

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF NOVEL COUMARINYL
AZO-CHALCONES

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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF NOVEL COUMARINYL
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Special dedication to my beloved parents, Hassan Asmuri and Hanizah Zainuddin.

My siblings, my teachers, my supervisors, my beloved friends and all lab members.

*For all your love, care support, and believe in me.
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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*-chromen-2-one (**123**), 3-((2*E*)-(3-(2'-hydroxy-5'-((4"-methylphenyl)diazenyl)phenyl)acryloyl)-6-bromo-2*H*-chromen-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)benzaldehyd (**118**), 2-hidroksi-5-((4'-klorofenil)diazenil)benzaldehyd (**119**), dan 2-hidroksi-5-((4'-metilfenil)diazenil)benzaldehyd (**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*-kromen-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
<i>J</i>	- Coupling constant
KOH	- Potassium hydroxide
L	- Liter
MeOH	- Methanol
MgO	- Magnesium oxide
MW	- Microwave
NaOH	- Sodium hydroxide

POCl_3	- Phosphorus 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 categorised 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 ($\text{N}(\text{CH}_2\text{CH}_3)_2$, 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), ^1H , ^{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 ($\text{N}(\text{CH}_2\text{CH}_3)_2$, 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 ($\text{N}(\text{CH}_2\text{CH}_3)_2$, OH, Br and OCH_3) on the coumarin rings.

REFERENCES

1. Lacy, A., & O'Kennedy, R. (2004). Studies on coumarins and coumarin-related compounds to determine their therapeutic role in the treatment of cancer. *Current Pharmaceutical Design*. **10** (30), 3797-3811.
2. Rohini, K., & Srikumar, P. S. (2014). Therapeutic role of coumarins and coumarin-related compounds. *Journal of Thermodynamics & Catalysis*. **5** (2), 1-3.
3. Venugopala, K. N., Rashmi, V., and Odhav, B. (2013). Review on natural coumarin lead compounds for their pharmacological activity. *BioMed Research International*. 1-14.
4. Dyrager, C. (2012). Design and synthesis of chalcone and chromone derivatives as novel anticancer agents. (Doctoral dissertation, University of Gothenburg).
5. Syam, S., Abdelwahab, S. I., Al-Mamary, M. A., and Mohan, S. (2012). Synthesis Of Chalcones With Anticancer Activities. *Molecules*. **17**(6), 6179-6195.
6. Vazquez-Rodriguez, S., Figueroa-Guñez, R., Matos, M. J., Santana, L., Uriarte, E., Lapier, M., Diego Maya, j., & Olea-Azar, C. (2013). Synthesis of coumarin–chalcone hybrids and evaluation of their antioxidant and trypanocidal properties. *Medicinal Chemistry Communications* **4** (6), 993-1000.

7. Mazzone, G., Malaj, N., Galano, A., Russo, N., & Toscano, M. (2015). Antioxidant properties of several coumarin–chalcone hybrids from theoretical insights. *RSC Advances*. **5** (1), 565-575.
8. Christie, R. M., and Lui, C. H. (1999). Studies of fluorescent dyes: part 1. an investigation of the electronic spectral properties of substituted coumarins. *Dyes and Pigments*. **42** (1), 85-93.
9. Ahmed K.A., E.-M. M. M., Abdel-Mottaleb M.S.A., Mohamed S. Attia and El-Saadany S. (2013). Synthesis and evaluation of novel fluorescent dyes using microwave. *Research Journal of Chemical Sciences*. **3** (4), 3-18.
10. Bezwada, R. S. (2008). Chemistry of Coumarins. *Indofine Chemical Company*.
11. Deshpande, H. A., Chopde, H. N., Pandhurnekar, C. P., & Batra, R. J. (2013). Synthesis, characterization and testing of biological activity of some novel chalcones derivatives of coumarin. *Chemical Science Transactions*. **2** (2), 621-627.
12. Ojala, T. (2001). *Biological screening of plant coumarins* (pp. 42-45). Helsinki, Finland: University of Helsinki.
13. Dong, F., Jian, C., Kai, G., Qunrong, S., & Zuliang, L. (2008). Synthesis of coumarins via pechmann reaction in water catalyzed by acyclic acidic ionic liquids. *Catalysis Letters*. **121** (3-4), 255-259.
14. Patil, C. B., Mahajan, S. K., & Katti, S. A. (2009). Chalcone-A Versatile Molecule. *Journal of Chemical and Pharmaceutical Research*. **1** (25), 11-22.

15. Suwito, H., Jumina, M., Kristanti, A. N., & Puspaningsih, N. N. T. (2014). Chalcones: Synthesis, structure diversity and pharmacological aspects. *Journal of Chemical and Pharmaceutical Research*. **6** (5), 1076-1088.
16. Berar, U. (2012). Chalcones: compounds possessing a diversity in applications. *Orbital-The Electronic Journal of Chemistry*. **4** (3), 209-221.
17. Kumar, R., Saha, A., & Saha, D. (2012). A new antifungal coumarin from *Clausena excavata*. *Fitoterapia*. **83** (1), 230-233.
18. Lekphrom, R., Kanokmedhakul, S., Kukongviriyapan, V., & Kanokmedhakul, K. (2011). C-7 oxygenated coumarins from the fruits of *Micromelum minutum*. *Archives of Pharmacal Research*. **34** (4), 527-531.
19. Jamil, S., Abdullah, S. A., Lathiff, S. M. A., & Sirat, H. M. (2014). Tyrosinase Inhibitory Activity of Flavonoids from *Artocarpus Lowii* King. *Jurnal Teknologi*. **71**(1), 55-58.
20. Rees, K. A., Bermudez, C., Edwards, D. J., Elliott, A. G., Ripen, J. E., Seta, C., Tiong & Butler, M. S. (2015). Flemingian-Type Prenylated Chalcones from the Sarawak Rainforest Plant *Desmodium congestum*. *Journal of Natural Products*. **78** (8), 2141-2144.
21. Vekariya, R. H., & Patel, H. D. (2014). Recent Advances in the Synthesis of Coumarin Derivatives via Knoevenagel Condensation: A Review. *Synthetic Communications*. **44** (19), 2756-2788.
22. Song, A., Wang, X., & Lam, K. S. (2003). A convenient synthesis of coumarin-3-carboxylic acids via Knoevenagel condensation of Meldrum's acid with ortho-hydroxyaryl aldehydes or ketones. *Tetrahedron Letters*. **44** (9), 1755-1758.

23. Shockravi, A., Shargi, H., Valizadeh, H., & Heravi, M. M. (2002). Solvent free synthesis of coumarins. *Phosphorus, Sulfur, and Silicon and the related Elements*. **177** (11), 2555-2559.
24. Stanchev, S., Penkova, A., Retailleau, P., Avdeev, G., & Manolov, I. (2008). Crystal Structure of 3-Acetyl-6-methoxy-2*H*-1-benzopyran-2-one. *Analytical Sciences: X-ray Structure Analysis Online*. **24**, 183-184.
25. Borges, F., Roleira, F., Milhazes, N., Santana, L., & Uriarte, E. (2005). Simple coumarins and analogues in medicinal chemistry: occurrence, synthesis and biological activity. *Current Medicinal Chemistry*. **12** (8), 887-916.
26. Joshi, R., & Chudasama, U. (2008). Synthesis of coumarins via the pechmann condensation using inorganic ion exchangers as solid acid catalysts. *Jornal of Scientific and Industrial Research*. **67**, 1092-1097.
27. El-Fattah, M. A., El-Kady, M. Y., El-Rayes, S. M., & Khalil, M. (2010, November). Synthesis and Biological Evaluation of Some Coumarin Derivatives. In *14th International Electronic Conference of Synthetic Organic Chemistry*
28. Zanger, M., & McKee, J. R. (1995). *Small scale syntheses: a laboratory textbook of organic chemistry*. Wm. C. Brown Publishers.
29. Hatamjafari, F. (2014). Synthesis of Coumarin Derivatives Using Glutamic Acid Under Solvent-Free Conditions. *Oriental Journal of Chemistry*. **30** (2), 863-865.

30. Choudhary, A. N., & Juyal, V. (2011). Synthesis of chalcone and their derivatives as antimicrobial agents. *International Journal of Pharmacy and Pharmaceutical Sciences*. **3** (3), 12-128.
31. Hsieh, C. T., Hsieh, T. J., El-Shazly, M., Chuang, D. W., Tsai, Y. H., Yen, C. T., Wu, S. F., Wu, Y. C. & Chang, F. R. (2012). Synthesis of chalcone derivatives as potential anti-diabetic agents. *Bioorganic & Medicinal Chemistry Letters*. **22** (12), 3912-3915.
32. Gupta, R., Gupta, N., & Jain, A. (2010). Improved synthesis of chalcones and pyrazolines under ultrasonic irradiation. *Indian Journal of Chemistry. Section B, Organic Including Medicinal*. **49** (3), 351-355
33. Jayapal, M. R., & Sreedhar, N. Y. (2010). Synthesis and characterization of 4-hydroxychalcones by aldol condensation using $\text{SOCl}_2/\text{EtOH}$. *International Journal of Current Pharmaceutical Research*. **2** (4), 60-62.
34. Seedhar, N. Y., Jayapal, M. R., Prasad, K. S., & Prasad, P. R. (2010). Synthesis and characterization of 4-hydroxy chalcones using PEG-400 as a recyclable solvent. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. **1**, 480-485.
35. Jayapal, M., & Sreedhar, N. Synthesis of 2, 4-dihydroxy substituted chalcones using silica sulphuric acid reagent under solvent free conditions. (2011). *Asian Journal of Pharmaceutical and Clinical Research*. **4** (1), 106-108
36. Vazquez-Rodrigueza, S., Serraa, S., Santosb, Y., & Santanaa, L. (2010). Efficient synthesis of coumarin-chalcones hybrids as new scaffold with antibacterial interest. *Chemistry Letter*. **30** (2), 110-111.

37. Alaqel, S., Imran, M., El-Feky, S. A., & Khan, S. A. (2013). Synthesis and antimicrobial activity of 3-(substituted phenyl)-1-(7-substituted coumarin-3-yl) prop-2-ene-1-ones. *Journal of Chemical & Pharmaceutical Research*. **5** (12), 1089-1093.
38. Al-Ayed, A. S. (2011). Synthesis of new substituted chromen [4, 3-c] pyrazol-4-ones and their antioxidant activities. *Molecules*. **16** (12), 10292-10302.
39. Hamdi, N., Bouabdallah, F., Romerosa, A., & Benhassen, R. (2010). Expedious synthesis for α , β -unsaturated coumarin derivatives using boran chelates: A novel class of potential antibacterial and antioxidant agents. *Comptes Rendus Chimie*. **13** (10), 1261-1268.
40. Siddiqui, Z. N. (2015). A convenient synthesis of coumarinyl chalcones using $\text{HClO}_4\text{-SiO}_2$: A green approach. *Arabian Journal of Chemistry*.
41. Gholap, S. S., Deshmukh, U. P., & Tambe, M. S. (2013). Synthesis and in-vitro antimicrobial screening of 3-cinnamoyl coumarin and 3-[3-(1H-indol-2-yl)-3-aryl-propanoyl]-2H-chromen-2-ones. *Iranian Journal of Catalysis*. **3** (3), 171-176.
42. Sun, Y. F., & Cui, Y. P. (2008). The synthesis, characterization and properties of coumarin-based chromophores containing a chalcone moiety. *Dyes and Pigments*. **78** (1), 65-76.
43. Li, X., Zhao, Y., Wang, T., Shi, M., & Wu, F. (2007). Coumarin derivatives with enhanced two-photon absorption cross-sections. *Dyes and pigments*. **74** (1), 108-112.

44. Kok, T. W., & Basar, N. (2012). Coumarins *via* Knoevenagel condensation reaction (KCR) and Pechmann condensation reaction. *Jurnal Teknologi*. **57** (1), 83-98.
45. Guo, Q., Liu, M. L., Feng, L. S., Lv, K., Guan, Y., Guo, H. Y., & Xiao, C. L. (2011). Synthesis and in-vitro antimycobacterial activity of fluoroquinolone derivatives containing a coumarin moiety. *Arch. Pharm. Chem. Life Sci.* **344** (12), 802-809.
46. Odabaşoğlu, M., Albayrak, Ç., Özkanca, R., Aykan, F. Z., & Lonecke, P. (2007). Some polyhydroxy azo–azomethine derivatives of salicylaldehyde: Synthesis, characterization, spectroscopic, molecular structure and antimicrobial activity studies. *Journal of Molecular Structure*. **840** (1), 71-89.
47. Perumal, S., Pillai, S., Cai, L. W., Mahmud, R., & Ramanathan, S. (2012). Determination of minimum inhibitory concentration of *Euphorbia hirta* (L.) extracts by tetrazolium microplate assay. *Journal of Natural Products*. **5**, 68-76.
48. Marçal, F. J. B., Cortez, D. A. G., Ueda-Nakamura, T., & Nakamura, C. V. (2010). Activity of the extracts and neolignans from *Piper regnellii* against methicillin-resistant *Staphylococcus aureus* (MRSA). *Molecules*, **15** (4), 2060-2069.
49. Souza, S. M. D., Monache, F. D., & Smânia, A. (2005). Antibacterial activity of coumarins. *Zeitschrift fuer Naturforschung C*, **60**(9-10), 693-700.
50. Chapin, K. C. and Lauderdale, T. Reagents, Stains and Media: Bacteriology. In: Murray, P. R. Baron, E. J., Jorgensen, J. H., Tenover, M. C. and Tenover, R. C. (ed.). (2003) *Manual of Clinical Microbiology*. 8th. ed. Washington, D.C.: ASM Press. 358.

51. Eloff, J. N. (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta medica*. **64** (8), 711-713.