UNIVERSITI PUTRA MALAYSIA

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF NEW AZO COUMARIN COMPOUNDS

YOUSIF ISMAEL SAEED

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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF NEW AZO COUMARIN COMPOUNDS

By

YOUSIF ISMAEL SAEED

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

March 2018

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DEDICATION

*To my beloved **Father & Mother***
Who have always loved me unconditionally and whose good examples have taught me to work hard for the things that I aspire to achieve.

*To **Narmen**,*
My wonderful wife,
Who has been a wonderful support and encouragement during the challenges of study and life, I am really thankful for having you in my life, and I always express my love & respect for you.

To my dear children
***Mabast, Halbast & Liyan**, I always express me thankful to Allah for giving me these presents to enlighten my life*

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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YOUSIF ISMAEL SAEED

March 2018

Chairman : Professor Mansor B. Ahmad, PhD
Faculty : Science

The development of new heterocyclic based azo dye derivatives is becoming the significant attraction of medicinal chemists. Azo compounds consist of at least a conjugated chromophore azo (-N=N-) group in connection with one or more aromatic or heterocyclic systems. Finding that bacterial and fungal resistance and toxicity of drugs, there is a focus on the insistent need to discover new antibiotics to inhibit these human pathogenic infections. Therefore, there is an urgent need to design and develop new drugs by synthesis of analogues and modifications in existing compounds. For this purpose a new, 2-hydroxy-5-(aryl) diazenyl) benzaldehyde was obtained by the diazotization of different aromatic amines followed by coupling with salicylaldehyde, which is generally carried out at a low temperature. The new prepared compounds were reacted with ethylacetoacetate in the presence of piperidine and glacial acetic acid as catalyst via Knovenagel condensation to form new azo coumarin compounds. The structures of all the newly synthesized compounds have been characterized and confirmed by TLC and spectroscopic methods, such as FTIR, ^{1}H-NMR, ^{13}C-NMR and mass spectrometric analysis. Subsequently, the bioactivity evaluation was done for all synthesized drugs against different human pathogenic infections, such as two gram-positive bacteria (*Staphylococcus aureus* S273*, Bacillus subtilis* B29) and two gram-negative bacteria (*Escherichia coli* E266*, Acinetobacter anitratus* A9) with one fungal strain (*Aspergillus brilliance* ATCC 16404*)*. Furthermore, from the investigation of antimicrobial screening data it is clearly evident that most of the synthesized compounds exhibited significant to moderate antibacterial activity against tested microorganisms as compared to the standard drugs, whereas, some of them exhibited weak activity. Among all the new compounds tested 2-hydroxy-5- ((mercapto-1,3,4-thiadiazol-2-yl diazenyl) benzaldehyde, 3-acetyl-6-((5-mercapto-1,3,4-thiadiazol-2-yl) diazenyl)-2H-chrome-2-one and 2-hydroxy-5-((2,4- dinitrophenyl) diazenyl) benzaldehyde were more potent as compared to the standard drug streptomycin. In

addition, 2-hydroxy-5-((2,4-dinitrophenyl) diazenyl) benzaldehyde exhibited moderate potency against the tested fungus compared to nystatin. In general, the synthesized compounds were active against all bacteria strains and exhibited greater activity compared with the standard drug. Finally, it was concluded that the newly synthesized compounds were able to inhibit the growth of certain microbes, which were successfully characterized, and *in vitro* antimicrobial activities were explored so that they may be used for drug developments in the future.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

SINTESIS, PENCIRIAN DAN AKTIVITI BIOLOGI SEBATIAN BARU AZO KUMARIN

Oleh

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Mac 2018

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Pengembangan derivatif berasaskan azo pewarna heterosiklik baru menjadi tarikan ketara ahli kimia perubatan. Sebatian Azo terdiri daripada sekurang-kurangnya kumpulan azo kromofor konjugasi (-N = N-) yang berkaitan dengan satu atau lebih sistem aromatik atau heterosiklik. Oleh kerana rintangan bakteria dan kulat yang diperolehi dan ketoksikan ubat terdapat tumpuan kepada keperluan yang mendesak untuk mencari antibiotik baru bagi menghalang mereka bentuk dan membangunkan ubat-ubatan baru dengan sintesis analog dan pengubahsuaian sebatian sedia ada. Bagi tujuan ini, 2-hidroksi-5-(aril) diazenil) benzaldehid telah diperolehi melalui diazotizasiamina aromatik yang berbeza diikuti oleh gandingan dengan salisilaldehid yang biasanya dijalankan pada paras suhu rendah. Sebatian yang disediakan telah ditindakbalas dengan etilasetoasetat dengan kehadiran piperidin dan asid asetik glasial sebagai pemangkin melalui pemeluwapan Knovenagel bagi membentuk sebatian azo kumarin baru. Struktur semua sebatian yang baru disintesis telah dicirikan dan disahkan oleh kaedah TLC dan spektroskopik, seperti FTIR, ^{1}H-NMR, ^{13}C-NMR dan analisis spektrometer massa. Selepas itu, penilaian kebioaktifan diuji untuk semua ubat yang disintesis terhadap jangkitan patogen manusia yang berbeza seperti dua bacteria gram-positif *(Staphylococcus aureus S273, Bacillus subtilis* B29*)* dan dua bakteria gram-negatif (*Escherichia coli* E266*, Acinetobacter anitratus* A9*)* dengan satu jenis kulat (*Aspergillus brilliance* ATCC 16404). Tambahan pula, dari penyiasatan data penapisan antimikrob adalah jelas bahawa kebanyakan sebatian yang disintesis menunjukkan aktiviti anti-bakteria yang signifikan hingga sederhana terhadap mikroorganisma yang diuji dibandingkan dengan ubat-ubatan piawai, sedangkan sebahagiannya memperlihatkan aktiviti lemah. Di antara semua sebatian yang diuji 2-hidroksi-5- ((merkapto-1, 3, 4-tiadiazol-2-ildiazenil) benzaldehid, dan 3-asetil-6 - ((5-merkapto-1, 3, 4-tiadiazol-2-il) diazenil) -2H-krom-2-on dipaparkan aktiviti tinggi serta 2-hidroksi-5-(2,4-dinitrofenil) diazenil)

benzaldehid terhadap mikroorganisma yang diuji dibandingkan dengan streptomisin ubat piawai. Di samping itu, 2-hidroksi-5-((2, 4-dinitrofenil) diazenil) benzaldehid dipamerkan berkuasa sederhana terhadap kulat yang diuji berbanding dengan ubat Nystatin.

Secara umum, sebatian yang disintesis itu aktif terhadap semua jenis bakteria dan menunjukkan aktiviti hebat berbanding ubat standard. Akhirnya, kesimpulan dicapai bahawa sebatian-sebatian yang baru disintesis itu mampu menghalang pertumbuhan mikrob tertentu, yang berjaya dicirikan, dan aktiviti antimikrob mereka *Invitro* telah diteroka supaya mereka boleh digunakanuntuk pembangunan ubat-ubatan pada masa depan.

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In the name of Allah, the most gracious, the most merciful peace and blessings of prophet Mohammed. First and foremost, my sincere thanks and praise is due to the almighty Allah for giving me the life and health to witness the completion of this tedious and challenging program. My appreciation goes to my supervisory team chaired by Professor Mansor B. Ahmad for his patience, moral support, and academic guidance, finding time to go through the research work and for making valuable suggestions, despite his schedules which have contributed greatly in my effort to finish this work. I wish to express my deep and sincere gratitude to my two co-supervisors, Dr. Siti Mariam Mohd Nor and Dr. Siti Munirah Mohd Faudzi for their constructive criticisms and useful suggestions offered throughout the period of this work despite their tight schedules they guided me in many issues and helped me with kind patience. I would like to use this opportunity to express my profound gratitude and love to my father and my mother for they are showing me the right way in my life. Special gratitude to my sisters, brothers and also brothers and sister in-law for their prayers, support throughout my study. I am highly thankful to Prof. Dr. Issam Ahmad Mohammed for his guidance, sincere efforts and brilliant ideas to pursue my research work. I am deeply grateful to my lovely wife Narmen Hasan and my children for their patience and endurance during my absence really; they are the inspiration for all words I have written in this thesis. Finally, my special thanks also, go to my gratitude to Kurdistan Regional Government (KRG) for supporting me to have the opportunity to get a higher degree in Universiti Putra Malaysia (UPM).

I certify that a Thesis Examination Committee has met on 15 March 2018 to conduct the final examination of Yousif Ismael Saeed on his thesis entitled "Synthesis,Characterization and Biological Activity of New Azo Coumarin Compounds" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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This is to confirm that:

- the research conducted and writing of this thesis was under our supervision;
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Signature: _____
Name of Member of Supervisory Committee: Dr. Siti Mariam Binti Mohd Nor

Signature: _____
Name of Member of Supervisory Committee: Dr. Siti Munirah Mohd Faudz

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Δ	Chemical Shift
Ar	Aryl Group
Aq	Aqueous solution
^{13}CNMR	Carbon Nuclear Magnetic Resonance
d	Doublet
EVD	External Ventricular Drain
DMSO	Dimethylsulfoxide
EVD	External Ventricular Drain
FTIR	Fourier Transformation Infrared
J	NMR Coupling Constant
H1N1	Swine Flu Virus
HIV	Human Immunodeficiency Virus
^{1}HNMR	Proton Nuclear Magnetic resonance
m	meta
NA	Nutrient agar
m/z	Mass to Charge Ratio
PDA	Potato Dextrose Agar
s	Singlet
t	Triplet
TB	Tuberculosis
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
UV	Ultraviolet Light

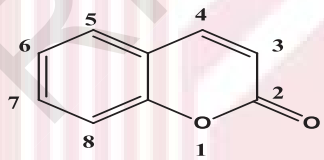
CHAPTER 1

INTRODUCTION

1.1 Coumarin

Heterocyclic chemistry is a major and fast developing research area in organic chemistry. Coumarin is considered as a heterocyclic organic compound, which plays a vital role in natural products and synthetic organic chemistry; this compound can be obtained from several plants, including the tonka bean (Bahekar and Shinde 2004). The curbed name of coumarins is derived from the category 'coumarou', the vernacular name of the tonka bean (*Dipteryx odorata* Willd, Fabaceae) (Jain and Joshi, 2012; Singh, 2017). The compound releases a sweet odour, and has thus been used for perfumes since 1882.

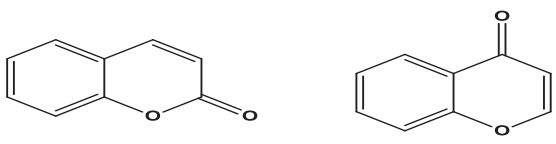
Coumarins and their derivatives are the simplest molecule family belonging to the group of benzopyrones, which consist of a benzene ring joined to a pyrone nucleus and classified under a vast class of phenolic substances (Ravi Shankar *et al.*, 2013). The chemical structure of coumarin (with an IUPAC name of 2H-chrome-2-one, also known as 2H-1-benzopyran-2-one) is shown in Figure 1.1.



Coumarin

Figure 1.1 : Structure of coumarin

Benzopyrone is a class under the benzopyrone family of compounds and consists of combined pyrone ring and benzene nucleus. Benzo-α-pyrone normally knew as coumarin, and the benzo-γ-pyrone of which the flavonoids are general members referred to as chromone (Figure 11.2).



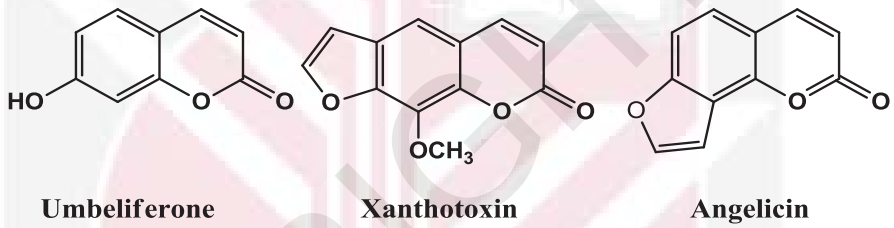
Coumarin **Chromone**

Figure 1.2 : Structures of coumarin and chromone

Coumarins are widely used as additives in food, perfumes, cosmetics, pharmaceuticals, dispersed fluorescent and laser dyes, insecticides and optical brighteners (Aslam *et al*., 2010). Many coumarins are found in plants and possess synthetic analogs, which have been shown to exert a remarkably broad spectrum of biological activities, including antibacterial (Nikpassand *et al*., 2014), antifungal, anticoagulant, anti-inflammatory, antitumor and anti-human immunodeficiency virus (anti-HIV) activities (Dekić *et al*., 2010).

1.1.1 Types of coumarin

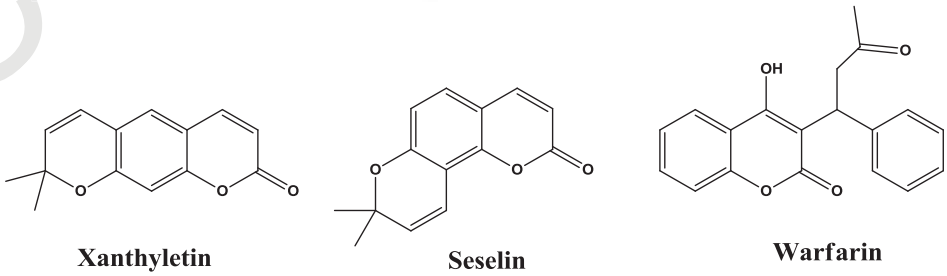
Coumarin can be divided into four different main groups. The first class is known as simple coumarin, which is alkoxylated, hydroxylated or alkylated on the benzene ring (e. g. Umbelliferone). The second class is referred to as the furanocoumarins, which contain a five-membered furan rig joined to a substituted coumarin moiety. Examples include linear furanocoumarins (e.g. Xanthotoxin) and Angular furanocoumarins (e. g. Angelicin) (Figure 1.3)



Umbeliferone **Xanthotoxin** **Angelicin**

Figure 1.3 : Examples of different classes of coumarin

The third class is known as pyranocoumarin, which is analogous to the furano coumarin, but harbours a six-membered ring. The compounds include the liner furanocoumarins such as xanthyletin and the angular furanocoumarins such as seselin. The last class comprises the pyrone-substituted coumarins. An example of this type is warfarin (Kayal *et al*., 2014) (Figure 1.4).



Xanthyletin **Seselin** **Warfarin**

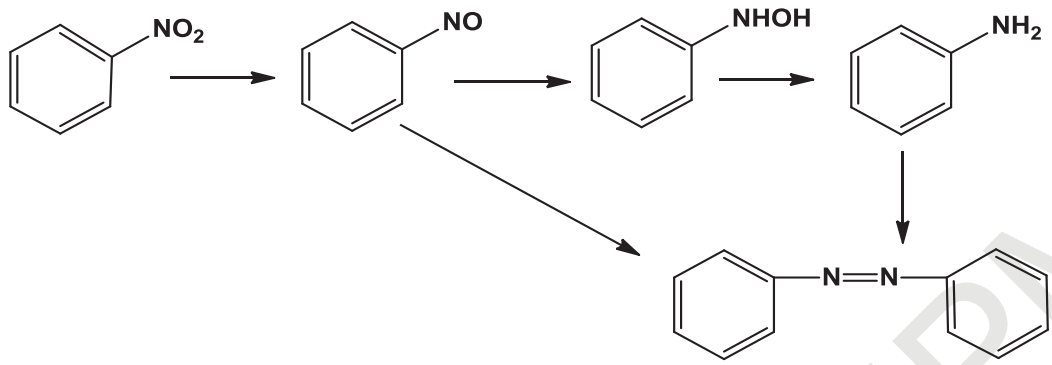
Figure 1.4 : Structures of seselin, xanthyletin, and warfarin

1.1.2 Sources of coumarin

To date, over 1300 kinds of coumarins have been recognized, essentially in the form of secondary metabolites in green plants, fungi and bacteria (Iranshahi *et al*., 2009). The tonka bean (*D. odorata*) is a special plant that contains coumarin (Gleye *et* al., 2003), when coumarin was first isolated from the tonka bean and reported by Vogel since 1820 (Nandy *et al*., 2012; Ibrahim *et al*., 2005). They are usually found free or in combination with sugars as glycosides (Molnar *et al.*, 2012.) in many higher plants, especially those of the Umbelliferae, Rosaceae and Rutaceae families. Also, some fundamental oils can be exist highly level of coumarins, such as cinnamon bark oil, cassia leaf oil, and lavender oil as well as found in some fruits such as bilberry, cloudberry, green tea and other foods like chicory (Asif, 2015) followed by the roots (*Ferulago campestris*), leaves (*Murraya paniculata*) (Venugopala *et al*., 2013). Moreover, Several microbial source species have been used to separate several coumarin types, such as novobiocin and coumermycin from *Streptomyces* and aflatoxins from *Aspergillus* (Lacy & O'Kennedy, 2004). Classically, various chemical procedures, such as the Perkin, Pechmann or Knoevenagel reactions, are carried out to synthesize coumarin (Borges *et al.*, 2005). Recently, other suitable methods, such as the Wittig, Kostanecki–Robinson and Reformatsky reactions, have been applied to produce coumarin (Nikpassand *et al*., 2013). In 1868, William Henry Perkin was the first chemist to successfully synthesize a series of coumarins.

1.2 Azo compounds

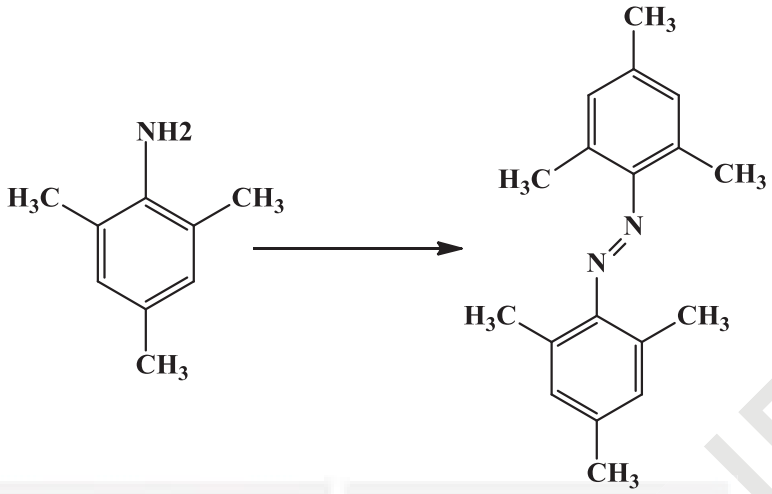
Azo compounds comprise a class of chemical compounds attracting scientific research attention (Otutu, 2013; Kirkan, B. & Gup, R., 2008). This class is a large group of dyes containing –N=N– connected with two or more aromatic or heterocyclic systems (Bamoniri *et al*.,2014; Asniza *et al*., 2009). Aromatic azo compounds are intermediates in the reduction of the nitro group to the amino or reverse reactions – the oxidation of the amino group to the nitro one (scheme 1.1). In both cases, an excess of a reducer or oxidant agent is used and the conditions are appropriately chosen to receive the product in the shortest time, with the best yield, and at minimum work and consumption costs. Thus, stopping the reaction sequence at the corresponding transition product (azo) as the main target and then its isolation conditions must be accurately selected (Węglarz-Tomczak & Górecki, 2012).



Scheme 1.1 :The general reduction reaction of the Nitro compounds to the amino derivatives

Methods of their synthesis rely on the use of a suitable oxidative/reductive reactions or diazotization/coupling reaction. Subsequent to these early investigations a number of modified procedures have been developed for syntheses of diazo-compounds, as direct method: a Metallic nitrite is added in the solution of strongly basic amines in aqueous mineral acids (Rasheed, 2011). Inverted method: Alkaline solutions of metallic nitrite and salt of sulphonated or carboxylated aryl amines when reacted with excess of cold mineral acid. Witt method: Metabisulphite is added in the solution of aryl amine in nitric acid. Knoevenagel method: Alkyl nitrite or ester of nitrous acid is added to a solution of salts of aryl amine in water or alcohol or any other inert solvent .General Method: Diazo compounds can be prepared by other methods as well which include:

1- Oxidation reactions for the synthesis of diazo compound (the reactions are carried out in attendance of oxidizing agents such as mercuric oxide, bromine, nitrous acid, mercuric acetate, nitrogen trioxide).
2- Reduction reaction for the synthesis of diazo compound (nitrous acid, hydroxylamine, acetyl chloride is reducing agents used to prepare diazo compounds). An instance of oxidative diazo-compound formation of azo compounds by oxidation method using $KMnO_4$ and $CuSO_4.5H_2O$ (Scheme1.2).



Scheme 1.2 : Preparation of 1,2-bis(2,4,6-trimethylphenyl) diazene

1.2.1 Types of azo compounds

From the chemistry viewpoint, the azo compounds can be classified into different types depending on the number of azo moieties present in the molecule (i.e. monoazo, diazo and triazo compounds) (Santra *et al*., 1999; Neha & Patni, 2016). Monoazo dyes (e.g. Orange II) contain only one N=N double bond (Figure 1.5). even as diazo and triazo dyes include two and three N=N double bonds such as acid black I (Figure 1.6) , correspondingly (Al-Rubaie and Mhessn, 2012),In reaction of diazonium salts with phenols, the product is substituted at the para position to the hydroxyl group. If this site is occupied the ortho isomer is obtained.

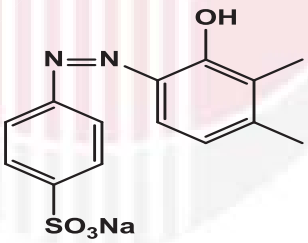


Figure 1.5 : Orange II

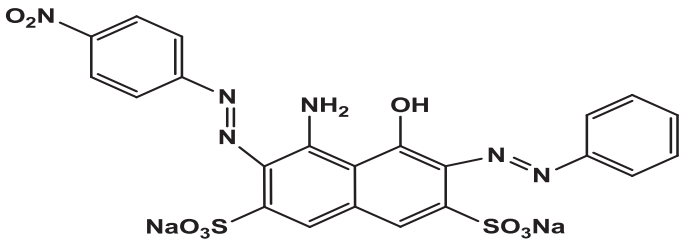


Figure 1.6 : Acid black I

1.2.2 Source and application of azo compounds

Azo dyes do not occur in nature and are produced only through chemical synthesis (Chakraborty, 2009; Prajapati *et al*., 2013). They are synthesized by diazotization reaction of a primary aromatic amine and coupled with one or more nucleophiles The modern industry started since the discovery of the 'Mauve' dyestuff by Henry Perkin in 1856 while searching for malaria treatment. In 1858, Griess discovered the diazotization and coupling of azo dyes in fibres (Mościpan *et al*., 2016). Literature survey shows that azo dyes have been used in dyeing textile fibres, biomedical studies and organic synthesis; as antibacterials (Daniel, 1962; Masoud *et al.*, 2003) and antifungals (Raghavendra *et al*., 2013); in medicines (Wainwright, 2008), cosmetics, paints and plastics; and in shipbuilding, the automobile industry, cable manufacture (Khdem &Mageed, 2013) and optical storage technology. Furthermore, azo dyes have been studied widely because of their excellent thermal and optical properties in applications such as optical recording medium (Sameih *et al*., 2008; Kirkan & Gup, 2008) toner, ink-jet printing and oil-soluble lightfast (Refat *et al.*, 2013). These substances are important intermediary products in organic synthesis and initiators in polymer chemistry (Swati *et al*., 2011; Bicer and Arat, 2009). Azo dyes occupy an integral part in microbiology (Wainwright and Wainwright, 2001). They are also identified to be involved in biological reactions, such as inhibition of DNA, RNA and protein synthesis; carcinogenesis; and nitrogen fixation (Al-Sheikh *et al.,* 2014; Park *et al*., 2007; District, 2012; Goyal *et al*., 1998).

1.3 Azo coumarin

Azo coumarin has become an important class of heterocyclic compounds in drug research. Combining coumarin with azo dyes to obtain a novel azo coumarin dye may attract interesting to study of heterocyclic coumarin moieties (Figure 1.7). In general, various ways are available for synthesizing azo coumarin. For instance, diazotization of primary aromatic amine then coupling with unsubstituted coumarin as coupling component (Atanasoaie *et al.*, 2009), or substituted coumarin (Yazdanbakhsh *et al*., 2007)) . The other route is by the diazotization of primary aromatic amines, followed by coupling with one or more nucleophiles (such as aldehyde) to form an azo compound and then adding an ester in the presence of piperidine via condensation reaction (Shao, 2010). They are used for various applications such as disperse dyes for dyeing polyester fabrics and evaluation of their fastness properties (Metwally *et al.,* 2013), and have an important roles in the anti-breast cancer activity (Ibrahim *et al*., 2016).The biological importance of azo-coumarins and their derivatives is well known for their use as s anti-viral, anticancer, anticoagulant, antioxidant activities antibiotics, antineoplastic, anti-diabetics, antibacterial and antifungal activities (Gopi *et al.*, 2011).

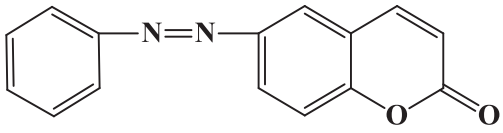


Figure 1.7 : General structure of azo coumarin

1.4 Problem statement

Infectious diseases, such as HIV, swine flu virus (H1N1), external ventricular drain infection, Ebola virus disease and tuberculosis, are the leading cause of sickness and fatality worldwide. Drug-resistant strains of bacterial and fungal pathogens have prompted the expedition for new drugs acting both as antibacterial and antifungal agents. Over the past decades, antibiotic resistant bacteria have been found increasingly which has become a global concern (Blez *et al*., 2013). The resistance to antimicrobial agents related to the effect changes of a chromosome or the exchange of genetic material via plasmids and transposons. Currently, almost all of the synthesized drug derivatives have been used for practical purposes, and synthetic medicinal chemistry has reached a broad scope. Generally, optimistic results of infectious disease research show that antimicrobial chemotherapy can prevent diseases and overcome the above-mentioned problem in the near future. The approach for the treatment of microbial infections has led towards the development of new drugs. It could be possible either by synthesis of analogues, modifications in existing compounds, or searching novel structures, for employing in the pursuit of biological assays. In the current work, we used convenient methods such as direct method to synthesis new azo compounds and Knovenagel condensation for azo coumarin compounds then compared their antibacterial and antifungal activities.

1.5 Objectives of study

1 - To synthesize a series of 5-(substituted-phenyl azo) -2-hydroxy-benzaldehyde intermediates and new azo coumarin compounds;

2 – To characterise the synthesized new compounds by spectroscopic methods, such as Fourier transform infrared (FTIR), ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR and mass spectrometry (MS).

3 - To investigate antibacterials and antifungals activities of the new synthesized compounds.

1.6 Significance of the research

One of the most threatened to the global are the infectious fatal diseases which are a leading cause of illness and death throughout the world. In recent years, the researchers are finding that bacteria are mutating and developing new strains that are resistant to known antibiotics. So the search is on for new antibiotics that will protect the human race from bacterial infection in the future. Malaysia as apart from the global, thus the Malaysian scientific communities are supporting the researchers for searching to design new compounds, which possess potential activities against microbial. Therefore, in this proposed research, the research will synthesize new azo compounds, which is promising to play a role to inhibit the bacteria as well as the fungi as proved from the literature survey. Furthermore, a simple technique with low cost of the raw materials will be used in synthetic route. In this regard, this research summarizes new azocoumarin compounds obtained by coupling coumarin with various diazotized aromatic amines. The use of coumarin as a coupling component is new; until now, the literature data mention that only the substituted coumarin derivatives were used in coupling reactions with diazotized amines.

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