. All the four compounds which had been identified as taxiphyllin (**17**), L-asparagine (**18**), 4-hydroxybenzaldehyde (**19**) and β-sitosterol (**14**) are the main extractives of the shoots of *D. giganteus* and *D. hamiltonii*.



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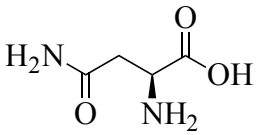

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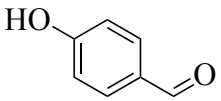
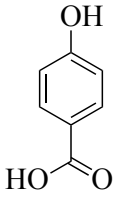


19

2.3 Previous Studies on *Dendrocalamus asper* Species

Zhang et al., (2018) reported that five secondary metabolites consisted of two phenolic compounds, p-hydroxybenzaldehyde (**19**) and 4-hydroxybenzoic acid (**20**), a ketosteroid, cholest-4-en-3-one (**21**) and two fatty acids; lauric acid (**22**) and palmitic acid (**23**) were isolated from the dichloromethane extract of *Dendrocalamus asper* bamboo shoots. Compound **19** was identified as one of the major second metabolism in shoots of *D. asper*. Compound **20** was used to test on potassium voltage-gated channel ($K_{V1.4}$). Compound **20** was able to inactive Kv1.4 at 10 μM by lowering the membrane potential and the abnormal neuronal firing being inhibited. Meanwhile, reduction of chloride current through γ-aminobutyric acid type A ($GABA_A$) of compound **19** on *Xenopus laevis* oocytes was evaluated by Zhang et al., (2017). The result showed that high concentration of compound **19** at 101.7 μM significantly reduced the GABA-induced chloride current of $GABA_A$ receptors. *Xenopus laevis* oocytes was used as in vitro model for the screening of compounds that modulate receptor activities.



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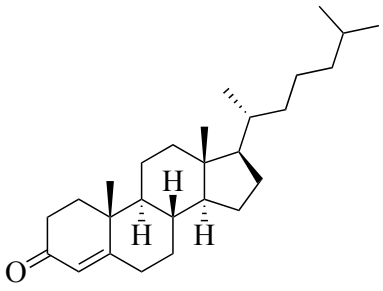

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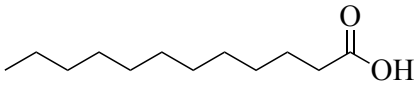

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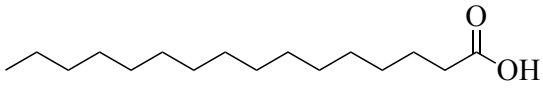
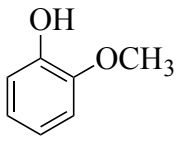


23

The chemical constituents in ethanolic and methanolic-ethanolic extracts of *D. asper* leaves were identified using pyrolysis-GC/MS (Mulyono *et al*., 2012). Pyrolysis-GC/MS predicted that methanol-ethanolic extract contained Guaiacol (0.43 %) (**24**), maltol (0.68 %) (**25**), 4-ethenyl-2-methoxyphenol (0.41 %) (**26**), 2,6-dimethoxyphenol (0.77 %) (**27**), phytol (7.89 %) (**28**) and 9,12,15-octadecatrien-1-ol (22.26 %) (**29**). Meanwhile, phenol (0.29 %) (**30**), 2,6-dimethoxyphenol (0.76 %) (**27**), 9,12,15-octadecatrienal (0.52 %) (**31**), myristaldehyde (0.36 %) (**32**), 4,8-dimethyl-1-nonanol (0.45 %) (**33**), hexahydrofarnesol (0.28 %) (**34**), and phytol (1.77 %) (**28**) contained in ethanolic extract of *D. asper* leaves.



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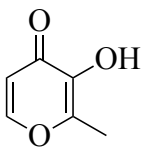

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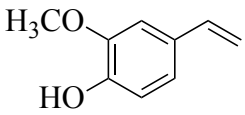

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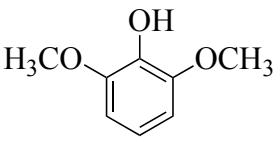

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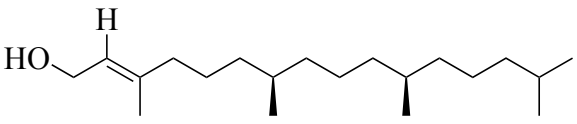

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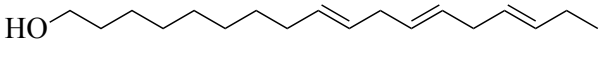

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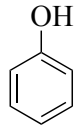

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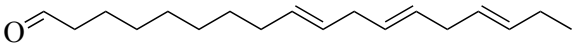

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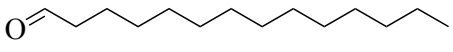

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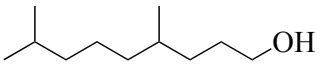

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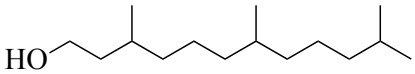
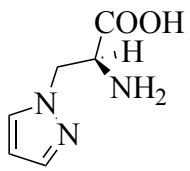


34

2.4 Previous Studies on Naturally Occurring of *N*-Heterocyclic Derivatives (Pyrazoles)

Pyrazole is a simple aromatic ring organic compound with the formula $C_3H_3N_2H$, which is a π-excessive heterocycle. This compound has a 5 membered ring containing three carbon atoms and two adjacent nitrogen atoms. Pyrazole can be found naturally and synthesized through condensation reaction. α-Amino-β-(pyrazolyl-*N*) propionic acid (**35**) was the first pyrazole discovered in nature in year 1959 by Noe et al., (1959) which was isolated from ethanol extract of watermelon seeds (*Citrullus vulgaris*). This compound is shown to have anti-diabetic activity.



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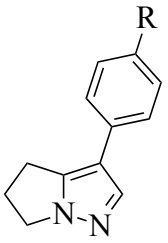
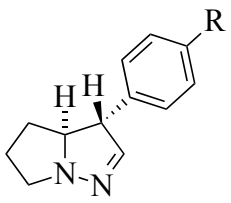
The root bark of *Newbouldia laevis* has resulted in the isolation of six pyrazole alkaloid compounds namely withasomnine (**36**), 4'-hydroxywithasomnine (**37**), 4'-

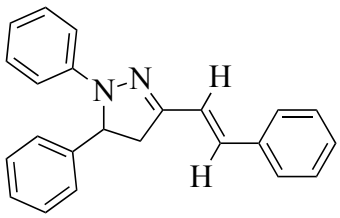
methoxywithasomnine (**38**), newbouldine (**39**), 4'-hydroxynewbouldine (**40**) and 4'-methoxynewbouldine (**41**) from methanol extract, in which compounds **38** and **41** were new natural products (Aladesanmi, Nia, & Nahrstedt, 1998). Compound **36** also originally isolated from the roots of Indian medicinal plants *Withania somnifera* in year 1966 (Schröter, Neumann, Katritzky, & Swinbourne, 1966). Meanwhile, another one novel alkaloid, 1,5-diphenyl-3-styryl-2-pyrazoline (**42**) was isolated from the aerial parts of *Euphorbia guyoniana* (Boudiar et al., 2010).



36 R = H
37 R = OH
38 R = OCH_3

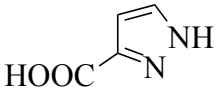


39 R = H
40 R = OH
41 R = OCH_3



42

In 1997, Parameswaran et al., (1997) reported that two novel pyrazole acids were isolated from methanol extract of the sponge *Tedania anhelans*. Two pyrazole acids identified spectroscopically as pyrazole-3(5)-carboxylic acid (**43**) and 4-methylpyrazole-3(5)-carboxylic acid (**44**), which are first time reported as natural products. Meanwhile, compound **44** also isolated from the butanol fraction of the marine sponge *Suberites vestigium* (Mishra, Wahidullah, & Kamat, 1998).



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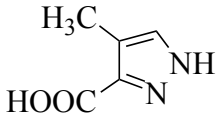
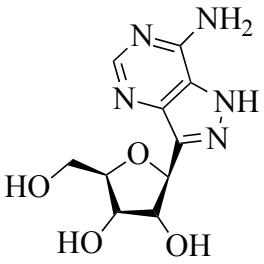


44

Two C-nucleoside antibiotics were isolated from culture filtrates of the rice mold *Nocardia interforma* which were formycin A (7-amino-3-(β-$_D$-ribofuranosyl)pyrazolo[4,3-*d*]-pyrimidine) (**45**) and formycin B (3-(β-$_D$-ribofuranosyl)pyrazolo[4,3-*d*]-pyrimidin-7-one) (**46**) (Hori et al., 1964). Other two C-nucleosides, pyrazomycin (**47**) and its α-α-anomer pyrazomycin B (**48**) were isolated from *Streptomyces candidus* fermentations. Compound **47** showed to inhibit an orotidine 5'monophosphate (OMP) decarboxylase but compound **48** did not inhibit OMP decarboxylase (Dis, Lehman, Jakubowski, Moyer, & Handschumacher, 1979; Elgemeie, Zaghary, Amin, & Nasr, 2005). Five fluviols (**49-53**) were isolated from strains of *Pseudomonas fluoresecens* var. pseudoiodinum (Smirnov, Kiprianova, Garagulya, Esipov, & Dovjenko, 1997).



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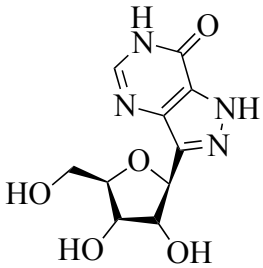

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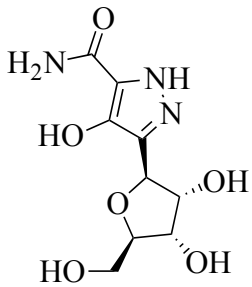

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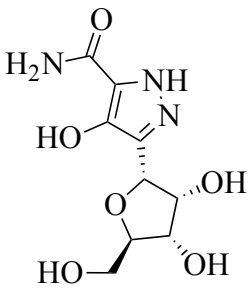

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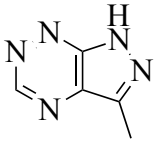

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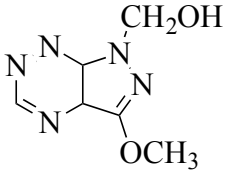

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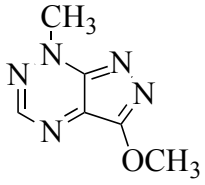

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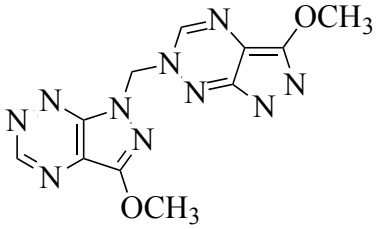

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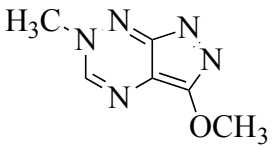

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2.5 Previous Studies on Pyrazoline Derivatives

2.5.1 Methods of the synthesis of pyrazoline derivatives

Pyrazoline was first discovered by Knorr in year 1883 (S. Jadhav, Kulkarni, Patil, Dhole, & Patil, 2016; Knorr, 1883). Pyrazolines is dihydropyrazole, heterocycles having an endocyclic double bond with two adjacent nitrogen atoms at position 1- and 2- within the five-membered ring, the most studied group of compounds in the azole family (Korrouchi et al., 2018) as shown in **Figure 2.1**.

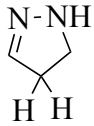
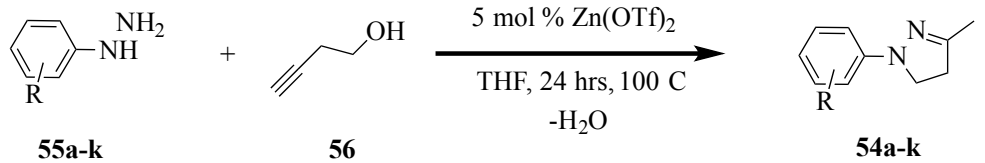


Figure 2.1. Chemical structure of 2-pyrazoline.

Alex et al., (2008) reported that synthesis of pyrazoline derivatives (**54a-k**) by using zinc triflate (5 mol %) as a catalyst in the reaction between phenylhydrazine derivatives (**55a-k**) and 3-butynol (**56**) in THF (3 mL) refluxed at 100 °C for 24 hrs to give excellent product yield (52-98 %) (**Scheme 2.1**) .

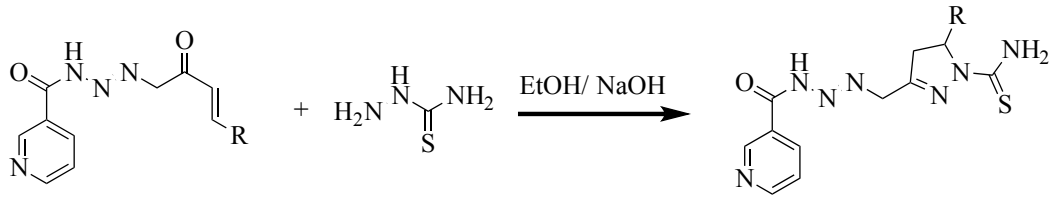


55a-k **56** **54a-k**

a R = H	**a** R = H (96 %)
b R = 4-CH_3	**b** R = 4-CH_3 (96 %)
c R = 2-CH_3	**c** R = 2-CH_3 (88 %)
d R = 4-Cl	**d** R = 4-Cl (98 %)
e R = 2-Cl	**e** R = 2-Cl (52 %)
f R = 4-Br	**f** R = 4-Br (99 %)
g R = 4-CN	**g** R = 4-CN (96%)
h R = 4-(O=S=O)CH_3	**h** R = 4-(O=S=O)CH_3 (87 %)
i R = 4-$CH(CH_3)_2$	**i** R = 4-$CH(CH_3)_2$ (99 %)
j R = 3-Cl,4-Cl	**j** R = 3-Cl,4-Cl (98 %)
k R = 3-Cl, 5-Cl	**k** R = 3-Cl, 5-Cl (97 %)

Scheme 2.1. Reaction of Arylhydrazine (**55a-k**) with 3-butyno1 (**56**) to various substituted pyrazolines (**54a-k**) with the percentage of yield (%)

According to Valarmathy et al., (2010) reported that 3-(β-picolinoylaminoazo methyl-5-aromatic substituted)-1-thioamide-2-pyrazoline derivatives (**57a-e**) were synthesised from the combination of 1-(β-picolinoylaminoazo)-3-benzylidene propan-2-one derivatives (**58a-e**) and thiosemicarbazide (**59**) with NaOH as catalyst in ethanol. (**Scheme 2.2**).



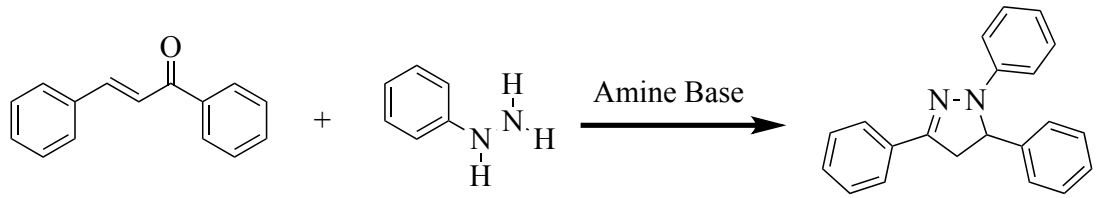
58a-e **59**

57a R = 4-$CH_3(C_2H_5)$
57b R = 2-$OH(C_2H_5)$
57c R = C_2H_5
57d R = 4-CH_3-5-$NH(C_2H_5)$
57e R = 4-OH-3-$OCH_3(C_2H_5)$

Scheme 2.2. Synthesis of 2-pyrazoline derivatives (**57a-e**)

Mahe´ et al., (2009) reported that high yield of 1,3,5-triphenyl-2-pyrazoline (**60**), 86 % and 93 % were synthesised between chalcone (0.25 mmol) (**61**) with acetylhydrazine (1.1 equiv) (**62**) in the presence 0.1 equiv and 0.2 equiv of 1,5,7-triazabicyclo[4.4.0]dec-5-one (TBD), respectively (**Scheme 2.3**). Percentage yield of

compound **60** was higher than other amine base. The results shown in **Table 2.1**. TBD is a cheap and commercially available guanidine as base catalyst to synthesize pyrazoline derivatives compared with several base catalysts such as 1,4-diazabicyclo[2.2.2]octane (DABCO), 4-dimethylaminopyridine (DMAP), quinuclidine, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), N,N,N'N'-tetramethylguanidine (TMG) and 7-methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene (MTBD).



61 **62** **60**

Scheme 2.3. Synthesis of 1,3,5-triphenyl-2-pyrazoline (**60**) by chalcone (**61**) with acetylhydrazine (**62**) in presence of various amine bases

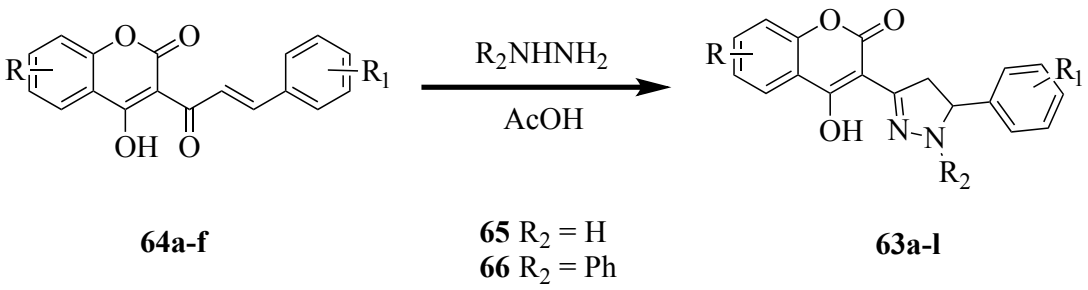
Table 2.1. Synthesis of 1,3,5-triphenyl-2-pyrazoline (**60**) with different amine base

Entry	Amine base (0.1 equiv)	Product (%)
1	-	0
2	DABCO	0
3	DMAP	0
4	Quinuclidine	0
5	DBU	5
6	TMG	3
7	MTBD	11
8	TBD	86
9	*TBD	93

Entry 1-8 = 0.1 equiv of TBD in dry toluene at 60 °C for 17 hours
*TBD = 0.2 equiv of TBD in dry toluene at 60 °C for 23 hours

3[1,3,5-Trisubstituted-4,5-dihydro-*1H*-3-pyrazolyl]-4-hydroxy-*2H*-2-chromenone derivatives (**63a-l**) was synthesised by reacting 4-hydroxycoumarin

chalcone derivatives (**64a-f**) in alcohol-glacial acetic acid with hydrazine hydrate (**65**)/ phenylhydrazine (**66**) as shown in **Scheme 2.4** and **Table 2.2** (Asad et al., 2014).



64a-f **65** R_2 = H, **66** R_2 = Ph **63a-l**

Scheme 2.4. Synthesis of compounds **63**(**a-l**)

Table 2.2. % Yields and melting points of various substituted pyrazoline compounds (**63a-l**) (Asad et al., 2014)

Compound **63**	R	R_1	R_2	Yield (%)	MP (°C)
a	6,7-H	2,4-Cl_2	H	72	244
b	6,7-H	2,4-Cl_2	Ph	63	192
c	6,7-$(Me)_2$	2,4-$(OH)_2$	H	71	248
d	6,7-$(Me)_2$	2,4-$(OH)_2$	Ph	73	200
e	6,7-$(Me)_2$	2,4-OH-3-MeO	H	73	288
f	6,7-$(Me)_2$	2,4-OH-3-MeO	Ph	75	204
g	6,7-H	4-CF_3	H	89	238
h	6,7-H	4-CF_3	Ph	77	158
i	6,7-$(Me)_2$	4-CF_3	H	74	240
j	6,7-$(Me)_2$	4-CF_3	Ph	83	180
k	6,7-H	2-Pyridyl	H	84	264
l	6,7-H	2-Pyridyl	Ph	78	188

1,3,5-Triarylpyrazolines (**67**) was synthesised through the condensation of chalcone (**61**) and *p*-[4-(tert-butyl)phenyl]hydrazine (**68**) in the presence of copper triflate and 1-butyl-3-methylimidazolium hexafluorophosphate,[bmim](PF_6) as a catalyst which was described by Rao et al., (2013). The pyrazole (**69**) was synthesized after oxidation *in situ* of compound **67** with good yield (82 %). The catalyst can be reused more than four cycles without much loss in the catalytic activity (**Scheme 2.5**).