NOVEL SYNTHESIS OF CYANOACETYLHYDRAZONE DERIVATIES AND THEIR ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES

PUAH PERNG YANG

THIS DISSERTATION IS SUBMITTED AS A PARTIAL REQUIREMENT TO OBTAIN DEGREE OF BACHELOR OF SCIENCE WITH HONOURS

INDUSTRIAL CHEMISTRY PROGRAMME
FACULTY OF SCIENCE AND NATURAL RESOURCES
UNIVERSITI MALAYSIA SABAH

2014

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS

JUDUL: NOVEL SYNTHESIS OF CYANOACETYLHYDRAZONE DERIVATIVES AND THEIR ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES.

IJAZAH: IJAZAH SARJANA MUDA DENGAN SAINS DENGAN KEPUJIAN KIMIA INDUSTRI

SAYA: PUAH PERNG YANG (HURUF BESAR) SESI PENGAJIAN: _____

Mengaku membenarkan tesis *(LPSM/Sarjana/Doktor Falsafah) ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:-

1. Tesis adalah hakmilik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. Sila tandakan (/)

[ ] SULIT (Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di AKTA RAHSIA RASMI 1972)

[ ] TERHAD (Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana Penyelidikan dijalankan)

[ ] TIDAK TERHAD

Yang

(TANDATANGAN PENULIS)

Alamat tetap: 46, Lorong Jantung 9,
Taman Jantung 14100,
Simpang Ampat,
Pulau Pinang.

Disahkan oleh:

NURULAIN BINTI ISMAIL
LIBRARIAN
UNIVERSITI MALAYSIA SABAH

(TANDATANGAN PENYELIA)

Dr. Mohammad Shaheen Khan.

NAMA PENYELIA

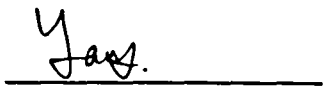
Tarikh: 27/6/2014.

Tarikh: _____

Catatan :- * Potong yang tidak berkenaan.
*Jika tesis ini SULIT atau TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.
*Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana Secara penyelidikan atau disertai bagi pengajian secara kerja kursus dan Laporan Projek Sarjana Muda (LPSM)

DECLARATION

I declare that this dissertation is based on my original work except for questions and summaries, each of which has been fully acknowledged.



PUAH PERNG YANG

(BS 1111 0551)

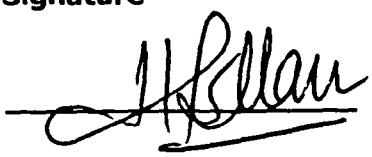
21ST May 2014

VERIFICATION

Signature

SUPERVISOR

(Dr. Mohammad Shaheen Khan)



ACKNOWLEDGEMENT

First of all, I would like to express my gratitude to God who gave me the spirit and strength in completing my dissertation. I would also like to thank all people who had assisted and supported me in the progress of this project. My best appreciation is for my supervisor, Dr. Mohammad Shaheen Khan who has been continuously giving me support and guidance to finish my dissertation with full focus and help.

I also wish to express my gratitude towards Universiti Malaysia Sabah for providing lab facilities to conduct my final year project. Also, to all the lab staff of Chemistry Department for providing technical support and help throughout my lab works.

Last but not least, sincere appreciation to my beloved family members and friends for their unconditional support and advice during my studies.

ABSTRACT

In this study, cyanoacetylhydrazone derivatives that possessing an azometine (-NHN=CH-) proton constitute an important class of compounds was synthesized and their biological activities were evaluated. The structures of newly synthesized compounds were confirmed by elemental analysis, FTIR spectral data. The range of the infrared region used is (4000~650 cm^{-1}). All synthesized compounds were tested and evaluated as antimicrobial and antiradical agents. In the determination of antimicrobial activity, the synthesized compounds showed no degree of inhibition against two types of bacterial namely *Streptococcus pneumonia* (G+) and *Escherichia coli* (G-) due to low percentage of purity of the synthesized compounds. A disk-diffusion method was use to evaluate the antimicrobial property of those compounds and Kirby-Bour disk-diffusion method was applied with modification. The antioxidant activity of synthesized compounds was determined by 1,1-Diphenyl–2-picrylhydrazyl (DPPH) assay. The synthesized compounds showed weaker antiradical activity (higher IC_{50}) than BHT which showed 0.0686 mg/mL. In conclusion, four types of cyanoacetylhydrazone compounds were synthesized and showed no inhibition in antimicrobial and lower potential in antioxidant properties. Hence, future study can be carried out to investigate and improve the effectiveness and purity of producing these compounds for better screening biological activities.

SINTESIS NOVEL TURUNAN CYANOACETYLHYDRAZONE DAN PENILAIAN AKTIVITI ANTIMIKROB DAN ANTIOKSIDAN

ABSTRAK

Dalam kajian ini, turunan cyanoacetylhydrazone memiliki sebuah proton azometine (-NHN=CH-) telah berjaya disediakan dan merupakan satu kelas yang penting untuk sebatian, berfungsi sebagai sasaran struktur dan aktiviti biologi telah dinilai. Struktur sebatian baru disintesis telah disahkan oleh analisis unsur, FTIR data spektrum. Rangkaian kawasan inframerah digunakan ialah dalam julat 4000 hingga 650 cm^{-1}. Sebatian wakil produk disintesis telah diuji dan dinilai sebagai agen antimikrob dan anti-radical. Dalam menentukan aktiviti antimikrob , sebatian yang telah disintesis tidak menunjukkan tahap perencatan terhadap dua jenis bakteria iaitu Streptococcus pneumonia *(G+) dan* Escherichia coli *(G-) kerana peratusan kesucian sebatian disintesis yang rendah. Satu kaedah cakera penyebaran adalah gunakan untuk menilai harta antimikrob daripada sebatian dan pengubahsiuaian Kirby-Bour kaedah cakera penyebaran turut digunakan. Aktiviti antioksidan sebatian disintesis telah ditentukan oleh 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay. Sebatian yang telah sintesis menunjukkan aktiviti antiradical yang lemah (lebih tinggi IC_{50}) daripada BHT yang menunjukkan 0.0686 mg/mL. Sebagai kesimpulan, empat jenis sebatian cyanoacetylhydrazone telah disintesiskan di mana perencatan dalam potensi anti-mikrob tidak ditunjukkan dan mempunyai potensi antioksidan yang rendah. Oleh itu, kajian pada masa hadapan dapat dijalankan untuk mengkaji dan meningkatkan keberkesanan dan ketulenan dalam menghasilkan sebatian tersebut untuk aktiviti biologi yang lebih baik.*

LIST OF CONTENTS

CHAPTER 3: METHODOLOGY

CHAPTER 4: RESULTS AND DISCUSSION

CHAPTER 5: CONCLUSION AND FUTURE RECOMMENDATION

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

LIST OF PHOTOS

LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

°C	Degree Celsius
%	Percentage
>	More than
&	And
wt	Weight
IR	Infrared
mol/L	Mol per liter
<	Less than
FTIR	Fourier Transform Infrared Spectroscopy
ml	Milileter
g	Gram
M	Molar
cm^{-1}	Per centimeter
kg	Kilogram
g/mole	gram per mole
H_2O	Water
α	Alpha
β	Beta
Cl	Chlorine
Br	Bromine
NO_2	Nitrogen Dioxide
C-N	Carbon-Nitrogen
N-N	Nitrogen-nitrogen
C=N	Carbon=Nitogen
-C=N-N-	Triatomic Group
KOH	Potassium Hydroxide
µg	Microgram
ml	Milliliter

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The essence of organic synthesis is the formation of both single and multiple bonds between two or more carbon atoms or between many combinations of carbon. It also consist of various heteroatoms, most notably oxygen, nitrogen, sulfur and phosphorus, which may or may not be present in the final products of a particular synthetic scheme (Donohoe *et al.,* 2014; Voinov & Grigor'ev, 2002; Zhang *et al.,* 2014). Along the way to a particular target, such bond-forming reactions have to properly set up and this involves manipulations of many initial functional groups into other forms to enable the bond formation to take place or to provide ways around selectivity or group incompatibilities, either with each other or with the necessary reaction conditions (Knight, 2013).

Synthetic organic chemistry is one of the most rapidly developing fields in chemistry. Worldwide, useful new reagents and reactions are reported in the chemical literature daily. Among them, carbon-carbon double bond formation is one of the most useful and fundamental reactions in synthetic organic chemistry, particularly in the synthesis of complex naturally occurring products, which show biological activity (Shen, 2006). The resistance towards available drugs is rapidly evolving into a major threat to the global heath security. To deal with this resistance, the need to design new compounds with better clinical efficacy has become one of the most important areas of today's research (Bala *et al.*, 2013).

1.1.1 Cyanoacetic Acid Hydrazide

Among several commercially available substituted hydrazines, cyanoacetic acid hydrazide has received the most attention recently. It is a versatile and convenient intermediate for the synthesis of various heterocyclic compounds. Figure 1.1 shows the chemical structure of cyanoacetic acid hydrazide (Martins *et al.*, 2008). The *β*-functional nitrile moiety of the molecule is a favorable unit for addition followed by cyclization or via cycloaddition with numerous reagents providing heterocyclic compounds of different ring sizes with one or several heteroatoms that are interesting as pharmaceuticals, as herbicides, as antibacterial agents, and as dyes. Their reactions with dinucleophiles normally result in the formation of polycyclic ring systems which may be the skeleton of important heterocylic compounds. As reported in previous publications, *β*-functional nitriles had been used as starting components for novel synthesis of azoles, azines, and azoloazines. Among the *β*-functional nitriles, cyanoacetic acid hydrazide and their analogues are especially important starting materials or intermediates for the synthesis of various nitrogen-containing heterocyclic compounds (Bondock *et al.*, 2006).

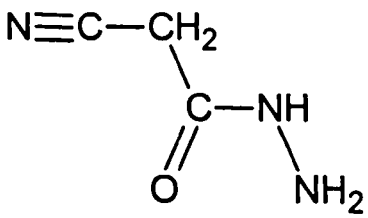


Figure 1.1 Structure of cyanoacetic acid hydrazide (Martins *et al.,* 2008).

1.1.2 Hydrazone

Hydrazones are a class of organic compounds which possess the structure $R_1R_2C=NNH_2$. They are related to ketone and aldehyde in which oxygen has been replaced with NNH_2 group. These azometine -NHN=CH- proton constitute an important class of compounds for new drug development. Hydrazones are formed by the reaction of hydrazine or hydrazide with aldehydes and ketones. They act as reactants in various important reactions such as hydrazone iodination, Shapiro reaction and Bamford-Stevans reaction to form vinyl compounds. They act as intermediate in Wolff-Kishner reaction (Ali *et al.*, 2012).

Hydrazones contain two nitrogen atoms of different nature and a C-N double bond that is conjugated with a lone electron pair of the terminal nitrogen atom. These structural fragments are mostly responsible for the physical and chemical properties of hydrazones. The C-atom in hydrazone has both electrophilic and nucleophilic character and both the N-atoms are nucleophilic although the amino type nitrogen is more reactive. Due to these properties hydrazones are widely used in organic synthesis. Figure 1.2 shows the classification of active centers (Belskaya *et al.*, 2010).

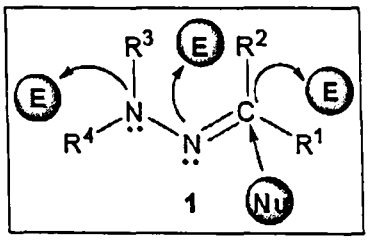
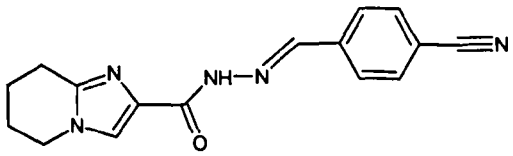
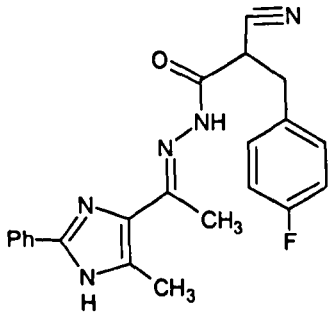


Figure 1.2 Classification of active centers (Belskaya *et al.*, 2010).

Hydrazones can also be synthesized by the Japp-Klingemann reaction (from β-ketoacids or β-ketoester's and aryldiazonium salts). The N,N'-dialkyl type of hydrazones can be hydrolysed, reduced and oxidized. This leads to the formation of amines by reduction of N-N bond. The C=N double bond in hydrazones are important compounds in drug design as they act as ligands for metal complexes, organocatalysis and synthesis of organic compounds. The chemical structure of hydrazone derivatives were showed in Figure 1.3 (Ali *et al.*, 2012).



N'-[(*E*)-(4-cyanophenyl)methylidene]-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine-2-carbohydrazide



2-cyano-3-(4-fluorophenyl)-*N*'-[(1*E*)-1-(5-methyl-2-phenyl-1*H*-imidazol-4-yl)ethylidene]propanehydrazide

Figure 1.3 Hydrazons derivatives (Ali *et al.,* 2012).

Hydrazones are present in many of the bioactive heterocyclic compounds that are of wide interest due to their diverse biological and clinical applications. This created interest in researchers who have synthesized variety of hydrazone derivatives and screened them for their various biological activities viz. antimicrobial, anticonvulsant, antiviral, antidepressant, antimycobacterial, analgesic, anti-inflammatory, anthelmintic, antiplatelet, antimalarial, vasodialator, antischistosomiasis, anti-HIV, antidiabetic, anticancer and trypanocidal activities. Hydrazones possessing an azometine –NHN=CH- proton constitute an important class of compounds as target structures and evaluated their biological activities. These observations have been guiding for the development of new hydrazones that possess varied biological activities. Hydrazide-hydrazone compounds are not only intermediates but they are also very effective organic compounds in their own right. When they are used as intermediates, coupling products can be synthesized by using the active hydrozen component of –CONHN=CH- azometine group (Negi *et al.*, 2012).

In addition, some of the new hydrazide-hydrazones which are recently synthesized were active against *M. tuberculosis* H37Rv between the concentrations of 0.78-6.25 µg/mL. Synthesis of a series of hydrazide-hydrzones via the reaction of cyanoacetic acid hydrazide with bromo(4-methoxyacetophenone) reported for sedative, antidepressant and analgesic activities (Negi *et al.*, 2012). Development of

novel chemotherapeutic agents is an important and challenging task for the medicinal chemists and many research programs are directed towards the design and synthesis of new drugs for their chemotherapeutic usage. Hydrazone compounds constitute an important class for new drug development in order to discover an effective compound against multidrug resistant microbial infection (Deep *et al.*, 2010).

1.2 Objectives of Research

The objectives of this study are;

i. to synthesize and characterize cyanoacetylhydrazone derivatives.

ii. to determine the antibacterial and antioxidant activities of the synthesized cyanoacetylhydrazone derivatives.

1.3 Scope of Study

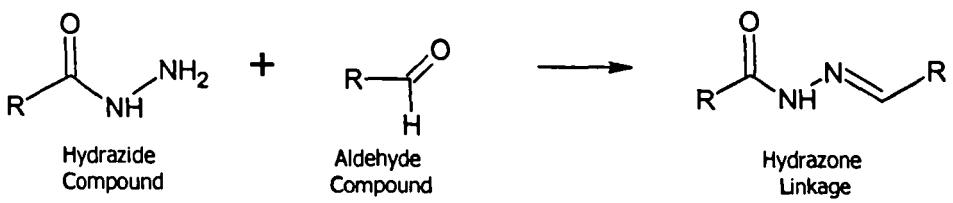
In this research, the cyanoacetylhydrazone is prepared by reacting acetophenone and cyanoacetic acid hydrazide. Cyanoacetylhydrazone are a very important group of analytical reagents for the determination of various biological assays. This has sparked a great interest for many researchers to synthesize vast varieties of hydrazone derivatives and screen for their various biological activities like anticonvulsant, antidepressant, analgesic, anti-inflammatory, antimicrobial, antioxidant and other biological activity. In this study, two entire new cyaoacetylhydrazone derivatives are synthesized and characterized. Biological test like antibacterial activity against Gram-negative and Gram-positive is assessed for these new synthesized products. Besides that, an antioxidant activity is also tested to develop the biological potential in pharmaceutical industry. In future, more cyanoacetylhydrazone derivatives can be further study and compare down to its atomic level to determine which functional group of the compound gives the main effect in the biological activity.

CHAPTER 2

LITERATURE REVIEW

2.1 Cyanoacetylhydrazone

Generally, Cyanoacetic acid hydrazide is used to synthesize hydrazide-hydrazone (Rafat M Mohareb *et al.*, 2011). Derivatives of hydrazine, particularly the hydrazide compounds formed from carboxylate groups, are able to react specifically with aldehyde or ketone functional groups in targeted molecules. A hydrazone linkage, which is a type of Schiff base, is created when reaction with either group takes place. Scheme 2.1 shows the reaction of hydrazide compound with aldehyde compound. A relatively stable bond is formed between the hydrazide compound and a ketone. However, the bond formed with an aldehyde group can be kinetically unstable (Hermanson, 2013).



Scheme 2.1 Formation of Hydrazone Linkage (Hermanson, 2013).

The reaction of cyanoacetic acid hydrazide with ω-bromo(4-methoxyacetophenone) gave the hydrazide-hydrazone derivative. These compounds reacted with either potassium cyanide or potassium thiocyanide to give the cyanide or thiocyanide derivatives respectively. The reaction of hydrazide-hydrazone

derivative with either hydrazine hydrate or phenyl hydrazine gave the hydrazine derivatives respectively. The latter compounds underwent a series of heterocyclization when react with different reagents to give 1,3,4-triazine and pyridinederivatives. These all the novel synthesized compounds show antidepressant, sedative and analgesic activities of the newly synthesized products were evaluated (Rafat & Mohamed, 2010).

Well known hydrazine derivatives, Cyanoacetic acid hydrazide or cyanoacetyl hydrazine, represents an important class of compounds that has greatly contributed in organic synthesis. Traditionally hydrazines have been employed as reagents for the derivatization and characterization of carbonyl compounds. However, in recent years the N-N linkage is found to be a key structural motif in various bioactive agents. Most significantly, an increase in number of N-N bond-containing heterocycles and peptidominetics have emerged in commercial applications, such as pharmaceutical and agricultural agents (Mohareb et al., 2011). Research shows that Cyanoacetic acid hydrazide is a vital starting material when it reacts with pregnenolone to form hydrazide-hydrazone derivative followed by its heterocyclizations to form novel heterocyclic ring systems attached to pregnenone showed high inhibitory effects against non-small cell lung cancer (NCI-H460) and breast adenocarcinoma (MCF-7), which are much higher than the doxorubicin (Mohareb & Al-Omran, 2012). Cyanoacetic acid hydrazide also act as starting material, which reacts with cycloalkanones to form cyanoacetohydrazones which is useful in the synthesis of 6-Amino-2-oxo-3,5-pyridinedicarbonitriles that possesses biological properties like antihypertensive, antihistaminic, anticancer, in addition to antibacterial or antifungal (Girgis *et al.*, 2003).

Cyanoacetic acid hydrazide is commercially available (Martins *et al.*, 2008). Cyanoacetic acid hydrazide, together with its derivatives, are important groups of starting material able to produce various types of compounds which have been reported to possess varied biological and pharmacological activity. Many publications and research has reported synthesis of different heterocyclic compounds, which utilizes cyanoacetic acid hydrazide as the key starting material. Some of the biological properties of heterocyclic compounds that were prepared from cyanoacetic acid hydrazide were reported (Abubshait, 2012). Aziz & Gomha had successfully

synthesized different series of 1,4-dihydropyridine derivatives by using cyanoacetohydrazide with different aromatic and heteromatic ketones as starting material. Screened for cytotoxic effects of the 1,4-dihydropyridine derivatives against human breast cell line MCF-7 had been done using Sulfo-Rhodamine-B stain (SRB) assay method (Aziz & Gomha, 2013). Eldin *et al.,* used cyanoacetic acid hydrazide in heterocyclic synthesis of a few annelated pyran derivatives, which shows latent functional substituents, making them highly promising for biological activity studies as well as for further chemical transformations (Eldin *et al.*, 1993). Doss *et al.,* reacted cyanoacetic acid hydrazide with Digitoxin and Digoxin to produce the hydrazide-hydrazone derivatives, which is helpful when synthesizing various type of coumarin, thiazole, thiophene and pyridine derivatives with potential biological activities (Doss *et al.*, 2001).

Cyanoacetic acid hydrazide can be used as an ambident nucleophile, which means that it can have properties of both *N*- and *C*- nucleophile. During treatment of cyanoacetic acid hydrazide with various reagents, there are five possible sites for the reaction or attack to take place; the nucleophile is able to attack the carbon of the carbonyl function (position 3) and the carbon atom of the nitrile function (position 5). On the other hand, the active methylene group (position 4) and amino groups (positions 1 and 2) are able to attack electrophiles. Figure 2.1 illustrates the five possible sites of cyanoacetic acid hydrazide (Bondock *et al.*, 2006).

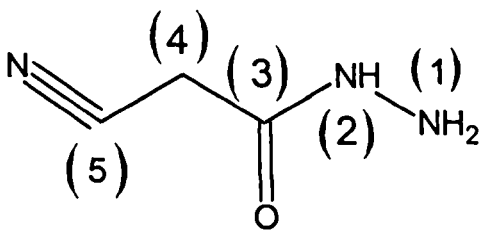
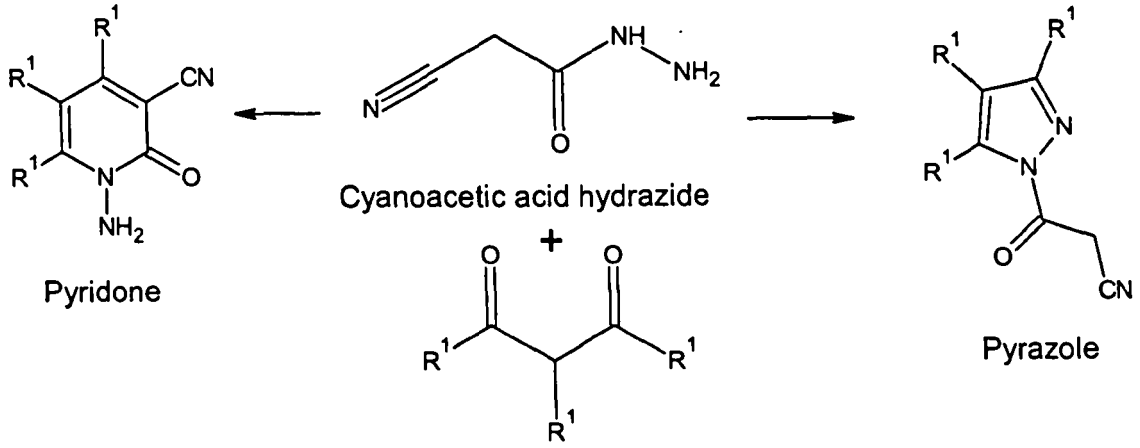


Figure 2.1 Cyanoacetic Acid Hydrazide (Bondock *et al.*, 2006).

The reactions of cyanoacetic acid hydrazide with wide number of reactants, both nucleophiles and electrophiles, are used to synthesize a variety of polyfunctional heterocyclic compounds with potential biological interest. Reports have shown that the main reaction involves the cyclocondensation reaction of cyanoacetic acid hydrazide with 1,3-dicarbonyl compounds. These reports have shown prove, where

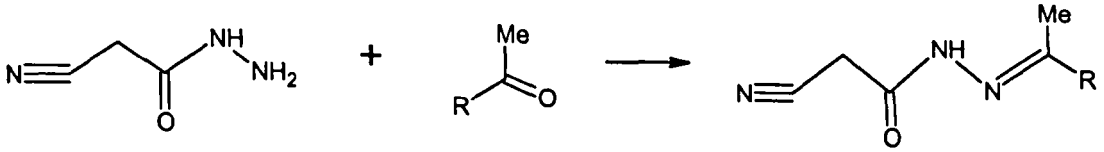
the product of this cyclocondensation reaction is dependent on the reaction conditions. Scheme 2.2 demonstrates the reaction of cyanoacetic acid hydrazide (Martins *et al.*, 2008).



Scheme 2.2 Reaction of Cyanoacetic acid hydrazide (Martins *et al.*, 2008).

2.2 Cyanoacetylhydrazone Derivatives

Cyanoacetohydrazones is said to possess interesting antimicrobial, antitumor, antihepatitis-C virus (HCV activity). Treatment of cyanoacetohydrazide with acetophenone derivatives in ethanol, with presence of catalytic amount of acetic acid gives 2-cyano-N-(1-substituted ethylidene) acetohydrazides respectively in good yields. Scheme 2.3 shows the formation of cyanoacetohydrazone. Reaction of cyanoacetohydrazide with different aromatic and heteroaromatic ketones yielded the corresponding hydrazones, which when reacted with ketene dithioacetal in the presence of KOH at room temperature, gives pyridinedicarbonitrile derivatives. The latter compounds, when refluxed with hydrazine hydrate, gives pyrazolo[4,3-*c*] pyridinecarbonitrile derivatives. The potential of these derivatives were then evaluated for their *in-vitro* cytotoxic activity against human breast cancer cell line (MCF7). Some of the compounds revealed significant activity compared to Doxorubicin as a reference drug (Aziz & Gomha, 2013).



Scheme 2.3 Formation of Cyanoacetohydrazone (Aziz & Gomha, 2013).

REFERENCES

Abdel-Magid, A. F., Carson, K. G., Harris, B. D., Maryanoff, C. A., & Shah, R. D. 1996. Reductive amination of aldehydes and ketones with sodium triacetoxyborohydride. Studies on direct and indirect reductive amination procedures. *The Journal of Organic Chemistry*, **61**(11): 3849-3862.

Abubshait, H. A. 2012. Synthesis and investigation of mass spectra of some nitrogen heterocycles and salycylaldazine derivatives. *Der Pharmacia Sinica*, **3**(2): 278-288.

Ali, M. R., Marella, A., Alam, M. T., Naz, R., Akhter, M., Shaquiquzzaman, M., Hooda, J. 2012. Review of biological activities of hydrazones. *Indonesian Journal of Pharmacy*, **23**(4): 193-202.

Aziz, H. M. A. E., & Gomha, S. M. 2013. Synthesis and *in vitro* anti-breast cancer Activity of some novel 1, 4-dihydropyridine derivatives. *International Journal of Pharmacy & Pharmaceutical Sciences*, **5**: 183-189.

Bala, S., Uppal, G., Kajal, A., Kamboj, S., & Sharma, V. 2013. Hydrazones as promising lead with diversity in bioactivity-therapeutic potential in present scenario. *International Journal of Pharmaceutical Sciences Review and Research*, **18**(1): 65-74.

Bashir, S., Erum, A., Kausar, R., Saleem, U., Ruqia-Tulain, U., & Alamgeer. 2012. Antimicrobial activity of some ethno-medicinal plants used in Pakistan. *Research in Pharmacy,* **2**(1): 42-45.

Belkheiri, N., Bouguerne, B., Bedos-Belval, F., Duran, H., Bernis, C., Salvayre, R., Baltas, M. 2010. Synthesis and antioxidant activity evaluation of a syringic hydrazones family. *European Journal of Medicinal Chemistry*, **45**(7): 3019-3026.

Belskaya, N. P., Dehaen, W., & Bakulev, V. A. 2010. Synthesis and properties of hydrazones bearing amide, thioamide and amidine functions. *Archive for Organic Chemistry*, **1**: 275-332.

Bondock, S., Tarhoni, A. E.-G., & Fadda, A. A. 2006. Utility of cyanoacetic acid hydrazide in heterocyclic synthesis. *Archive for Organic Chemistry*, **9**: 113-156.

Darwish, E. S., Fattah, A. M. A., Attaby, F. A., & Al-Shayea, O. N. 2014. Synthesis and antimicrobial evaluation of some novel thiazole, pyridone, pyrazole, chromene, hydrazone derivatives bearing a biologically active sulfonamide moiety. *International Journal of Molecular Sciences*, **15**(1): 1237-1254.

Deep, A., Jain, S., Sharma, P. C., Verma, P., Kumar, M., & Dora, C. P. 2010. Design and biological evaluation of biphenyl-4-carboxylic acid hydrazide-hydrazone for antimicrobial activity. *Acta Poloniae Pharmaceutica-Drug Research*, **67**(3): 255-259.

Demirbas, N., Demirbas, A., & Sancak, K. 2002. Synthesis and characterization of some 3-alkyl-4-amino-5-cyanomethyl-1,2,4-triazoles. *Turkish Journal of Chemistry*, **26**(5): 801-806.

Donohoe, T. J., Jones, C. R., & Winter, C. 2014. *Partial and Complete Reduction of Pyrroles, Furans, Thiophenes, and Their Benzo Analogs, Heterocycles containing more than one Heteroatom.* 2nd Edition. Comprehensive Organic Synthesis, Oxford, pp. 794-837.

Doss, S. H., Wardakhan, W. W., & Louca, N. A. 2001. The reaction of Digitoxin and Digoxin with cyanoacetic acid hydrazide: Synthesis of coumarin, thiazole,

thiophene and pyridine derivatives with potential biological activities. *Archives of Pharmacal Research*, **24**(5): 377-384.

Eldin, S. M., El-Din, A. A. M., & Basyouni, W. M. 1993. Cyanoacetic acid hydrazide in heterocyclic synthesis: A new route for the synthesis of several annelated pyran derivatives. *Archives of Pharmacal Research*, **16**(4): 318-321.

El-Sherif, A. A., Shoukry, M. M., & Abd-Elgawad, M. 2012. Synthesis, characterization, biological activity and equilibrium studies of metal(II) ion complexes with tridentate hydrazone ligand derived from hydralazine. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **98**(0): 307-321.

Ersan S, Nacak S and Berkem R. 1998. Synthesis and antimicrobial activity of N-[methyl benzylidene] acetohydrazides. *IL Farmaco*, **53**: 773-776.

Gaikwad, D. D., Dake, S. A., Kulkarni, R. S., Jadhav, W. N., Kakde, S. B., & Pawar, R. P. 2007. Efficient Baeyer–Villiger oxidation of ketones using molecular iodine/H_2O_2 as heterogeneous catalytic system. *Synthetic Communications*, **37**(23): 4093-4097.

Gaunt, M. J., Johansson, C. C. C., McNally, A., & Vo, N. T. 2007. Enantioselective organocatalysis. *Drug Discovery Today*, **12**(1–2): 8-27.

Girgis, A. S., Hosni, H. M., & Ahmed-Farag, I. 2003. A convenient regioselective synthesis of 6-amino-2-oxo-3,5-pyridinedicarbonitriles. *Zeitschrift für Naturforschung B*, **58**(7): 678-685.

Govindasami, T., Pandey, A., Palanivelu, N., & Pandey, A. 2011. Synthesis, characterization and antibacterial activity of biologically important vanillin related hydrazone derivatives. *International Journal of Organic Chemistry*, **1**: 71.

Hermanson, G. T. 2013. *Bioconjugate Techniques*. 3rd Edition. Boston, Academic Press.

Hudzicki. J. 2013. American society for microbiology ASM Microbel LibraryKirby-Bauer Disk Diffusion Susceptibility Test Protocol. Available online: http://www.microbelibrary.org/component/resource/laboratory-test/3189-kirby-bauer-disk-diffusion-susceptibility-test-protocol.

Kadhum, A. A. H., Al-Amiery, A. A., Musa, A. Y., & Mohamad, A. B. 2011. The antioxidant activity of new coumarin derivatives. *International Journal of Molecular Sciences*, **12**(9): 5747-5761.

Khan, S. A., Mullick, P. O. O. J. A., Pandit, S., & Kaushik, D. 2009. Synthesis of hydrazones derivatives of quinoxalinone and prospective antimicrobial and antiinflammatory agents. *Acta Poloniae Pharmaceutica - Drug Research*, **66**: 169-172.

Knight, D. W. 2013. *Organic Methodology and Organic Synthesis.* Reference Module in Chemistry, Molecular Sciences and Chemical Engineering: Elsevier.

Krishna, U. M., Martinez, A. W., Caves, J. M., & Chaikof, E. L. 2012. Hydrazone self-crosslinking of multiphase elastin-like block copolymer networks. *Acta Biomaterialia*, **8**(3): 988-997.

Küçükgüzel, S. G., Mazi, A., Sahin, F., Öztürk, S., & Stables, J. 2003. Synthesis and biological activities of diflunisal hydrazide–hydrazones. *European Journal of Medicinal Chemistry*, **38**(11–12): 1005-1013.

Kumar, D., Judge, V., Narang, R., Sangwan, S., De Clercq, E., Balzarini, J., & Narasimhan, B. 2010. Benzylidene/2-chlorobenzylidene hydrazides: Synthesis, antimicrobial activity, QSAR studies and antiviral evaluation. *European Journal of Medicinal Chemistry*, **45**(7): 2806-2816.

Kumar, D., Maruthi Kumar, N., Ghosh, S., & Shah, K. 2012. Novel Bis (Indolyl) Hydrazide–Hydrazones as Potent Cytotoxic Agents. *Bioorganic & medicinal chemistry letters*, **22**(1): 212-215.

Kumar, D., Narang, R., Judge, V., Kumar, D., & Narasimhan, B. 2012. Antimicrobial evaluation of 4-methylsulfanyl benzylidene/3-hydroxy benzylidene hydrazides and QSAR studies. *Medicinal Chemistry Research*, **21**(3): 382-394.

Laxmi, J., Palanichamy, V., Reddy, N. N., Rajsekaran, C., Kumari, D., & Mitra, H. 2012. Standardization of cultivation parameters for the extraction of carotenoid from Pink Pigmented Facultative Methylotrophic (PPFM) Bacteria. *Asian Journal of Pharmaceutical & Clinical Research*, **5**(2).

Liu, Y., Li, J., Du, J., Hu, M., Bai, H., Qi, J., Chao, G., Tian, W. T., Hong, S., Ling, J. J. & Ji, G. P. 2011. Accurate assessment of antibiotic susceptibility and screening resistant strains of a bacterial population by linear gradient plate. *Science China Life Sciences*, **54**(10): 953-960.

Machakanur, S. S. 2012. A study on star molecular architectures with 1, 3, 5-teiazine and cyclotriphosphazene cores.

Martins, M. A. P., Moreira, D. N., Frizzo, C. P., Longhi, K., Zanatta, N., & Bonacorso, H. G. 2008. Reaction of β-Alkoxyvinyl halomethyl ketones with cyanoacetohydrazide. *Journal of the Brazilian Chemical Society*, **19**(7): 1361-1368.

Mohareb, R. M., & Al-Omran, F. 2012. Reaction of pregnenolone with cyanoacetylhydrazine: Novel synthesis of hydrazide-hydrazone, pyrazole, pyridine, thiazole, thiophene derivatives and their cytotoxity evaluations. *Steroids*, **77**(14): 1551-1559.

Mohareb, R. M., & El-Khair, A. A. 2011. Novel synthesis of hydrazide-hydrazone and their uses for the synthesis 1, 3, 4-oxadiazine, 1, 2, 4-triazine, pyrazole and pyridazine derivatives with antimicrobial and antifungal activities. *International Journal of Applied Biology and Pharmaceutical Technology*, **2**(4): 434-446.

Mohareb, R. M., & Hana, H. Y. 2008. Synthesis of progesterone heterocyclic derivatives of potential antimicrobial activity. *Acta Pharmaceutica*, **58**(1): 29-42.

Mohareb, R. M., & Mohamed, A. A. 2010. The reaction of cyanoacetylhydrazine with Ω-bromo(4-Methyl)acetophenone: Synthesis of heterocyclic derivatives with antitumor activity. *Molecules,* **15**(5): 3602-3617.

Mohareb, R. M., Fleita, D. H., & Sakka, O. K. 2011. Novel synthesis of hydrazide-hydrazone derivatives and their utilization in the synthesis of coumarin, pyridine, thiazole and thiophene derivatives with antitumor activity. *Molecules*, **16**: 16-27.

Mohareb, R. M., Ibrahim, R. A., & Moustafa, H. E. 2010. Hydrazide-hydrazones in the synthesis of 1,3,4-oxadiazine, 1,2,4-triazine and pyrazole derivatives with antitumor activities. *Open Organic Chemistry Journal*, **4**: 8-14.

Mohareba, R. M., & abou El-Khairb, A. 2011. Novel synthesis of hydrazide-hydrazone and their uses for the synthesis 1, 3, 4-oxadiazine, 1, 2, 4-triazine, pyrazole and pyridazine derivatives with antimicrobial and antifungal activities. *International Journal of Applied Biology and Pharmaceutical Technology*, **2**(4): 434-446.

Negi, V. J., Sharma, A. K., Negi, J. S., & Ram, V. 2012. Biological activities of hydrazone derivatives in the New Millennium. *International Journal of Pharmaceutical Chemistry*, **2**(4): 100-109.

Nguyen, R., & Huc, I. 2003. Optimizing the reversibility of hydrazone formation for dynamic combinatorial chemistry. *Chemical Communications*, **8**: 942-943.

Othman, M., Loh, H. S., Wiart, C., Khoo, T. J., Lim, K. H., & Ting, K. N. 2011. Optimal methods for evaluating antimicrobial activities from plant extracts. *Journal of Microbiological Methods*, **84**(2): 161-166.

Özmen, Ü. Ö., & Olgun, G. 2008. Synthesis, characterization and antibacterial activity of new sulfonyl hydrazone derivatives and their nickel (II) complexes. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **70**(3): 641-645.

Patel Rajesh, M., & Patel Natvar, J. 2011. In vitro antioxidant activity of coumarin compounds by DPPH, Super oxide and nitric oxide free radical scavenging methods. *Journal of Advanced Pharmacy Education & Research*, **1**: 52-68.

Patil, B. R., Machakanur, S. S., Badiger, D. S., Hunoor, R. S., Gudasi, K. B., Nethaji, M., & Annie Bligh, S. W. 2011. Hexa-arm star shaped hydrazone derivatives from hexakis(4-formylphenoxy)-cyclotriphosphazene core. *Journal of Molecular Structure*, **1003**(1–3): 52-61.

Pavia, D., Lampman, G., Kriz, G., & Vyvyan, J. 2009. *Introduction to Spectroscopy*, International. 4th Edition. Cengage Learning, pp. 25-84.

Prathap, K. J., Himaja, M., Mali, S. V., Ranjitha, A., Karigar, A., & Sikarwar, M. 2010. Synthesis and antioxidant activity of novel ketone hydrazones bearing 5-(pyridine-3-yl)-1, 3, 4-thiadiazole. *Journal of Pharmacy Research*, **3**(10).

Refat, H. M., & Fadda, A. A. 2013. Synthesis and antimicrobial activity of some novel hydrazide, benzochromenone, dihydropyridine, pyrrole, thiazole and thiophene derivatives. *European Journal of Medicinal Chemistry*, **70**(0): 419-426.

Reller, L. B., Weinstein, M., Jorgensen, J. H., & Ferraro, M. J. 2009. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *Clinical Infectious Diseases*, **49**(11): 1749-1755.

Rollas, S., & Küçükgüzel, S. G. 2007. Biological activities of hydrazone derivatives. *Molecules*, **12**(8): 1910-1939.

Sandtnerová, K. L. R., & BÍlik, V. 1976. Hydrazones and their derivatives. II.* Preparation of pure D-erythrose. *Chemical Papers - Chemicke Zvesti*, **30**(4): 564-567.

Shen, Y. 2006. Synthetic Utility of Fluorinated β-Keto-Phosphonium Salts,-Phosphonates and Related Compounds. *Advances in Organic Synthesis*, **523**(572): 523-572.

Singh, M., & Raghav, N. 2011. Biological activities of hydrazones: a review. *International Journal of Pharmacy and Pharmaceutical Sciences*, **3**(4): 26-32.

Stuart, B. 2004. *Infrared Spectroscopy*. John Wiley & Sons, Inc, pp. 80-82.

Tirkey, V., Mishra, S., Dash, H. R., Das, S., Nayak, B. P., Mobin, S. M., & Chatterjee, S. 2013. Synthesis, characterization and antibacterial studies of ferrocenyl and cymantrenyl hydrazone compounds. *Journal of Organometallic Chemistry*, **732**(0): 122-129.

Tirzitis, G., & Bartosz, G. 2010. Determination of antiradical and antioxidant activity: basic principles and new insights. *Acta Biochimica Polonica*, **57**: 139-42.

Ulsoy N, Capan G, Otük G and Kiraz M. 1999. Synthesis and antimicrobial activity of new 6-phenylimidazo [2, 1-b] thiazole derivatives. *Bollettino Chimico Farmaceutico*, **139**(4): 167-172.

Voinov, M. A., & Grigor'ev, I. A. 2002. A route to the synthesis of previously unknown α-heteroatom substituted nitrones. *Tetrahedron Letters*, **43**(13): 2445-2447.

Wardakhan, W. W., EL-SAYED, N. N., & Mohareb, R. M. 2013. Synthesis and anti-tumor evaluation of novel hydrazide-hydrazone derivatives. *Acta pharmaceutica*, **63**(1): 45-57.

Zhang, Z., Kaplan, M. J., & Antilla, J. C. 2014. *Addition of Heteroatom Nucleophiles to CO and CN pi Bonds.* 2nd Edition. Comprehensive Organic Synthesis, Oxford, pp. 653-696.