Original Article

MICROWAVE ASSISTED SYNTHESIS OF HETEROCYCLIC COMPOUND AND ITS ANTICANCER ACTIVITY

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Abstract

The chemistry of heterocyclic compounds has been an interesting field of study for a long time. The synthesis of novel Oxadiazole derivatives and investigation of their chemical and biological behavior have gained more importance in recent decades for biological, medical and agricultural reasons. Azole derivatives are the important class of the heterocyclic compounds. Derivatives of 1,3,4 oxadiazole 2,5 disubstituted position have been reported to possess considerable pharmacological activities. Synthesize oxadiazole with various substitution from starting material 1 hydroxy 2 -napthoicacid. 1, 3,4 oxadiazole which were synthesized in laboratory as part of an ongoing program of discovering small molecules with multiple beneficial effects in metabolic syndrome. An effective synthesis of different novel 2, 5-disubstituted- 1,3,4 oxadiazole derivatives by nucleophilic addition elimination reaction, nucleophilic substitution reaction and cyclization reaction. 1-hydroxy- 2- naphthoic acid with concentrated sulphuric acid and ethanol resulted the formation of carboxylate, and again refluxing in ethanol and hydrazine hydrate resulted the formation of carbohydrazide intermediate. when treated with cynogen bromide,1,4 diaxone and sodium bicarbonate and ethanol the formation of compound 1,3,4 oxadiazole, derivatives were synthesized by using different aromatic aldehyde. Results demonstrated that microwave method is more eco-friendly than conventional method.

Keywords: 1,3,4-oxadiazole derivatives, antimicrobial activity, anticancer activity.

INTRODUCTION

Hetrocyclic compound are widely distributed in nature which are essential to life and industry also. Many hetrocyclic compounds are biosynthesized by plants and animal. Oxidiazole is considered to be derived from furan by replacement of two methane groups by two pyridine type nitrogen. Heterocyclic compounds having five membered ring containing two carbon atom, one oxygen, two nitrogen and two double bond such as oxadiazole. 1,3,4 oxadiazole derivatives have played a vital role in the medicinal chemistry.[1] Hetrocyclic compounds are organic compounds containing at least one carbon, and at least one element other than carbon, such as sulfur ,oxygen, or nitrogen within a ring structure. A Large size of hetrocyclic comprises of those fused with benzene rings like quinoline, benzothiophene, indol, and benzofuran respectively .fusion of two benzene rings gives rise to a third large family of compound, respectively the acridine,dibenzothiophene ,carbazole and dibenzofuran. Many of the drugs currently available in the market contain several heterocycles, with nitrogen atom, as major backbone having potential pharmacological activity.[2]

1,3,4-Oxadiazole

Compounds containing the 1,3,4-oxadiazole has drawn interest due to the unique chemical structure and large variety of biological properties. Oxadiazoles have often been described as bioisosteres for amides and esters. Due to increased hydrolytic and metabolic stabilities of the oxadiazole ring, improved pharmacokinetic and in vivo performance are often observed. which makes this heterocycle an important structural moiety for the pharmaceutical industry. As a result of these characteristics, oxadiazoles have often been the target of many drug discovery programs as tyrosine kinase inhibition, histamine H3 antagonism, hypocholesterolemic agents, antiviral agents, muscarinic receptor antagonists, anti-inflammatory agents, antimicrobial, cytotoxic activities, antitumor , antineoplastic properties , tumor-selective.[3]

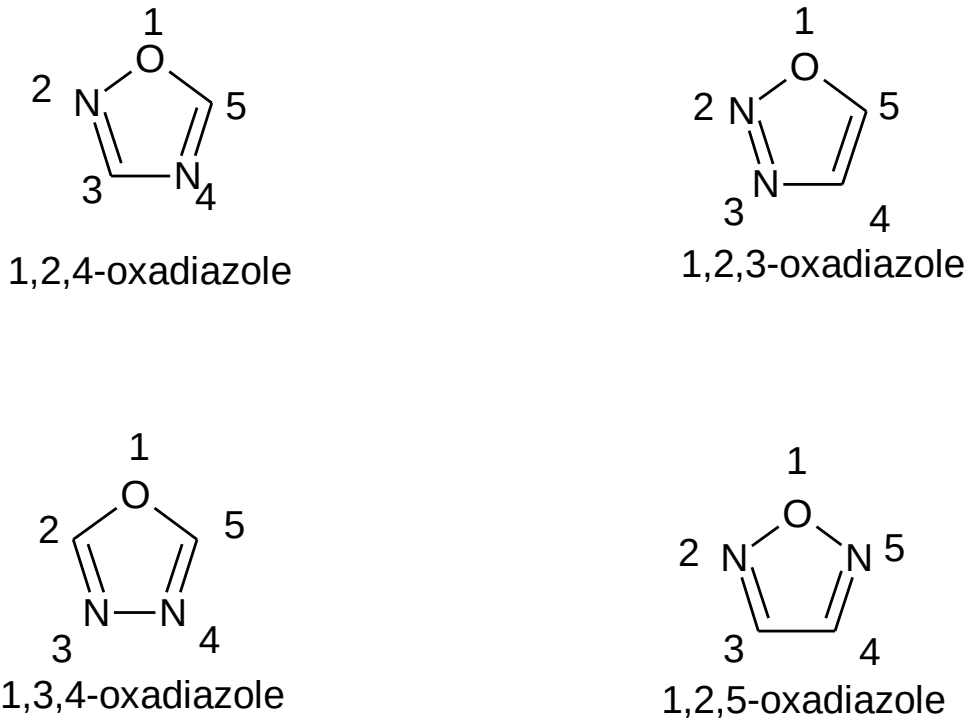


Fig. 1 Four isomers of oxadiazole

Microwave

Microwave have been used to speed up chemical reactions in the laboratories which led scientists to investigate the mechanism of microwave dielectric heating and to identify the advantages of the technique for chemical synthesis. During recent years, microwaves have been extensively used for carrying out chemical reactions and have become a useful non-conventional energy source for performing organic synthesis. The first recorded application of microwave energy in organic synthesis is the aqueous emulsion polymerization of butyl acrylate, acrylic acid and methacrylic acid using pulsed electromagnetic radiation. The start of the rapid growth of microwave assisted procedures in organic synthesis was ignited in 1986 by pioneering papers by Gedye and co-workers and Giguere and co- workers. [4]During the last two decades, the activity in this new technique has experienced exponential growth and has been extensively reviewed. Kappe and Dallinger have reported the impact of microwaves on drug discovery. Even microwave-assisted reactions under solvent-free conditions promoted the synthesis of Zincke's salt and its conversion to chiral pyridinium salts in water and microwave- assisted organic transformations using benign reaction media have also been reported.[5]

Role of electromagnetic spectrum in microwave

Microwave heating refers the use of electromagnetic waves ranges from 0.01m to 1m wave length of certain frequency to generate heat in the material. These microwaves lie in the region of the electromagnetic spectrum between millimeter wave and radiowave i.e. between I.R and radio wave. They are defined as those waves with wavelengths between 0.01 meter to 1 meter, corresponding to frequency of 30GHz to0.3GHz.The basic principle behind the heating in microwave oven is due to the interaction of charged particle of the reaction material with electromagnetic wavelength of particular frequency. The phenomena of producing heat by electromagnetic irradiation are either by collision or by conduction, sometime by both. All the wave energy changes its polarity from positive to negative with each cycle of the wave. This cause rapid orientation and reorientation of molecule, which cause heating by collision. [6]If the charge particles of material are free to travel through the material (e.g. Electron in a sample of carbon), a current will induce which will travel in phase with the field.

Comparison of Conventional Vs Microwave Method

- Conventional heating :

In this method of heating, reactants are slowly activated by a conventional external heat source. Heat is driven into the substance, passing first through the walls of the vessel in order to reach the solvent and the reactants. This is a slow and inefficient method for transferring energy into the reacting system.

- Microwave heating :

Here, microwaves couple directly with the molecules of the entire reaction mixture, leading to a rapid rise in the temperature. Since the process is not limited by the thermal conductivity of the vessel, the result is an instantaneous localized superheating of any substance that will respond to either dipole rotation or ionic conductivity. Only the reaction vessel contents are heated and not the vessel itself; better homogeneity and selective heating of polar molecules might be achieved. [7]

The acceleration of chemical reactions by microwave exposure results from the interactions between the material and electromagnetic field leading to the thermal and specific (non-thermal) effects. For microwave heating, the substance must possess a dipole moment. A dipole is sensitive to external electric field and tries to align itself with the field by rotation. If submitted to an alternating current, the electric field is inversed at each alternant and therefore dipoles tend to move together to follow the inversed electric field.

Advantage and Disadvantage of Microwaves

Advantages

- High purity of products
- Less side-products
- Improved yields
- Simplified and improved synthetic procedure
- Wider usable range of temperature
- Higher energy efficiency
- Sophisticated measurement and safety technology
- Modular systems enable changing from mg to kg scale.

Disadvantages

- Heat force control is difficult
- Water evaporation
- Closed container is dangerous because it could be burst

Cancer

Cancer is a disease of the cells in the body. There are many different types of cell in the body, and many different types of cancer which arise from different types of cell. What all types of cancer have in common is that the cancer cells are abnormal and multiply out of control.[8]

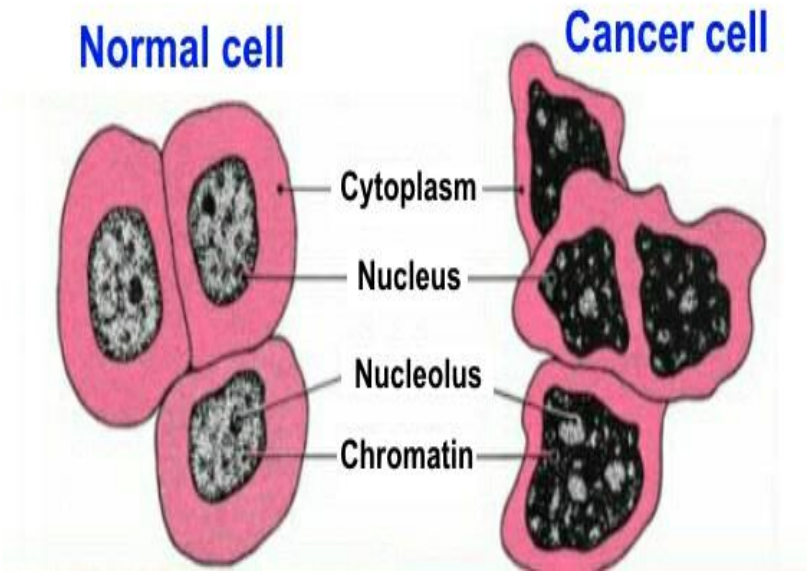


Fig. 2 Normal cell Vs Cancer Cell.

Normal body cells

The body is made up from millions of tiny cells. Different parts of the body such as organs, bones, muscles, skin, and blood are made up from different specialised cells. All cells have a centre called a nucleus. The nucleus in each cell contains thousands of genes which are made up from a chemical called DNA. The genes are like codes which control the functions of the cell. For example, different genes control how the cell makes proteins, or hormones, or other chemicals. Certain genes control when the cell should multiply, and certain genes even control when the cell should die. Most types of cell in the body divide and multiply from time to time. As old cells wear out or become damaged, new cells are formed to replace them. Some cells normally multiply quickly. For example, you make millions of red blood cells each day as old ones become worn out and are broken down. Some cells do not multiply at all once they are mature - for example, brain cells. Normally, body only makes the right number of cells that are needed.[9]

Abnormal cells

Sometimes a cell becomes abnormal. This occurs because one (or more) gene in the cell becomes damaged or altered. The abnormal cell may then divide into two, then four, then eight, and so on. Lots of abnormal cells may then develop from the original abnormal cell. These cells do not know when to stop multiplying. A group of abnormal cells may then form. If this group of cells gets bigger, it becomes a large clump of abnormal cells called a tumour.

Types of cancer

1. Carcinomas - Cancer that arises from ectoderm or endoderm at tissue. (Ectoderm involves skin and its appendages, nerve tissue. Endoderm involves intestinal system & organs).

2. Sarcomas - Cancer that arises from mesodermal tissue . (mesoderm involves muscles, bone, cartilage, and connective tissue).

3. Teraloma - cancer that arises from cells of ectoderm, endoderm or mesoderm.

4. Blastoma - Certain types of cancer that have primitive appeaarance resembling embroyonic structure. Neuroblastoma of nerve tissues, myoplasma of muscles tissues.

5. Cancer of blood- There are two types, leukaemia i.e. WBC count more than 105 to 106 per mm3(Normal WBC caunt is 7500 per mm3) &ploycythemiai.e increase in RBC caunt.[10]

Anticancer evaluation

The MCf-7 cell line was maintained in DMEM medium supplemented with 10 % fetal bovine serum. The cells were plated at a density of 1 × 104 cells per well in a 96-well plate, and cultured for 24 h at 37°C. The cells were subsequently exposed to 01 mM . The plates were incubated for 24 h, and cell proliferation was measured by adding 10 µL of MTT (thiazolyl blue tetrazolium bromide) dye (5 mg/ml in phosphate-buffered saline) per well. The plates were incubated for a further 4 h at 37°C in a humidified chamber containing 5% CO2. Formazan crystals formed due to reduction of dye by viable cells in each well were dissolved in 200 µl DMSO, and absorbance was read at 490 nm. The results were compared with the standard drug inhibitors 5 flurouracil (20µg/ml).[11]

In 1990, Anastas P. and Warner J. [12] defined green chemistry (GC): “The design of chemical products and processes that reduce or eliminate the use and generation of hazardous substances.” Human society is continually facing such environmental matters and problems as ozone depletion, air pollution, global climate transform, soil and water pollution, acid rain, natural resources and an addition of hazardous waste. There are twelve principles of GC. Anastas P. and Warner J. first published GC in their book, green chemistry: Theory and Practice, in 1998. Both serve as members of the california green chemistry science advisory panel.

Cancer, one of the leading cause of, socioeconomic burden and mortality in humans, accounting for approximately 9.6 million deaths worldwide yearly with future predictions suggest that by 2030, 13 million people will die from cancer each year (World Health Organization).

Since ancient times, natural products are the prime source of therapeutics for various human diseases. Furthermore, natural compounds have been vital in drug development as they possess a range of biological activities and pharmacological features including their easy availability, less expensive and non-toxic action on normal cells that supports their potential as putative anticancer agent (Dutta et al., 2019)[13]

Generally, combination of natural products gives synergetic and or additive outcome and recently we have also showed that curcumin synergistically enhanced the anticancer action of cisplatin. Moreover, natural products also sensitize cancer cells to anti-cancer drugs which is another important mechanism relevant in achieving optimal cancer therapeutic outcomes with minimal adverse effects (Lin et al., 2020, Khan et al., 2020).[14]

Objectives

- Synthesis of 2, 5 disubstituted 1,3,4 oxadiazole derivatives.
- Screening of synthesized compounds for anticancer and anti- microbial activities etc.
- To study physicochemical data of compounds by Conventional Method
- To study physicochemical data of compounds by Microwave Method

MATERIAL AND METHOD

A research methodology is a universal way to addressing a study subject through data collection, data evaluation, and results based on the findings of the study. A research technique is a plan for carrying out a research study. The methodical gathering and analysis of facts and information for the advancement of knowledge in any area may be loosely defined as research. The goal of the study is to use systematic techniques to find solutions to intellectual and practical problems.The current study is descriptive in nature and is based on secondary data gathered from a variety of sources, including books, education, and development, journals, scholarly articles, government publications, and printed and online reference materials.

RESULT AND DISCUSSION

Table 1: Drugs currently available in the market containing several Heterocyclic ring and their use

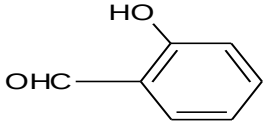
Hetrocyclic Ring	Drugs	Use
Imidazole	Clotrimazole, Miconazole	Antifungal
Triazole	Fluconazole	Antifungal
Benzimidazole	Mebendazole, Albendazole	Anthelmentic
Thiadiazole	Acetazolamide	Diuretic
Oxazole	Sulfoxazole	Anti-bacterial sulphonamide
1,3,4-oxadiazole	Isradipine	Ca++ channel blocker

From above table we can see the drugs available in the market containing several Heterocyclic ring and their use such as Clotrimazole, Miconazole, Fluconazole, Mebendazole, Albendazole, Acetazolamide, Sulfoxazole, Isradipine [15]

Table 2: Anticancer activity of synthetic compound 4a1- 4a10against MCF-7 cell line (Human breast cancer)

Compounds	Absorbance	% inhibition
Negative control	0.538	-
Positive control	0.103	80.85
$4a_1$	0.220	59.10
$4a_2$	0.073	86.43
$4a_3$	0.101	81.22
$4a_4$	0.130	75.83
$4a_5$	0.156	71.00
$4a_6$	0.019	83.08
$4a_7$	0.077	85.68
$4a_8$	0.172	68.02
$4a_9$	0.193	64.12
$4a_{10}$	0.114	78.81

Table: 3 Structures of aldehydes

Compounds	Aromatic Aldehydes
$4a_1$	

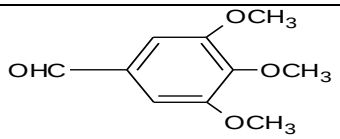
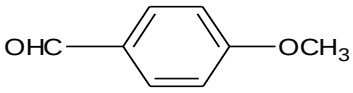
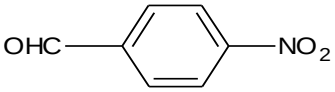
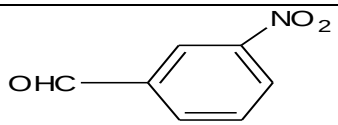
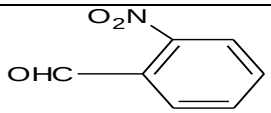
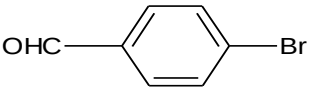
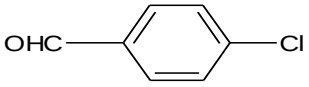
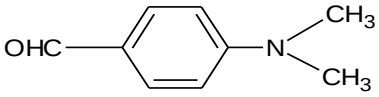
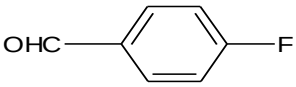
4a$_2$	
4a$_3$	
4a$_4$	
4a$_5$	
4a$_6$	
4a$_7$	
4a$_8$	
4a$_9$	
4a$_{10}$	

Table 4: Physicochemical data of compounds by conventional method.

Compounds	**Molecular formula**	**MW**	**MP** (^{0}C)	**%Yeild**	**Time(hrs)**	**Rf Value**	**Mobile Phase**
(1)	$C_{13}H_{12}O_3$	216	80-82 ^{0}C	67.67%	10-12hrs	0.54	hexane:E.A,3:1
(2)	$C_{11}H_{10}N_2O_2$	202	182-184^{0}C	64.63%	8-10hrs	0.56	hexane:E.A,3:1
(3)	$C_{12}H_9N_3O_2$	215	118-120^{0}C	63.85%	4 hrs	0.73	hexane:E.A,3:1
(4)	$C_{19}H_8N_3O_2$	310	210-212^{0}C	64.77%	2 hrs	0.77	hexane:E.A,3:1
$4a_1$	$C_{19}H_9N_3O_3$	327	199-200^{0}C	65.75%	2 hrs	0.75	hexane:E.A,3:1
$4a_2$	$C_{19}H_{19}N_3O_5$	369	200-202^{0}C	64.70%	2 hrs	0.7	hexane:E.A,3:1
$4a_3$	$C_{20}H_{15}N_3O_2$	329	180-182^{0}C	63.33%	2 hrs	0.69	hexane:E.A,3:1

$4a_4$	$C_{19}H_{13}N_4O4$	361	199-200^{0}C	66.66%	2 hrs	0.7	hexane:E.A,3:1
$4a_5$	$C_{19}H_{13}N_4O4$	361	199-200^{0}C	6.9.69%	2 hrs	0.5	hexane:E.A,3:1
$4a_6$	$C_{19}H_{13}N_4O4$	361	200-202^{0}C	66.10%	2 hrs	0.55	hexane:E.A,3:1
$4a_7$	$C_{19}H_{12}N_3O_2Br$	394	180-182^{0}C	61.11%	2 hrs	0.72	hexane:E.A,3:1
$4a_8$	$C_{19}H_{12}N_3O_2Cl$	349	219-220^{0}C	62.5%	2 hrs	0.64	hexane:E.A,3:1
$4a_9$	$C_{21}H_{18}N_4O_2$	358	138-140^{0}C	66.66%	2 hrs	0.72	hexane:E.A,3:1
$4a_{10}$	$C_{19}H_{12}N_3O_2F$	305	140-142^{0}C	64.28%	2 hrs	0.8	hexane:E.A,3:1

Physicochemical data of compounds by conventional method is as shown in table 4 [16]

Table 5: Physicochemical data of compounds by Microwave Method.

Compounds	**Molecular formula**	**MW**	**MP(^{0}C)**	**% Yield**	**Time (min)**	**Rf Value**	**Mobile Phase**
(1)	$C_{13}H_{12}O_3$	216	80-82^{0}C	77.16%	4 min	0.54	hexane:E.A,3:1
(2)	$C_{13}H_{10}N_2O_2$	202	182-184^{0}C	73.35%	4 min	0.56	hexane:E.A,3:1
(3)	$C_{14}H_9N_3O_2$	215	118-120^{0}C	72.44%	4 min	0.73	hexane:E.A,3:1
(4)	$C_{19}H_8N_3O_2$	310	210-212^{0}C	75.23%	4 min	0.77	hexane:E.A,3:1
$4a_1$	$C_{19}H_9N_3O_3$	327	199-200	70.11%	4 min	0.5	hexane:E.A,3:1
$4a_2$	$C_{19}H_{19}N_3O_5$	369	200-202^{0}C	82.35%	4 min	0.6	hexane:E.A,3:1
$4a_3$	$C_{20}H_{15}N_3O_2$	329	180-182^{0}C	88.66%	4 min	0.6	hexane:E.A,3:1
$4a_4$	$C_{19}H_{13}N_4O4$	361	199-200^{0}C	75.75%	4 min	0.7	hexane:E.A,3:1
$4a_5$	$C_{19}H_{13}N_4O4$	361	199-200^{0}C	72.72%	4 min	0.54	hexane:E.A,3:1
$4a_6$	$C_{19}H_{13}N_4O4$	361	200-202^{0}C	72.70%	4 min	0.55	hexane:E.A,3:1
$4a_7$	$C_{19}H_{12}N_3O_2Br$	394	180-182^{0}C	77.77%	4 min	0.72	hexane:E.A,3:1
$4a_8$	$C_{19}H_{12}N_3O_2Cl$	349	219-220^{0}C	75%	4 min	0.6	hexane:E.A,3:1
$4a_9$	$C_{21}H_{18}N_4O_2$	358	138-140^{0}C	72.72%	4 min	0.7	hexane:E.A,3:1
$4a_{10}$	$C_{19}H_{12}N_3O_2F$	305	140-142^{0}C	71.42%	4 min	0.6	hexane:E.A,3:1

CONCLUSION

Microwave-assisted synthesis is a good technique in the field of green chemistry and manages a flexible platform for heterocycle ring formation. MW-assisted reactions have quickly become a robust and efficient tool in synthetic organic chemistry. In recent years a large number of reports appeared through MW-assisted synthesis of S-containing heterocycles. Microwave irradiation has been successfully applied in organic chemistry. Spectacular accelerations, higher yields under milder reaction conditions and higher product purities have all been reported. Advantages of microwave dielectric heating are highlighted by comparison with conventional thermal conditions.Heterocyclic compounds are one of the largest family of organic compounds with broad range of applications in medicinal chemistry and agrochemicals product. Bis-heterocycle and their families have had a significant development of new anticancer drugs according to the literature data published in recent years. Bis-heterocyclic compounds are present in various pharmaceuticals with unique physicochemical properties and have been confirmed as major pillars of medicinal chemistry. Anticancer research has been focused on these molecules' versatility and dynamic core structure. The presented review aims to summarize different bis-heterocyclic active compounds and their beneficial functions as drug targets in cancer therapy and chemoprevention.

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