SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF NOVEL COUMARINYL AZO-CHALCONES

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Special dedication to my beloved parents, Hassan Asmuri and Hanizah Zainuddin.

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ABSTRACT

Coumarinyl azo-chalcone is one of the coumarin derivatives containing moiety of chalcone. This coumarinyl azo-chalcones were prepared by introduction of substituents ($N(CH_2CH_3)_2$, OH, Br and OCH_3) on coumarin ring. The coumarinyl azo-chalcones **(122-126)** were synthesised by Claisen-Schmidt condensation reaction of coumarin and benzaldehydes derivatives. The coumarin precursors have been prepared through Knoevenagel Condensation reaction yielded 3-acetyl-7-(diethylamino)coumarin **(111)**, 3-acetyl-7-hydroxycoumarin **(112)**, 3-acetyl-6-bromocoumarin **(113)** and 3-acetyl-7-methoxycoumarin **(114)**. Meanwhile, benzaldehydes derivatives were prepared by coupling reaction of azo compound under cold condition, afforded 2-hydroxy-5-((4'-nitrophenyl)diazenyl)benzaldehyde **(118)**, 2-hydroxy-5-((4'-chlorophenyl)diazenyl)benzaldehyde **(119)**, and 2-hydroxy-5-((4'-methylphenyl)diazenyl)benzaldehyde **(120)**. Five coumarinyl azo-chalcones were synthesised and identified as 3-((2*E*)-(3-(2'-hydroxy-5'-((4''-nitrophenyl)diazenyl)phenyl)acryloyl)-6-bromo-2*H*-chromen-2-one **(122)**, 3-((2*E*)-(3-(2'-hydroxy-5'-((4''-chlorophenyl)diazenyl)phenyl)acryloyl)-6-bromo-2*H*-chrom-en-2-one **(123)**, 3-((2*E*)-(3-(2'-hydroxy-5'-((4''-methylphenyl)diazenyl)-phenyl)acryloyl)-6-bromo-2*H*-chrom-en-2-one **(124)**, 3-((2*E*)-(3-(2'-hydroxy-5'-((4''-chlorophenyl)diazenyl)phenyl)acryloyl)-7-methoxy-2*H*-chromen-2-one **(125)**, and 3-((2*E*)-(3-(2'-hydroxy-5'-((4''-methylphenyl)diazenyl)phenyl)acryloyl)-7-methoxy-2*H*-chromen-2-one **(126)**. The compounds obtained were characterized by infrared and nuclear magnetic resonance (1D and 2D NMR) spectroscopy. All coumarinyl azo-chalcones **(122-126)** were tested for the antibacterial activity against Gram-positive and Gram-negative bacteria. Coumarinyl azo-chalcone **(124)** showed moderate activity towards all Gram-positive and Gram-negative bacteria.

ABSTRAK

Kumarinil azo-kalkon adalah salah satu terbitan kumarin yang mempunyai struktur kalkon. Kumarinil azo-kalkon ini telah dihasilkan dengan memasukkan kumpulan gantian ($N(CH_2CH_3)_2$, OH, Br and OCH_3) pada lingkaran kumarin. Kumarinil azo-kalkon **(122-126)** telah dihasilkan melalui tindak balas kondensasi Claisen-Schmidt antara pelbagai terbitan kumarin dan benzaldehid. Kumarin telah disintesis terlebih dahulu melalui tindak balas kondensasi Knoevenagel bagi menghasilkan 3-asetil-7-(dietilamino)kumarin **(111)**, 3-asetil-7-hidroksikumarin **(112)**, 3-asetil-6-bromokumarin **(113)** dan 3-asetil-7-metoksikumarin **(114)**. Sementara itu, terbitan benzaldehid telah dihasilkan melalui tindak balas gandingan sebatian azo di dalam keadaan sejuk bagi menghasilkan 2-hidroksi-5-((4'-nitrofenil)diazenil)benzaldehid **(118)**, 2-hidroksi-5-((4'-klorofenil)-diazenil)benzaldehid **(119)**, dan 2-hidroksi-5-((4'-methylfenil)diazenil)benzaldehid **(120)**. Lima kumarinil azo-kalkon telah dihasilkan dan dikenali sebagai 3-((2*E*)-(3-(2'-hidroksi-5'-((4''-nitrofenil)diazenil)fenil)akriloil)-6-bromo-2*H*-kromen-2-on **(122)**, 3-((2*E*)-(3-(2'-hidroksi-5'-((4''-klorofenil)diazenil)fenil)akriloil)-6-bromo-2*H*-kromen-2-on **(123)**, 3-((2*E*)-(3-(2'-hidroksi-5'-((4''-metilfenil)diazenil)fenil)akriloil)-6-bromo-2*H*-kro-men-2-on **(124)**, 3-((2*E*)-(3-(2'-hidroksi-5'-((4''-klorofenil)diazenil)-fenil)akriloil)-7-metoksi-2*H*-kromen-2-on **(125)**, dan 3-((2*E*)-(3-(2'-hidroksi-5'-((4''-metilfenil)diazenil)fenil)akriloil)-7-metoksi-2*H*-chromen-2-on **(126)**. Sebatian yang dihasilkan telah dicirikan dengan menggunakan spektroskopi infra merah dan resonan magnet nukleus (RMN 1D dan 2D). Kesemua kumarinil azo-kalkon **(122-126)** yang dihasilkan telah diuji aktiviti antibakteria terhadap Gram-positif dan Gram-negatif bakteria. Kumarinil azo-kalkon **(124)** menunjukkan aktiviti sederhana terhadap semua Gram-positif dan Gram-negatif bakteria.

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LIST OF ABBREVIATIONS/SYMBOLS

δ	-	Chemical shift
ATR	-	Attenuated
^{13}C	-	Carbon-13
$CHCl_3$	-	Chloroform
CH_2Cl_2	-	Dichloromethane
CH_3CN	-	Acetonitrile
cm^{-1}	-	per centimeter
d	-	doublet
dd	-	doublet of doublet
DMSO	-	Dimethyl sulfoxide
EtOH	-	Ethanol
EtOH (abs)	-	Absolute ethanol
EtOAc	-	Diethyl ether
h	-	Hour
HCl	-	Hydrochloric acid
H_2SO_4	-	Sulfuric acid
H_2O	-	Water
HMBC	-	Heteronuclear Multiple Bond Correlation
HMQC	-	Heteronuclear Multiple Quantum Coherence
Hz	-	Hertz
IR	-	Infrared
J	-	Coupling constant
KOH	-	Potassium hydroxide
L	-	Liter
MeOH	-	Methanol
MgO	-	Magnesium oxide
MW	-	Microwave
NaOH	-	Sodium hydroxide

$POCl_3$	-	Phosporus oxychloride
lit.	-	Literature
µg	-	Microgram
mg	-	Milligram
ml	-	Milliliter
m.p.	-	Melting point
ppm	-	part per million
R_f	-	Retention factor
t	-	triplet
TLC	-	Thin Layer Chromatography

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Coumarin also known as ‘Coumarou’ is one of the organic compounds that can be found either in synthetic form or naturally isolated from the natural sources such as plant or animal [1]. Coumarin can be categoriesed as a member of the benzopyrone group, contain aromatic ring bonded to pyrone ring [2]. Coumarin is a phenolic substance which contains of α-pyrone rings with fused benzene [3].

Chalcone is a major class of flavonoids, widely distributed throughout the plants such as fruits, vegetables, soy and tea [4]. Chalcone consist of open-chain flavonoids with the two aromatic rings linked by a three-carbon α, β-unsaturated carbonyl system. Chalcone can be identified as the most important precursors in the biosynthesis of flavonoids and isoflavonoids [4-5].

Coumarin-chalcone is one of the coumarin derivatives containing chalcone moiety. Coumarin-chalcone is new innovation inspired by Vazquez-Rodriguez and collaborators to synthesis coumarin-chalcone using coumarin and chalcone moieties as the starting materials [6]. According to Vazquez-Rodriguez, the idea to hybridise coumarin and chalcone moieties is to improve and enhance antioxidant and trypanocidal activity against *Trypanosoma cruzi*. This is due to both coumarin and chalcone have been reported to show good pharmalogical properties such as antimicrobial, antitumor, antioxidant, antimalarial and antifungal activities [6-7].

Moreover, coumarin-chalcone derivatives give several important commercial fluorescent brightening agents and used as fluorescent dyes for synthetic fibres [8]. Fluorescent dyes which has been synthesised from coumarin-chalcone containing electron donating group at carbon 7-position of the coumarin rings enhance and improve the fluorescent properties of coumarin-chalcone [9]. The application of coumarin-chalcone had been reported either in perfumery, cosmetics, agrochemical or pharmaceutical industries [10].

1.2 Problem Statement

Synthesis of coumarin-chalcone is not well explored by the researcher, however these analogous have diverse applications in various industry include cosmetics, perfumery and pharmaceutical industries. Based on previous study, coumarinyl azo-chalcone with no substituents attached at coumarin ring and antimicrobial assays was successively studied by Harshal A. Deshpande *et al.* [11]. Thus, modification of coumarinyl azo-chalcone by the introduction of substituents ($N(CH_2CH_3)_2$, OH, Br and OCH_3) on coumarin ring is conducted to investigate its antibacterial activity.

1.3 Objectives of the Study

The purposes of this study are :

1. To synthesise and characterise coumarinyl azo-chalcone with the presence of substituents ($N(CH_2CH_3)_2$, OH, Br and OCH_3) on coumarin ring.

2. To evaluate antibacterial assay of coumarinyl azo-chalcone derivatives.

1.4 Scope of the Study

Based on the previous study, unsubstituted coumarinyl azo-chalcone was successfully synthesised. Thus this study focus on the modification of unsubstituted coumarinyl azo-chalcone by introducing substituents ($N(CH_2CH_3)_2$, OH, Br and OCH_3) on the coumarin ring. New coumarinyl azo-chalcones were synthesised by Claisen-Schmidt condensation reaction. The antibacterial assay was tested against two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli).* The synthesised compounds were elucidated by spectroscopic techniques using infrared (IR), ^{1}H, ^{13}C, COSY, HMQC and HMBC NMR spectroscopy.

1.5 Significance of Study

The purpose of this study is to synthesis coumarinyl azo-chalcone containing substituents ($N(CH_2CH_3)_2$, OH, Br and OCH_3) on the coumarin ring. The implication of this study is to observe the antibacterial activity of modified coumarinyl azo-chalcone by introduction of substituents ($N(CH_2CH_3)_2$, OH, Br and OCH_3) on the coumarin rings.

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