Studies of 1,3,4-oxadiazole derivatives

Part-I

Studies of 1,3,4-oxadiazole derivatives

2.1.1 Introduction:

Oxadiazoles is a five-member heterocyclic compound having two carbon atoms, two nitrogen atoms, one oxygen atom and two double bonds. The first monosubstituted 1,3,4-oxadiazoles were reported in 1955 by two independent laboratories\textsuperscript{1,2}. The basic skeleton is shown in following figure.

Oxadiazole is an important heterocyclic ring present in variety of biologically active molecules inclusive of fungicidal, bactericidal, anticancer, antitubercular activities, etc. Oxadiazole moiety is derived from furan by replacing two –CH= group with 2 pyrimidine typed nitrogen (-N=). So there should be possibility of four oxadiazole isomers reliant on the nitrogen atom position in the ring as follows.

Basic information

Oxadiazole is a heterocyclic nucleus, which gains heavy interest by many research scholars regarding invention of novel remedial molecules. There are possibly four isomers of oxadiazole in which 1,3,4-oxadiazole have enormous importance. Variety of therapeutically active agents e.g. raltaggravir as HIV-integrase inhibitor, furamizole as nitrofuran anti-bacterial, nesapidil as antihypertensive agents, anti-microbial, anticancer activity, etc. are based on 1,3,4-oxadiazole moiety. The 1,3,4-oxadiazole exhibit variety of reactions such as
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electrophilic substitution, nucleophilic substitution, thermal and photochemical reactions.

- **Structural parameters of 1,3,4-oxadiazole**

  1,3,4-Oxadiazole is an aromatic molecule with resonance energy 167.4 kJ/mol. 1,3,4-oxadiazole ring is symmetrical and planar with the following structural parameters:

<table>
<thead>
<tr>
<th>Bond lengths (Å)</th>
<th>Bond angles (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N3-N4 = 1.399</td>
<td>C2-O-C5 = 102.0</td>
</tr>
<tr>
<td>C2-N3 = 1.297</td>
<td>O-C2-N = 113.4</td>
</tr>
<tr>
<td>N4-C5 = 1.297</td>
<td>C2-N3-N4 = 105.6</td>
</tr>
<tr>
<td>O-C2 = 1.348</td>
<td>N3-N4-N5 = 105.6</td>
</tr>
<tr>
<td>O-C5 = 1.348</td>
<td>O-C5-C4 = 113.4</td>
</tr>
</tbody>
</table>

- **Chemical features of oxadiazole moiety**

  Oxadiazole is a very weak base because there is an inductive effect of extra heteroatom. We know oxadiazole consists of the two pyridine type nitrogen (-N=) hence, reduction in aromaticity of oxadiazole ring, which in turn leads the oxadiazole ring to exhibit the conjugated diene character.

  There is no or very less scope of electrophilic substitutions at the carbon atom in oxadiazole ring due to less electron density on the same carbon atom. Rather, electrophilic attack can occurs at nitrogen, but again there must be association of electron-releasing groups in oxadiazole ring, whereas for nucleophilic substitution like in halogen substituted oxadiazole there is replacement of halogen atom by nucleophiles.
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- Brief descriptions on reactions of oxadiazole:

**A) Reaction with electrophile:**

If we see the following reaction it proves that, low $\pi$-electron density on the carbon atom electrophile attacks favourably at 3rd position and results in 1,3,4-oxadiazolium salts.

**B) Reaction with nucleophile:**

In case of nucleophiles the carbon atoms in 1,3,4-oxadiazole ring have low $\pi$-electron density, which gain access to the attack of nucleophiles on this carbon atom and reveals that the reaction progress either with substitution of nucleophile or ring cleavage. The halogen or sulfonyl group substituted 1,3,4-oxadiazole moiety at 2nd position can easily endure nucleophilic substitution reaction.

Most of 1,3,4-oxadiazoles are best obtained by synthesis from acyclic precursors. Such reactions are ‘one bond’ or ‘two bond’ cyclization. Different methods for the synthesis have been cited in literature$^{3-8}$. 2,5-Disubstituted-1,3,4-oxadiazole derivatives have been tested for various pharmacological activities,
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such as antibacterial\(^9\), antiinflammatory\(^10\), analgesic\(^11\), antiviral and anticancer\(^12\), antihypertensive\(^13\), anticonvulsant\(^14\), antiproliferative\(^15\), cardiovascular\(^16\), herbicidal\(^17\), hypoglycemic\(^18\), hypnotic and sedative\(^19\), MAO inhibitor\(^20\), Insecticidal\(^21\), etc.

Some 1,3,4-oxadiazoles possessing insecticidal activity were synthesized by Xiumian Zheng et al.\(^22\) Takahiko Inoue et al.\(^23\) have reported oxadiazole useful as prolyl aminopeptidase inhibitor. H. Liszkiewicz. et al.\(^24\) have screened oxadiazole for their antimicrobial activity. Fuloria et al.\(^25\) have reported the synthesis of 1-(2-aryl-5-phenethyl-1,3,4-oxadiazol-3(2H)-yl)ethanones by reacting N-(substituted benzyldene)-3-phenyl propionohydrazides with acetic anhydride. Virginija Jakubkiene et al.\(^26\) have screened 1,3,4-oxadiazoles for their antiinflammatory activity. Song Cao et al.\(^27\) have investigated some oxadiazole possessing insecticidal activity. S. Guniz Kucukguzel et al.\(^28\) have discovered oxadiazole derivatives and reported their antimycobacterial activity. A. Ali et al.\(^29\) have prepared 5-(2-(2-fluorophenoxy)phenyl)-1,3,4-oxadiazol-2-amine as anticonvulsant agent. Meria Grazia Mamolo et al.\(^30\) have synthesized 3-substituted-5-(pyridine-4-yl)-3H-1,3,4-oxadiazole-2-one and studied their antimycobacterial activity.

Recently, Ronald Kim et al.\(^31\) have discovered oxadiazole derivatives useful as protease inhibitors. Amir M. et al.\(^32\) have synthesized 2,5-disubstituted 1,3,4-oxadiazole derivatives and their antiinflammatory activity. A. A. El-Emam et al.\(^33\) have investigated some oxadiazole derivatives possessing antimicrobial and anti-HIV-1-activity. S. A. F. Rostom et al.\(^34\) have reported oxadiazoles as potential antitumor and anti-HIV agents. Afshin Zarghi et al.\(^35\) have synthesized substituted-5-(2-benzylxyphenyl)-1,3,4-oxadiazoles possessing anticonvulsant activity. M. T. Khan et al.\(^36\) have synthesized 2,5-disubstituted-1,3,4-oxadiazoles useful as tyrosinase inhibitors.

The syntheses of following derivatives have been discussed in this chapter and it is divided into three parts namely:
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Part I  Studies of 1,3,4-oxadiazole derivatives

Section I  2-((4-acetyl-5-aryl-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-o-tolylquinazolin-4(3H)-one

Section II  2-((4-acetyl-5-aryl-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-m-tolylquinazolin-4(3H)-one

Section III  2-((4-acetyl-5-aryl-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-p-tolylquinazolin-4(3H)-one

Part II  Studies of 4-thiazolidinone derivatives

2-(3-(2,4-dimethylphenyl)-4-oxo-3,4-dihydroquinazolin-2-ylthio)-N-(2-arylthiazolidin-4-oxo-3-yl)acetamide

Part III  Studies of dihydropyrimidine derivatives

Section I  4-aryl-N-(2,4-dimethylphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide

Section II  4-aryl-N-(2,4-dimethylphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide

Spectroscopic analysis, thermal analysis (TGA/DTA) and biological activities of these compounds are discussed in this chapter.

2.1.2 InfraRed (IR) spectral analysis

Information about the structure of a molecule could frequently be obtained from its absorption spectrum. The atomic and electronic configuration of a molecule is responsible for the position of absorption bands. The most structural information of organic molecules could be obtained from their IR spectra. The masses of the atoms and forces holding them together are of such magnitudes that usual vibration of the organic molecules interact with electromagnetic radiation to absorb and radiate in the IR region. During the absorption, it is necessary for the molecule to undergo a change in dipole moment. IR spectroscopy is an excellent method for the quantitative analysis because the spectrum of a compound is unique except for optical isomers. It is most useful for the identification, purity
Studies of 1,3,4-oxadiazole derivatives and gross structure detail. This technique is often faster than any other analytical methods.

The IR spectrums of synthesized compounds were scanned on a FT-IR-Thermonicolate IR 200 spectrophotometer over the frequency range from 4000-400 cm\(^{-1}\) by KBr pellet method.

### 2.1.3 Nuclear Magnetic Resonance (NMR) spectral analysis

This section of the chapter includes spectral data of synthesized compounds. Some nuclei spin about their axes in a manner to that of electrons spin. In the presence of externally applied magnetic field, a spinning nucleus can only assume a limited number of stable orientations. NMR occurs, when a spinning nucleus in lower energetic orientation in a magnetic field absorbs sufficient electromagnetic radiation and excites to a higher energetic orientation. The excitation energy varies with the type and environment of the nucleus. NMR spectroscopy can be used for the quantitative chemical analysis. NMR spectroscopy consists of measuring the energy that is required to change a spinning nucleus from a stable orientation to a less stable orientation in the magnetic field. Different spinning nuclei in the magnetic field absorb different frequency of radiation to change their orientations. The frequencies at which absorption occur can be used for qualitative analysis.

NMR spectrometer was invented in 1945 by Falix Bloch (Stanford University) and Edward Purcell. They shared the Noble prize (1952) in Physics for their work.

\(^1\)H NMR spectra of synthesized compounds were recorded on Brucker DRX 300 (200 MHz) spectrophotometer by using CDCl\(_3\) as a solvent and TMS as an internal standard. Chemical shifts were recorded in (\(\delta\) ppm) and coupling constant (\(J\)) were recorded in Hertz (Hz). The solvent CHCl\(_3\) appeared at about 7.266 \(\delta\) ppm as a separate peak. Number of protons found in NMR is concomitant with the theoretical value.
2.1.4 Mass spectral analysis

Mass spectrometry is used by organic chemists to characterize organic molecules in two principal ways:

1) To measure exact molar mass and from this, exact molecular formulae can be determined.
2) To indicate within a molecule the points at which it prefers to fragment.

From this, the presence of certain structural units in the organic compound can be recognized.

The commonly used technique for obtaining the mass spectrum of an organic compound is electron impact (EI) technique. Other techniques chemical ionization (CI) and the fast atom bombardment (FAB), are used for organic compounds, while electro spray ionization (ESI) and matrix assisted laser desorption ionization (MALDI) are used for peptide/proteins.

Mass spectra of synthesized compounds were taken over Shimadzu GC-MS-QP 2010 spectrometer by using EI (0.7 kV) detector. Though molecular ion peak is low in abundance but it gives valuable information about identification of the compound. Molecular ion further undergoes into various fragments, which are very useful in establishing the structure. Pyrolysis of the compound is a complex process and involves a variety of reactions namely ionization, decomposition, branching, cross linking, rearrangement, recombination, etc. Up on loss of electron results into molecular ion (M+), and ultimately converted into low molecular mass substances.

2.1.5 Thermal analysis

During last few years, the methods of thermal analysis have been widely accepted in analytical chemistry to study various types of compounds such as drugs\(^{37,38}\), dyes\(^{39-41}\), fertilizers\(^{42,43}\), polymers\(^{44-47}\), pharma materials\(^{48,49}\), organic\(^{50,51}\), inorganic\(^{52,53}\), vitamine\(^{54}\), metallo-organic\(^{55}\) etc compounds have been reported. Several thermal methods have been recognized, which differ in the properties measured and temperature programs\(^{56}\).
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M. H. Yi et al.\textsuperscript{57} have synthesized soluble polyimides from 1,1-bis (4-aminophenyl)cyclohexane and various aromatic dianhydrides by the conventional polycondensation reaction followed by chemical imidization as well as high-temperature one-step polymerization. The thermal stabilities of the polyimides were evaluated by Thermo Gravimetric Analysis (TGA) as well as Differential Scanning Calorimetry (DSC) under nitrogen atmosphere, the polyimides exhibited excellent thermal stability. They are stable up to 500 °C, and the residual weights at 800 °C were above 30%, which is comparable to the typical soluble polyimides derived from dianinophenylindane (DAPI) produced by Ciba Geigy. The glass transition temperatures (\(T_g\)’s) measured by differential scanning calorimetry (DSC) ranged from 290 to 372 °C. They also concluded that the polyimides prepared from BPDA (biphenyl-tetracarboxylic acid dianhydride) had relatively high \(T_g\)’s, because of their rigid structure. The \(T_g\)’s of the polymers were decreased with an increase lower series; ODPA (3,3’,4,4’-oxydiphtalic dianhydride) < HFDA (hexafluoroisopropylidene-2,2 bis phthalic ether) < BTDA (3,3’,4,4’-benzophenontetraacrylyc anhydride) < PMDA (1,2,4,5-benzene tetracarboxylic dianhydride ) < BPDA (biphenyltetraacrylyc dianhydride). It was also found that the \(T_g\)’s were increased with the number of methyl substituents because of the increasing restriction on the main-chain rotational motion.

M. H. Yi et al.\textsuperscript{58} have synthesized 2,2-bis(4-aminophenyl)cyclohexane derivatives. They synthesized diamines containing polycycloalkane structures between two benzene rings by HCl-catalyzed condensation reaction of aniline hydrochloride and corresponding polycycloankanone derivatives. The polyimides were synthesized from the obtained diamines with various aromatic dianhydrids by one-step polymerization in m-cresol. They reported that the polyimides showed good thermal stabilities and solubility. The glass transition temperatures were observed in the range of 323-363 °C, and all of the polymers were stable up to 400 °C under nitrogen atmospheres.
2.1.5.1 Effect of various operating parameters

(i) Atmosphere

The atmosphere associated with any thermal analysis, which composed of gases those are introduced from outside and those is evolved from the samples.

The presence or absence of such gases may have a strong influence on the results. These gases may react with the sample or with each other, and change the reaction mechanism or product composition. Inert atmosphere and vacuum will influence decomposition processes as well. In vacuum, primary decomposition of gases will tend to be pumped away from the sample before the molecules collide with the surface and undergoes secondary reactions. When these molecules collide with inert gas molecules, they may undergo homogeneous reaction or may be reflected back to the sample surface and react there.

(ii) Container geometry

The container geometry influences the gaseous environment and heat transfer to the samples. Even with a flowing gaseous atmosphere, a deep narrow container will limit the contact between the samples surface and gas, whereas a shallow, broad container will promote the contact.

(iii) Container material

It is reasonable to expect that in some cases the container material will react with material being tested or some of the products.

(iv) Sample size

Two major effects are associated with the sample size, namely surface and bulk effects. In carrying out degradation studies, it is customary to reduce fill particle size until the rate of decomposition becomes independent of size.

(v) Heating rate

In the case where only kinetic considerations are significant and increase in the rate of temperature rise will cause the process to be displayed to a higher temperature because the sample will have been at the lower temperatures for a
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shorter length of time. The rate of change of the measured parameters will also be greater for faster heating.

2.1.5.2 Differential Scanning Calorimetry (DSC) and Differential Thermal Analysis (DTA)

Physical transformation\textsuperscript{59} such as glass transition, cold crystallization, crystallization from melt, crystalline disorientation, and melting can be studied by differential scanning calorimetry (DSC) and differential thermal analysis (DTA).

DSC is a method where by the energy necessary to establish a zero temperature difference between a substance and a reference material is recorded as a function of temperature or time. When an endothermic transition occurs, the energy input to the sample in order to maintain a zero temperature difference, because this energy input is precisely equivalent in magnitude to the energy absorbed during the transition in direct calorimetric measurement. DSC provides useful information about crystallinity, stability of crystallites, glass transition temperature, cross linking, kinetic parameters such as the activation energy, the kinetic order and heat of polymerization, etc.

DTA is more versatile and gives data of more fundamental nature than TGA. This technique involves recording of difference in temperature between a substance and reference material against either time or temperature as the two specimens are subjected to identical temperature regimes in an environment heated or cooled at a programmed heating rate. Any transition, which the sample undergoes results in absorption or liberation of energy by the sample with a corresponding deviation of its temperature from that of the reference.

In DTA, as soon as the sample reaches the temperature of the change of its state (chemical or physical), the differential signal appears as a peak. The number, position, shape and nature (exothermic or endothermic) of the DTA peaks give information about glass transition temperature, crystalline rearrangement, melting, curing, degradation, decomposition of chemical substance, etc.
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2.1.5.3 Thermo Gravimetric Analysis (TGA)

Different substances decompose over different ranges of temperature yielding different proportion of volatile and residues. Thermo gravimetry is useful analytical technique for recording weight loss of a test sample as a function of temperature, which may be used for understanding the chemical nature of the substance. Thus, the weight of a substance in an environment heated or cooled at a controlled rate is recorded as a function of time or temperature.

There are three types of thermogravimetry:

(i) Static or isothermal thermogravimetry
(ii) Quasistatic thermogravimetry and
(iii) Dynamic thermogravimetry

Most of the studies of chemical substance are carried out with dynamic thermogravimetry generally. Normally, the sample starts losing weight at a very slow rate up to a particular temperature and thereafter, the rate of loss becomes large over a narrow range of temperature. After this temperature, the loss in weight levels off. TGA curves are characteristic for a given substance because of unique sequence of physico-chemical reactions, which occur over definite temperature ranges. The change in weight is a result of the rupture and/or formation of various physical and chemical bonds at elevated temperatures that lead to evaluation of volatile products in the formation of heavier reaction products. Pyrolysis of many substances yields TG curves.

The weight of the sample decreases slowly as reaction begins and then decreases rapidly over a comparatively narrow range of temperature and finally levels off as the reaction is completed. The shape of the curve depends on the kinetic parameters: order of reaction ($n$), frequency factor ($A$) and activation energy ($E_a$). The values of these parameters have been shown to be of major importance to elucidate the mechanism of degradation\textsuperscript{60,61}.

Reich and Levi\textsuperscript{62} have described several temperature characteristics for qualitative assessment of relative thermal stability of polymers:
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1. Initial decomposition temperature ($T_o$)
2. Temperature for 10% weight loss ($T_{10}$)
3. Temperature for maximum rate of decomposition ($T_{max}$)
4. Half volatilization temperature ($T_s$)
5. Differential decomposition temperature and
6. Integral procedural decomposition temperature (IPDT) with dynamic heating

$T_o$ and $T_{10}$ are some of the main criteria of the thermal stability of a given substance.

For the estimation of kinetic parameters from TG traces, several so called exact methods have been proposed. All these methods involve two important assumptions that thermal and diffusion barriers are negligible and that Arrhenius equation is valid. Since, small quantities of materials are employed in TG studies, thermal and diffusion barriers would be negligible. Since, the shape of any TG curve is dependent on the nature of apparatus and the way in which it is used. Most kinetic treatments are based on relationship of the type:

$$\frac{dC}{dt} = k f(C) \ldots (2.1)$$

Where C = Degree of conversion, t = time; k = rate constant, and f(C) = temperature independent function of C

The constant k is generally assumed to have the Arrhenius form

$$k = A e^{-Ea/RT} \ldots (2.2)$$

C is defined as the conversion with respect to initial material

$$C = 1 - \frac{W}{W_o} \ldots (2.3)$$

Where, $W_o$ = Initial weight of the material and W= weight of the material at any time.

The residual weight fraction is given by $\frac{W}{W_o} = (1-C)$ and the rate of conversion is given by

$$\frac{dC}{dt} = -\left(\frac{1}{W_o}\right)\frac{dW}{dt} \ldots (2.4)$$

For homogeneous kinetics, the conversion would be assumed to have the form
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\[ f(C) = (1-C)^n \quad \text{(2.5)} \]

Where \( n \) = order of the reaction

Upon substituting Eqns. 2.2 and 2.5 into Eqn. 2.1

\[ \frac{dC}{dt} = A \, e^{-E_a/RT} \, (1-C)^n \quad \text{OR} \quad \frac{dC}{dT} = \left( \frac{A}{\beta} \right) \left( e^{-E_a/RT} \right) (1-C)^n \quad \text{(2.6)} \]

Where \( \beta \) = Rate of heating

2.1.5.4 Methods of single heating rate

(i) Freeman-Carroll and Anderson-Freeman methods

Freeman-Carroll has developed the following relation to analyze TGA data at single heating rate:

\[ \frac{\Delta \ln \left( \frac{dC}{dt} \right)}{\Delta \ln (1-C)} = n - \frac{E_a}{R} \left( \frac{\Delta (1/T)}{\Delta \ln (1-C)} \right) \quad \text{(2.7)} \]

A plot of L.H.S. against \( \Delta (1/T) / \Delta \ln (1-C) \) would yield a straight line with slope equal to \( -\frac{E_a}{R} \) and the intercept equal to \( n \). Using Eqn. 2.7 Anderson-Freeman derived the Eqn. 2.8

\[ \Delta \ln \left( \frac{dC}{dt} \right) = n \Delta \ln (1-C) - \frac{E_a}{R} \Delta \left( \frac{1}{T} \right) \quad \text{(2.8)} \]

According to Eqn. (2.8), the plot of \( \Delta \ln (dC/dt) \) against \( \Delta \ln (1-C) \) for equal interval of \( \Delta (1/T) \) would be a straight line with slope equal to \( n \) and the intercept equal to \( -\frac{E_a}{R} \Delta (1/T) \).

(ii) Sharp-Wentworth method

For a first order process \( (n = 1) \), Sharp-Wentworth derived the following relation to analyze TGA data.

\[ \log \left( \frac{dC}{dt} \right) = \log \left( \frac{A}{\beta} \right) - \frac{E_a}{2.303 R} \frac{1}{T} \quad \text{(2.9)} \]

Where, \( C \) = fraction of polymer decomposed at temperature \( T \), \( \beta \) = rate of heating, \( A \) = Frequency factor and \( E_a \) = activation energy of the process. The plot of \( \log \left( \frac{[dC/dt]}{(1-C)} \right) \) against \( 1/T \) would be a straight line with slope equal to \( -(E_a / 2.303 \, R) \) and the intercept equal to \( \log (A/\beta) \).
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(iii) Chatterjee method\textsuperscript{65}

Chatterjee has developed the following relation for the determination of $n$ from TG curves based on weight units.

$$n = \frac{\log \left( \frac{dW}{dt} \right)_1 - \log \left( \frac{dW}{dt} \right)_2}{\log W_1 - \log W_2} \quad \ldots (2.10)$$

Where, $W_1$ and $W_2$ are the weight of samples.

(iv) Horowitz-Metzger method\textsuperscript{66}

The value of $E_a$ can be determined from a single TG curve according to Horowitz–Metzger method

$$\ln \left[ \ln (1 - C)^{-1} \right] = \frac{E_a}{R T_s} \theta \quad \ldots (2.11)$$

Where, $C$ = fraction of the compound decomposed at time $t$, $E_a$ = activation energy, $T_s$ = Temperature at which the rate of decomposition is maximum and $\theta = T - T_s$

The frequency factor $A$ and entropy change $\Delta S$ can be determined respectively according to Eqns. 2.12 and 2.13.

$$\ln E - \ln \left( R T_s^2 \right) = \ln A - \ln \beta - \frac{E}{R T_s^2} \quad \ldots 2.12$$

$$A = \frac{k_b}{h} e^{\Delta S/R} \quad \ldots 2.13$$

Where, $k_b$ is Boltzmann constant.
2.1.6 Biological evaluation

- Antibacterial activity

Man is closely influenced by the activities of microorganisms. Microorganisms are a part of our lives in more ways than most of us understand. They have shaped our present environment and their activities will greatly influence our future. Microorganisms should not be considered separate from human beings but should be considered as a part of our life. Microorganisms have a profound beneficial influence on our daily life. They are employed in the manufacture of dairy products, certain foods, in processing of certain medicines and therapeutic agents in manufacture of certain chemicals and in numerous other ways.

Despite the established useful functions and potentially valuable activities of microorganisms, these microscopic forms of life may be best known as agents of food spoilage and causal agents of human beings viz. Acquired Immune Deficiency Syndrome (AIDS), herpes, legionnaires disease, influenza, jaundice, tuberculosis, typhoid, dermatomycoses, dysentery, malaria etc. in human beings. Animals (infected with brucellosis, tularemia, etc.) and plants (infected with mildews, rusts, smuts, cankers, leaf spots, etc.) have also been known to be victims of microbial pathogens. So far as is known, all primitive and civilized societies have experienced diseases caused by microbes, frequently with disastrous results. Moreover, microorganisms have played profound roles in warfare, religion and the migration of populations.

Control of microbial population is necessary to prevent transmission of disease, infection, decomposition, contamination and spoilage caused by them. Man’s personal comforts and convenience depend to a large extent on the control of microbial population.

2.1.6.1 Bacteria: In 1928, a German scientist C.E. Chrenberg first used the term “bacterium” to denote small microscopic organism with a relatively simple and primitive form of the cellular organization known as “prokaryotic”.
Danish physician, “Gram”, in 1884, discovered the famous “Gram staining” which is an important method of identification of bacteria. The categories of all the different types of bacteria can be divided in two broad divisions “Gram positive” and “Gram negative”. The gram positive bacteria resist decolonization with acetone, alcohol and remain stained dark purple (Methyl violet), while gram negative bacteria are decolorized. The gram staining behavior depends on the structure of the bacterial cell wall. Gram staining method is the widely acceptable method and is used frequently now a day.

The microorganisms are capable of producing diseases in host are known as ‘pathogenic’. Most of the microorganisms present on the skin and mucous membrane are non pathogenic and are often referred to as “commensals” or if they live on food residues as in intestine, they may be called “saprophytes”. Generally, the pathogenic cocci and bacilli are gram positive and the pathogenic coco bacilli are gram negative.

For evaluation of antibacterial activity in our case, we have used *Staphylococcus aureus* and *Streptococcus pyogenes* from gram-positive group of bacteria and *Escherichia coli* and *Pseudomonas aeruginosa* from gram-negative group of bacteria.

2.1.6.2 **Antifungal activity**

It has been estimated that the life span of humans has increased by almost a decade since the discovery of antimicrobial agents against of microbial infections. A consequence of our success with antibacterial agents and improved medical care is the increase in the number of fungal infections.

The incidence of fungal infections has increased dramatically in the past 20 years partly due to increase in the number of people whose immune systems are compromised by AIDS, aging, organ transplantation or cancer therapy. Accordingly, the increase in rates of morbidity and mortality of infections has been now recognized as a major problem. In response, the pharmaceutical industry has developed a number of novel less toxic antifungal for clinical use.
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Its increased use often for prolonged periods, has led to the recognition of the phenomenon of acquired antifungal resistance amongst previously susceptible strains or species and to the increased incidence of infections with less common species, with intrinsic resistance to one or more of the available antifungal.

Fungi are non photosynthetic eukaryotes growing either as colonies of single cells (yeasts) or as filamentous multicellular aggregate (molds). Most fungi live as saprophytes in soil or on dead plant material and are important in the mineralization of organic matter. A smaller number produce disease in human and animals. The in vitro methods used for detections of antifungal potency are similar to those used in antibacterial screening. As with bacteria, it is easy to discover several synthetic and natural compounds that, in small quantity, can retard or prevent growth of fungi in culture media.

For evaluation of antifungal activity in our case, we have used Candida albicans, Aspergillus niger and Aspergillus clavatus group of fungi.

2.1.7 Evaluation Techniques

The evaluation of antimicrobial activity followings methods are used\textsuperscript{68-70}.

1. Turbidometric method
2. Agar streak dilution method
3. Serial dilution method
4. Broth dilution method, which are of four types:

- Agar diffusion method
- Agar cup method
- Agar ditch method
- Paper disc method

Broth dilution method, a widely used non-automated in vitro bacterial susceptibility test has been used to evaluate antimicrobial activity. It is classic method that yields a quantitative result for the amount of antimicrobial agent that is needed to inhibit growth of microorganisms. It is carried out in tubes.

- Macro-dilution method in tubes
- Micro-dilution format using plastic trays

In the present protocol we have used the Micro-dilution format.
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2.1.7.1 Determination of Minimal Bactericidal Concentrations (MBC) by Broth Dilution Method:

Materials and Method:

1) All the synthesized drugs were used for antibacterial tests.
2) All necessary controls, *viz.* drug, vehicle, broth of organism were used.

✓ The drug Gentamicin, Ampicillin, Chloramphenicol, Ciprofloxacin and Norfloxacin were used as control.
✓ Mueller Hinton Broth was used as nutrient medium to grow the stains and dilute the drug suspensions for test. All MTCC cultures were tested against above-mentioned known and unknown drugs.

3) Serial dilution technique was followed by micro method as per NCCLS – 1992 manual.

4) Inoculum size: Inoculum size for test strain was adjusted to $10^8$ CFU/mL (colony forming unit) for bacteria and $10^6$ spores/mL for fungi.

5) The strains used for screening of antibacterial and antifungal activities were, the strains were procured from Institute of Microbial Technology (IMT), Chandigarh.

The following stains procured for IMT – Chandigarh were used for screening antibacterial and anti fungal activities.

- *Staphylococcus aureus* (Gram positive) MTCC– 96
- *Streptococcus pyogenes* (Gram positive) MTCC– 442
- *Escherichia coli* (Gram negative) MTCC– 443
- *Pseudomonas aeruginosa* (Gram negative) MTCC – 1688
- *Candida albicans* (Fungus) MTCC – 227
- *Aspergillus niger* (Fungus) MTCC – 282
- *Aspergillus clavatus* (Fungus) MTCC – 1323

DMSO was used as diluent / vehicle to get desired concentration of drugs to test upon standard bacterial strains.

2.1.7.1.1 Minimal Bactericidal Concentrations (MBC)

The main advantage of the ‘Broth Dilution Method’ for MBC determination lies in the fact is that it can readily be converted to determine the MBC as well.
Studies of 1,3,4-oxadiazole derivatives

1) Serial dilutions were prepared in primary and secondary screening.
2) The control tube containing no antibiotic is immediately subcultured (before incubation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and incubated at 37 °C for 24 h.
3) The MBC of the control organism is read to check the accuracy of the drug concentrations.
4) The lowest concentration inhibiting growth of the organism is recorded as the MBC.
5) All the tubes not showing visible growth (in the same manner as control tube described above) are subcultured and incubated overnight at 37 °C.
6) The amount of growth from the control tube before incubation (which represents the original inoculum) was compared.
7) Subcultures may show: Similar number of colonies indicating bacteriostasis, a reduced number of colonies - indicating a partial or slow bactericidal activity and No growth - if the whole inoculum has been killed. The test must include a second set of the same dilutions inoculated with an organism of known sensitivity.

2.1.7.1.2 Minimal Fungicidal Concentration (MFC)

Broth dilution method for MFC determination lies in the fact that it can readily be converted to determine the MFC as well. Serial dilution where primary and secondary screening and the material and method was just followed like a bactericidal activity. The growth, inhibition is measured and compound is applied in the method to determine the activity in μg/mL concentration.

Methods used for primary & secondary screening:

Each synthesized drug was diluted obtaining 2000 μg/mL concentration, as a stock solution.

♦ **Primary screening:** In primary screening 1000 μg/mL, 500 μg/mL, 250 μg/mL and 125 μg/mL concentrations of the synthesized drugs were taken.
Studies of 1,3,4-oxadiazole derivatives

The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms.

Secondary screening: The drugs found active in primary screening were similarly diluted to obtain 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.250 µg/mL, 3.125 µg/mL and 1.5625 µg/mL concentrations.

10 µL suspension from each well was further inoculated on appropriate media and growth was noted after 24 and 48 hrs. The lowest concentration, which showed no growth after spot subculture was considered as MIC (Bacteria) for each drugs.

Reading Results:

The highest dilution showing at least 99% inhibition is taken as MBC. The result of this test is affected by the size of the inoculum. The test mixture should contain 10⁸ organism/mL.

The standard drug:

The standard drugs used in the present study are Gentamicin, Ampicillin, Chloramphenicol, Ciprofloxacin and Norfloxacin for evaluating antibacterial activity which showed (0.25, 0.05, 0.5 & 1 µg/mL MBC against S.aureus, S.pyogenes, E.coli and P.aeruginosa respectively. Nystatin and Gresofulvin are used as the standard drug for antifungal activity which showed 100 µg/mL MFC against all the species, used for the antifungal activity.

<table>
<thead>
<tr>
<th>DRUG (MBC) in µg/mL</th>
<th>E.coli MTCC 443</th>
<th>P.aeruginosa MTCC 1688</th>
<th>S.aureus MTCC 96</th>
<th>S.pyogenes MTCC 442</th>
</tr>
</thead>
<tbody>
<tr>
<td>GENTAMYCIN</td>
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<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>AMPICILLIN</td>
<td>100</td>
<td>--</td>
<td>250</td>
<td>100</td>
</tr>
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<td>CHLORAMPHENICOL</td>
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<td>50</td>
</tr>
<tr>
<td>CIPROFLOXACIN</td>
<td>25</td>
<td>25</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>NORFLOXACIN</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DRUG (MFC) in µg/mL</th>
<th>C. albicans MTCC 227</th>
<th>A. niger MTCC 282</th>
<th>A. clavatus MTCC 1323</th>
</tr>
</thead>
<tbody>
<tr>
<td>NYSTATIN</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>GRESEOFULVIN</td>
<td>500</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

{29}
Studies of 1,3,4-oxadiazole derivatives

Part – I

Section - I

Synthesis of
2-((4-acetyl-5-aryl-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-o-tolylquinazolin-4(3H)-one

Scheme - 2.1
Studies of 1,3,4-oxadiazole derivatives

Experimental procedure

2-Mercapto-3-o-tolylquinazolin-4(3H)-one (1)

A mixture of carbon disulphide (0.030 mole) and o-toluidine (0.012 mole) was added drop wise to a refluxing mixture of anthranilic acid (0.010 mole) and potassium hydroxide (0.012 mole) in methanol (15 mL). The mixture was refluxed on a water bath for about 10 h, and then poured over ice to get a solid product. Dissolved in 10% potassium hydroxide solution and filtered, con. HCl was added to the filtrate. The white precipitate obtained was filtered and washed with cold 50% methanol. The product is crystallized from methanol, to get compound (1). Yield-75% m.p. 275 °C (Found: C, 67.10; H, 4.50; N, 10.42%. Caled for C_{15}H_{12}N_{2}OS: C, 67.14; H, 4.51; N, 10.44%)

Ethyl 2-(4-oxo-3-o-tolyl-3,4-dihydroquinazolin-2-ylthio)acetate (2)

A mixture of compound (1) (0.1 mole) and ethyl chloro acetate (0.1 mole) in dry acetone (35 mL), in the presence of K_{2}CO_{3} (0.15 mole) was refluxed on a water bath for about 12 h. The reaction mixture was poured in ice to get a solid product, washed with methanol (50%). The product is crystallized from ethanol to get compound (2). Yield-77% m.p. 156 °C (Found: C, 64.37; H, 5.10; N, 7.87%. Caled for C_{19}H_{18}N_{2}O_{3}S: C, 64.39; H, 5.12; N, 7.90%)

2-(4-Oxo-3-o-tolyl-3,4-dihydroquinazolin-2-ylthio)acetohydrazide (3)

A mixture of compound (2) (0.1 mole) and hydrazine hydrate (0.1 mole) was placed in a RBF and ethanol (40 mL) was added as a solvent. The reaction mixture was refluxed for 8 h, followed by cooling at room temperature and product is separated, filtered and washed with water, recrystallized from ethanol to get compound (3). Yield-73% m.p. 155 °C (Found: C, 59.95; H, 4.71; N, 16.43%. Caled for C_{17}H_{16}N_{4}O_{2}S: C, 59.98; H, 4.74; N, 16.46%)

(E)-N’-benzylidene-2-(4-oxo-3-o-tolyl-3,4-dihydroquinazolin-2-ylthio)acetohydrazide (4H)

A mixture of compound (3) (0.01 mole) and acetophenone (0.01 mole) in ethanol in was refluxed. During the reflux 2-3 drops of acetic acid was added and
Studies of 1,3,4-oxadiazole derivatives

refluxed continued for 6 h. After completion of reaction the product was filtered and washed it with water and methanol (50%) mixture and the product was recrystallized from methanol to get compound (4H). Yield 75% m.p. 223 °C (Found: C, 67.27; H, 4.70; N, 13.07%. Caled. For C$_{24}$H$_{20}$N$_{4}$O$_{2}$S: C, 64.85; H, 4.54; N, 12.60%).

2-((4-Acetyl-5-methyl-5-phenyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-o-tolylquinazolin-4(3H)-one (5H) (PD$_{1-H}$)

A mixture of Schiff-base (4H) 2.15 g (0.005 mole) and acetic anhydride (10 mL) were taken into a 100 mL round bottom flask. The mixture was refluxed for 4 h on oil bath. The progress of the reaction was monitored with the aid of TLC. The excess of acetic anhydride was distilled off and remaining mixture was poured in the ice cold water to get main product. Product was separated by filtration and crystallized from chloroform - n-hexane to afford the title compound PD$_{1-H}$.

Other compounds of the series (PD$_{1-A}$ to PD$_{1-G}$) were prepared by using a similar method and their analytical data are shown in Table - 2.1.
Studies of 1,3,4-oxadiazole derivatives

Analytical data of

2-((4-acetyl-5-aryl-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-o-toly1quinazolin-4(3H)-one (PD_{I-A} - PD_{I-H})

![Image of chemical structure]

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Ar</th>
<th>Molecular Formula</th>
<th>% Yield</th>
<th>M.P. °C</th>
<th>C %</th>
<th>H %</th>
<th>N %</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Req</td>
<td>Obs</td>
<td>Req</td>
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<tr>
<td>PD_{I-A}</td>
<td>4-OCH_{3}-C_{6}H_{4}</td>
<td>C_{28}H_{26}N_{4}O_{5}S</td>
<td>68</td>
<td>258</td>
<td>65.35</td>
<td>65.31</td>
<td>5.09</td>
</tr>
<tr>
<td>PD_{I-B}</td>
<td>4-Cl-C_{6}H_{4}</td>
<td>C_{27}H_{23}N_{4}O_{5}S</td>
<td>72</td>
<td>260</td>
<td>62.48</td>
<td>62.52</td>
<td>4.47</td>
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<tr>
<td>PD_{I-C}</td>
<td>2-OH-C_{6}H_{4}</td>
<td>C_{27}H_{23}N_{4}O_{5}S</td>
<td>63</td>
<td>248</td>
<td>64.78</td>
<td>64.76</td>
<td>4.83</td>
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<tr>
<td>PD_{I-D}</td>
<td>4-Br-C_{6}H_{4}</td>
<td>C_{27}H_{22}BrN_{4}O_{5}S</td>
<td>58</td>
<td>282</td>
<td>57.55</td>
<td>57.58</td>
<td>4.11</td>
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<td>PD_{I-E}</td>
<td>4-NO_{2}-C_{6}H_{4}</td>
<td>C_{27}H_{22}N_{4}O_{5}S</td>
<td>65</td>
<td>264</td>
<td>61.24</td>
<td>61.23</td>
<td>4.38</td>
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<td>PD_{I-F}</td>
<td>3-NO_{2}-C_{6}H_{4}</td>
<td>C_{27}H_{22}N_{4}O_{5}S</td>
<td>60</td>
<td>268</td>
<td>61.24</td>
<td>61.21</td>
<td>4.38</td>
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<tr>
<td>PD_{I-G}</td>
<td>4-CH_{3}-C_{6}H_{4}</td>
<td>C_{28}H_{26}N_{4}O_{5}S</td>
<td>66</td>
<td>270</td>
<td>67.45</td>
<td>67.49</td>
<td>5.26</td>
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<tr>
<td>PD_{I-H}</td>
<td>-C_{6}H_{5}</td>
<td>C_{27}H_{26}N_{4}O_{5}S</td>
<td>70</td>
<td>290</td>
<td>66.92</td>
<td>66.92</td>
<td>4.99</td>
</tr>
</tbody>
</table>

Table - 2.1
Studies of 1,3,4-oxadiazole derivatives

Figure 2.1 IR spectra of 2-((4-acetyl-5-methyl-5-phenyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-o-tolylquinazolin-4(3H)-one (PD14H)
Studies of 1,3,4-oxadiazole derivatives

Table-2.2 IR spectral data of 2-((4-acetyl-5-methyl-5-phenyl-4,5-dihydro-1,3,4 oxadiazol-2-yl)methylthio)-3-o-tolylquinazolin-4(3H)-one (PD\textsubscript{I-H})

<table>
<thead>
<tr>
<th>Vibration mode</th>
<th>Frequency in cm\textsuperscript{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed</td>
</tr>
<tr>
<td>C-H str. (asym.)</td>
<td>2923</td>
</tr>
<tr>
<td>C-H str. (sym.)</td>
<td>2852</td>
</tr>
<tr>
<td>C=O str. of amide</td>
<td>1685</td>
</tr>
<tr>
<td>C=O str. of -CH\textsubscript{3}</td>
<td>1714</td>
</tr>
<tr>
<td>-CH\textsubscript{3} bending vib.</td>
<td>1384</td>
</tr>
<tr>
<td>C-H str. (aromatic)</td>
<td>3070</td>
</tr>
<tr>
<td>C=C str. Ring skelatal</td>
<td>1596</td>
</tr>
<tr>
<td>-CH\textsubscript{2} bending</td>
<td>1477</td>
</tr>
<tr>
<td>C-H inplane bending</td>
<td>1085</td>
</tr>
<tr>
<td>C-N str.</td>
<td>1275</td>
</tr>
<tr>
<td>C-O-C str.</td>
<td>1018</td>
</tr>
<tr>
<td>C-H o.o.p. bending(1,2-disub.)</td>
<td>759</td>
</tr>
<tr>
<td></td>
<td>750.13</td>
</tr>
<tr>
<td></td>
<td>684</td>
</tr>
</tbody>
</table>

The IR data of PD\textsubscript{I-H} clearly shows a C=N stretching band at 1688 cm\textsuperscript{-1}, C-O absorption band at 1018 cm\textsuperscript{-1} and C-N stretching band at 1275 cm\textsuperscript{-1}, which indicates ring closure of the 1,3,4-oxadiazole. The presence of stretching band at 759 cm\textsuperscript{-1} indicate 1,2-disubstituted and 755 cm\textsuperscript{-1} and 684 cm\textsuperscript{-1} indicate mono substituted benzene ring is present in synthesized compound.
Studies of 1,3,4-oxadiazole derivatives

Figure 2.2 1H NMR spectra of 2-((4-acetyl-5-methyl-5-phenyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-o-tolylquinazolin-4(3H)-one (PD1-H)
Studies of 1,3,4-oxadiazole derivatives

Table 2.3 ¹H NMR spectral data of 2-((4-acetyl-5-methyl-5-phenyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-o-tolylquinazolin-4(3H)-one (PD₁1₁)

<table>
<thead>
<tr>
<th>Signal No.</th>
<th>Signal position (δ ppm)</th>
<th>Relative No. of Protons</th>
<th>Multiplicity</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.63</td>
<td>3</td>
<td>s</td>
<td>-CH₃(l)</td>
</tr>
<tr>
<td>2</td>
<td>2.18</td>
<td>3</td>
<td>s</td>
<td>-CH₃(k)</td>
</tr>
<tr>
<td>3</td>
<td>2.54</td>
<td>5</td>
<td>s</td>
<td>-CH₂+CH₃(e, j)</td>
</tr>
<tr>
<td>4</td>
<td>7.46-7.52</td>
<td>9</td>
<td>m</td>
<td>Ar-H(d, f, l, g, m, m’, n, n’, o)</td>
</tr>
<tr>
<td>5</td>
<td>7.56-7.59</td>
<td>1</td>
<td>t</td>
<td>Ar-H(h)</td>
</tr>
<tr>
<td>6</td>
<td>7.71-7.74</td>
<td>1</td>
<td>t</td>
<td>Ar-H(b)</td>
</tr>
<tr>
<td>7</td>
<td>7.98-8.01</td>
<td>1</td>
<td>t</td>
<td>Ar-H(c)</td>
</tr>
<tr>
<td>8</td>
<td>8.43-8.45</td>
<td>1</td>
<td>d</td>
<td>Ar-H(a), j=1.2</td>
</tr>
</tbody>
</table>

It can be seen from the chemical structure of compound PD₁1₁ that proton of the phenyl ring attached to the carbons C-d, C-f, C-I, C-g, C-m, C-m’, C-n, C-n’ and C-o appeared as a multiplet at 7.46-7.52 δppm. Chemical shift in the aromatic region with triplet at 7.56-7.59 δppm, 7.71-7.74 δppm and 7.98-8.01 δppm corresponds to C-h, C-b and C-c, respectively. The protons, which were present in methylene (C-j) (present in oxadiazole nucleus) and methyl group of C-e (attached to phenyl ring), both appeared as a singlet at 2.54 δppm. The other two methyl group C-l and C-k are appeared as a singlet at 1.63 δppm and 2.18 δ ppm, while the proton of C-a appeared as a doublet at 8.43-8.45 δppm (J=1.2 Hz).
Studies of 1,3,4-oxadiazole derivatives

Figure 2.3 Mass spectral analysis of 2-((4-acetyl-5-methyl-5-phenyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-o-tolyquinazolin-4(3H)-one (PD4H)
Studies of 1,3,4-oxadiazole derivatives

Possible fragmentation of compound PD_{1-H}
Thermal study of compound PD_{1-H}:

The thermal behaviour of PD_{1-H} is also characterized on the basis of TG and DTA methods. DTA and TG thermogram of PD_{1-H} is shown in Figure 2.4. It is evident from Figure 2.4 that PD_{1-H} is thermally stable up to about 295 °C and followed a single step degradation involving about 71.5% weight loss over the temperature range from 295–360 °C leaving 28.5% residue above 450 °C. The maximum weight loss is observed at about 330 °C. The characteristic temperatures for the assessment of the relative thermal stability of PD_{1-H} is initial decomposition temperature (T_0), temperature of 10% weight loss (T_{10}), temperature of maximum weight loss (T_{max}), temperature of final decomposition (T_f), % weight at the end of the reaction are 295 °C, 310 °C, 330 °C, 360 °C and 28.5%. The DTA technique provides much useful information about physicochemical changes occurring during the heating of the organic materials. DTA thermogram showed that reaction is endothermic.

Associated Kinetic parameters such as order of reaction (n), activation energy (E_a), frequency factor (A) and entropy change (ΔS^*) have been determined according to Freeman-Anderson method. Anderson-Freeman plots are shown in Figure 2.5. The determined least square values (R^2 = 0.987) of E_a, n, A and ΔS^* are 4918.6 kJmol^{-1}, 0.27, 7.22×10^{-4} s^{-1} and -310.91 JK^{-1} respectively. Degeneration process is a complex process and involving a variety of reactions such as cross linking, rearrangement, branching etc. Negative magnitude of ΔS^* confirmed that transition state is much in orderly state, while positive value of ΔS^* suggested that intermediate step is less stable than reactant.
Studies of 1,3,4-oxadiazole derivatives

Figure-2.4 TGA and DTA thermograms of 2-((4-acetyl-5-methyl-5-phenyl-4,5
dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-o-tolylquinazolin-4(3H)-one (PD_{1-H}) at the heating rate of 10 °C/min in an N\textsubscript{2} atmosphere
Studies of 1,3,4-oxadiazole derivatives

Figure-2.5: The Anderson-Freeman plot for thermal degradation of PD_{I-H}

\[ y = 5.916x + 0.277 \]
\[ R^2 = 0.987 \]

Table-2.4: Antimicrobial activity of 2-((4-acetyl-5-aryl-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl) methylthio)-3-o-tolylquinazolin-4(3H)-one

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Ar</th>
<th>Minimal Bactericidal Concentrations (MBC) in µg/mL</th>
<th>Minimal Fungicidal Concentrations (MFC) in µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. coli MTCC 443</td>
<td>P. aeruginosa MTCC 1688</td>
<td>S. aureus MTCC 96</td>
</tr>
<tr>
<td>PD_{I-A}</td>
<td>4-OCH_3-C_6H_4</td>
<td>200</td>
<td>250</td>
</tr>
<tr>
<td>PD_{I-B}</td>
<td>4-Cl-C_6H_4</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>PD_{I-C}</td>
<td>2-OH-C_6H_4</td>
<td>62.5</td>
<td>100</td>
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<tr>
<td>PD_{I-D}</td>
<td>4-Br-C_6H_4</td>
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<td>200</td>
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<tr>
<td>PD_{I-E}</td>
<td>4-NO_2-C_6H_4</td>
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<td>PD_{I-F}</td>
<td>3-NO_2-C_6H_4</td>
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<td>100</td>
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<tr>
<td>PD_{I-G}</td>
<td>4-CH_3-C_6H_4</td>
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<td>200</td>
</tr>
<tr>
<td>PD_{I-H}</td>
<td>-C_6H_5</td>
<td>200</td>
<td>62.5</td>
</tr>
</tbody>
</table>
Antimicrobial activity:

By visualizing the antibacterial data it could be observed that compound PD\(_{1-C}\) was good active, compounds PD\(_{1-B}\) and PD\(_{1-F}\) were moderately active and compound PD\(_{1-C}\) was highly active against *E. coli* compared with ampicillin as standard drug. The compound PD\(_{1-C}\) showed moderately active towards *E. coli* compared with chloramphenicol as standard drug.

The compound PD\(_{1-H}\) was moderately active against *P. aeruginosa* compared with chloramphenicol as standard drug. All the compounds were inactive towards *P. aeruginosa* with ampicillin standard antibacterial drugs.

The compounds PD\(_{1-B}\), PD\(_{1-F}\) and PD\(_{1-H}\) were good active, compounds PD\(_{1-C}\), PD\(_{1-D}\) and PD\(_{1-E}\) were highly active against *S. aureus* compared with ampicillin used as standard drug. All the compounds were inactive towards *S. aureus* compared with chloramphenicol as standard drug.

The compound PD\(_{1-D}\) was good active and compound PD\(_{1-E}\) was moderately active towards *S. pyogenus* compared with ampicillin as standard drug.

The compounds PD\(_{1-A}\), PD\(_{1-E}\) and PD\(_{1-F}\) were good active and compounds PD\(_{1-C}\) was highly active towards *C. albicans* compared with chloramphenicol as standard drug. All the compounds were inactive towards *C. albicans* compared with Nystatin as standard drug.

All the compounds were inactive towards *A. niger* and *A. Clavatus* in comparison of both standard antifungal drugs.

Looking to the structure activity relationship it can be concluded that remarkable inhibition was observed in compounds bearing Ar = 2-methoxy, 4-bromo, 4-nitro and 4-methyl substituents.
Studies of 1,3,4-oxadiazole derivatives

Section – II

Synthesis of
2-((4-acetyl-5-aryl-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-m-tolylquinazolin-4(3H)-one

Scheme - 2.2
Studies of 1,3,4-oxadiazole derivatives

Experimental procedure

2-Mercapto-3-m-tolylquinazolin-4(3H)-one (1)

A mixture of carbon disulphide (0.030 mole) and m-toluidine (0.012 mole) was added drop wise to a refluxing mixture of anthranilic acid (0.010 mole) and potassium hydroxide (0.012 mole) in methanol (15 mL). The mixture was refluxed on a water bath for about 10 h, and then poured over ice to get a solid product. Dissolved in 10% potassium hydroxide solution and filtered, con. HCl was added to the filtrate. The white precipitate obtained was filtered and washed with cold methanol (50%). The product is recrystallized from methanol, to get compound (1). Yield-72% m.p. 265 °C (Found: C, 67.18; H, 4.55; N, 10.41%. Calc. for C_{15}H_{12}N_{2}O_{2}S: C, 67.14; H, 4.51; N, 10.44%).

Ethyl 2-(4-oxo-3-m-tolyl-3,4-dihydroquinazolin-2-ylthio)acetate (2)

A mixture of compound (1) (0.1 mole) and ethyl chloro acetate (0.1 mole) in dry acetone (35 mL), in the presence of K_{2}CO_{3} (0.15 mole) was refluxed on a water bath for about 12 h. The reaction mixture was poured in ice to get a solid product, washed with methanol (50%). The product is crystallized from ethanol to get compound (2). Yield-70% m.p. 160 °C (Found: C, 64.36; H, 5.09; N, 7.93%. Calc. for C_{19}H_{18}N_{2}O_{3}: C, 64.39; H, 5.12; N, 7.90%).

2-(4-Oxo-3-m-tolyl-3,4-dihydroquinazolin-2-ylthio)acetohydrazide (3)

A mixture of compound (2) (0.1 mole) and hydrazine hydrate (0.1 mole) was placed in a RBF and ethanol (40 mL) was added as a solvent. The reaction mixture was refluxed for 8 h, followed by cooling at room temperature and product is separated, filtered and washed with water, crystallized from ethanol to get compound (3). Yield-69% m.p. 172 °C (Found: C, 59.94; H, 4.77; N, 16.44%. Calc. for C_{17}H_{16}N_{4}O_{2}S: C, 59.98; H, 4.74; N, 16.46%).

(E)-N’-(4-chlorobenzylidene)-2-(4-oxo-3-m-tolyl-3,4-dihydroquinazolin-2-ylthio)acetohydrazide (4B)

A mixture of compound (3) (0.01 mole) and p-chloro acetophenone (0.01 mole) in ethanol in was refluxed. During the reflux 2-3 drops of acetic acid
Studies of 1,3,4-oxadiazole derivatives

was added and refluxed continued for 6 h. After completion of reaction the product was filtered and washed it with water and methanol (50%) mixture and the product was crystallized from methanol and chloroform (1:1 v/v) solution to get compound (4B). Yield 76% m.p. 236 °C (Found: C, 62.30; H, 4.16; N, 12.07%. Calc. For C_{24}H_{19}ClN_{4}O_{2}S: C, 62.27; H, 4.14; N, 12.10%).

2-((4-Acetyl-5-(4-chlorophenyl)-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl) methylthio)-3-m-tolylquinazolin-4(3H)-one (5B) (PDII-B).

A mixture of Schiff-base (4B) 2.15 g (0.005 mole) and acetic anhydride (10 mL) were taken into a 100 mL round bottom flask. The mixture was refluxed for 4 h on oil bath. The progress of the reaction was monitored with the aid of TLC. The excess of acetic anhydride was distilled off and remaining mixture was poured in the ice cold water to get main product. Product was separated by filtration and crystallized from chloroform:n-hexane to afford the title compound PDII-B.

Other compounds of the series (PDII-A, PDII-C to PDII-G) were prepared by using a similar method and their analytical data are shown in Table 2.5.
Studies of 1,3,4-oxadiazole derivatives

Analytical data of 2-((4-acetyl-5-aryl-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-m-tolyquinazolin-4(3H)-one (PDII-A-PDII-H)

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Ar</th>
<th>Molecular Formula</th>
<th>% Yield</th>
<th>M.P. °C</th>
<th>% C</th>
<th>% H</th>
<th>% N</th>
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<tr>
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<td>4-CH3-C6H4</td>
<td>C28H26N4O5S</td>
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<td>198</td>
<td>65.35</td>
<td>65.33</td>
<td>5.09</td>
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<tr>
<td>PDII-B</td>
<td>4-Cl-C6H4</td>
<td>C27H23ClN4O5S</td>
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<td>258</td>
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<td>62.49</td>
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<td>C27H23N4O5S</td>
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<td>260</td>
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<td>61.25</td>
<td>4.38</td>
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<td>PDII-G</td>
<td>4-CH3-C6H4</td>
<td>C28H26N4O5S</td>
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<td>266</td>
<td>67.45</td>
<td>67.44</td>
<td>5.26</td>
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</table>

Table - 2.5
Studies of 1,3,4-oxadiazole derivatives

Figure 2.6 IR spectra of 2-((4-acetyl-5-(4-chlorophenyl)-5-methyl-4,5-dihydro 1,3,4-oxadiazol-2-yl)methylthio)-3-m-tolylquinazolin-4(3H)-one (PDII-B)
Studies of 1,3,4-oxadiazole derivatives

Table-2.6 IR spectral data of 2-((4-acetyl-5-(4-chlorophenyl)-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-m-tolylquinazolin-4(3H)-one (PD_{II-B})

<table>
<thead>
<tr>
<th>Vibration mode</th>
<th>Frequency in cm(^{-1})</th>
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<tr>
<td></td>
<td>Observed</td>
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<tr>
<td>C-H str. (asym.)</td>
<td>2922</td>
</tr>
<tr>
<td>C-H str. (sym.)</td>
<td>2880</td>
</tr>
<tr>
<td>C=O str. of amide</td>
<td>1683</td>
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<tr>
<td>C=O str. of –CH(_3)</td>
<td>1721</td>
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<tr>
<td>-CH(_3) bending vib.</td>
<td>1391</td>
</tr>
<tr>
<td>C-H str. (aromatic).</td>
<td>3075</td>
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<tr>
<td>C=C str. Ring skeletal</td>
<td>1576</td>
</tr>
<tr>
<td>-CH(_2) bending</td>
<td>1476</td>
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<tr>
<td>C-H inplane bending</td>
<td>1088</td>
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<tr>
<td>C-N str.</td>
<td>1277</td>
</tr>
<tr>
<td>C-O-C str.</td>
<td>1029</td>
</tr>
<tr>
<td>C-H o.o.p. bending (1,3-disub.)</td>
<td>680, 759, 882</td>
</tr>
<tr>
<td>C-H o.o.p. bending (p-sub.)</td>
<td>821</td>
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<tr>
<td>C-Cl str.</td>
<td>680</td>
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</table>

The IR data of PD_{II-B} clearly shows a C=O stretching of amide band at 1683 cm\(^{-1}\), C-O absorption band at 1029 cm\(^{-1}\) and C-N stretching band at 1277 cm\(^{-1}\), which indicates ring closure of the 1,3,4-oxadiazole. The presence of stretching band at 680, 759 and 882 cm\(^{-1}\) indicate 1,3-di substituted and 821 cm\(^{-1}\) indicate 1,4-di substituted benzene ring is present in synthesized compound.
Studies of 1,3,4-oxadiazole derivatives

Figure-2.7 $^1$H NMR spectra of 2-((4-acetyl-5-(4-chlorophenyl)-5-methyl-4,5-
dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-$m$-tolylquinazolin-4(3H)-
one (PD$_{II-B}$)
Studies of 1,3,4-oxadiazole derivatives

Table 2.7 $^1$H NMR spectral data of 2-((4-acetyl-5-(4-chlorophenyl)-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-$m$-tolylquinazolin-4(3$H$)-one (PD$\text{II-B}$)

<table>
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<tr>
<th>Signal No.</th>
<th>Signal position ($\delta$ ppm)</th>
<th>Relative No. of Protons</th>
<th>Multiplicity</th>
<th>Inference</th>
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<tr>
<td>1</td>
<td>1.66</td>
<td>3</td>
<td>s</td>
<td>-CH$_3$(l)</td>
</tr>
<tr>
<td>2</td>
<td>2.42</td>
<td>3</td>
<td>s</td>
<td>-CH$_3$(f)</td>
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<tr>
<td>3</td>
<td>2.53</td>
<td>5</td>
<td>s</td>
<td>-CH$_2$+CH$_3$(j, k)</td>
</tr>
<tr>
<td>4</td>
<td>7.06</td>
<td>1</td>
<td>s</td>
<td>Ar-H(e)</td>
</tr>
<tr>
<td>5</td>
<td>7.42-7.57</td>
<td>7</td>
<td>m</td>
<td>Ar-H(d, g, I, m, m’, n, n’)</td>
</tr>
<tr>
<td>6</td>
<td>7.69-7.72</td>
<td>1</td>
<td>t</td>
<td>Ar-H(h)</td>
</tr>
<tr>
<td>7</td>
<td>7.95-7.99</td>
<td>2</td>
<td>t</td>
<td>Ar-H(b, c)</td>
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<tr>
<td>8</td>
<td>8.43-8.44</td>
<td>1</td>
<td>d</td>
<td>Ar-H(a)</td>
</tr>
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</table>

It can be seen from the chemical structure of compound PD$\text{II-B}$ that proton of the phenyl ring attached to the carbons C-d, C-g, C-I, C-m, C-m’, C-n and C-n’ appeared as a multiplet at 7.42-7.57 δppm. Chemical shift in the aromatic region with triplet at 7.69-7.72 δppm and 7.95-7.99 δppm corresponds to C-h and C-b, C-c as a triplet respectively. Chemical shift at 7.06 δppm correspond to C-e as a singlet. The protons, which were present in methylene (C-j) (present in oxadiazole nucleus) and methyl group of C-k (attached to phenyl ring), both appeared as a singlet at 2.53 δppm. The other two methyl group C-l and C-f are appeared as a singlet at 1.66 δppm and 2.42 δppm, while the proton of C-a appeared as a doublet at 8.43 δppm.
Studies of 1,3,4-oxadiazole derivatives

Figure 2.8 Mass spectral analysis of 2-((4-acetyl-5-(4-chlorophenyl)-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-\textit{m}-tolylquinazolin-4(3\textit{H})-one (PDII-B)
Studies of 1,3,4-oxadiazole derivatives

Possible fragmentation of compound PDII-B:
Thermal study of compound PDII-B:

The thermal behaviour of PDII-B is also characterized on the basis of TG and DTA methods. DTA and TG thermogram of PDII-B is shown in Figure 2.9. It is evident from figure 2.9 that PDII-B is thermally stable up to about 270 °C and followed a single step degradation involving about 61.80% weight loss over the temperature range from 270–385 °C leaving 38.2% residue above 400 °C. The maximum weight loss is observed at about 315 °C. The characteristic temperatures for the assessment of the relative thermal stability of PDII-B is initial decomposition temperature (To), temperature of 10% weight loss (T10), temperature of maximum weight loss (Tmax), temperature of final decomposition (Tf), % weight at the end of the reaction are 270 °C, 295 °C, 315 °C, 385 °C and 38.2%. The DTA technique provides much useful information about physicochemical changes occurring during the heating of the organic materials. DTA thermogram showed that reaction is endothermic.

Associated Kinetic parameters such as order of reaction (n), activation energy (Ea), frequency factor (A) and entropy change (ΔS*) have been determined according to Freeman-Anderson method. Anderson-Freeman plots are shown in Figure 2.10. The determined least square values (R² = 0.985) of Ea, n, A and ΔS* are 6061.74 kJmol⁻¹, 0.29, 0.12×10⁻⁴ s⁻¹ and -306.39 JK⁻¹, respectively. Degeneration process is a complex process and involving a variety of reactions such as cross linking, rearrangement, branching etc. Negative magnitude of ΔS* confirmed that transition state is much in orderly state while positive value of ΔS* suggested that intermediate step is less stable than reactant.
Figure-2.9 TGA and DTA thermograms of 2-((4-acetyl-5-(4-chlorophenyl)-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-\textit{m}\textquoteright-tolylquinazolin-4(3\textit{H})-one (PDII-B) at the heating rate of 10 °C/min in an N\textsubscript{2} atmosphere.
Studies of 1,3,4-oxadiazole derivatives

Figure 2.10  The Anderson-Freeman plot for thermal degradation of PD_{II-B}

\[ y = 7.291x + 0.293 \]
\[ R^2 = 0.985 \]

Table 2.8  Antimicrobial activity of 2-((4-acetyl-5-aryl-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-\textit{m}-tolylquinazolin-4(3\textit{H})-one

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Ar</th>
<th>Minimal Bactericidal Concentrations (MBC) in (\mu g/\text{mL})</th>
<th>Minimal Fungicidal Concentrations (MFC) in (\mu g/\text{mL})</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E. coli MTCC 443</td>
<td>P. aeruginosa MTCC 1688</td>
</tr>
<tr>
<td>PD_{II-A}</td>
<td>4-OCH$_3$-C$_6$H$_4$</td>
<td>250</td>
<td>125</td>
</tr>
<tr>
<td>PD_{II-B}</td>
<td>4-Cl-C$_4$H$_4$</td>
<td>500</td>
<td>250</td>
</tr>
<tr>
<td>PD_{II-C}</td>
<td>2-OH-C$_6$H$_4$</td>
<td>200</td>
<td>125</td>
</tr>
<tr>
<td>PD_{II-D}</td>
<td>4-Br-C$_5$H$_4$</td>
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</tr>
<tr>
<td>PD_{II-E}</td>
<td>4-NO$_2$-C$_6$H$_4$</td>
<td>500</td>
<td>250</td>
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<tr>
<td>PD_{II-F}</td>
<td>3-NO$_2$-C$_6$H$_4$</td>
<td>62.5</td>
<td>125</td>
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<tr>
<td>PD_{II-G}</td>
<td>4-CH$_3$-C$_6$H$_4$</td>
<td>100</td>
<td>250</td>
</tr>
<tr>
<td>PD_{II-H}</td>
<td>-C$_6$H$_5$</td>
<td>125</td>
<td>200</td>
</tr>
</tbody>
</table>
Studies of 1,3,4-oxadiazole derivatives

**Antimicrobial activity:**

By visualizing the antibacterial data it could be observed that compound PD$_{II-G}$ was good active, compound PD$_{II-H}$ was moderately active and compound PD$_{II-F}$ was highly active towards *E. coli*. used ampicillin as standard drug. The compound PD$_{II-F}$ showed moderately active towards *E. coli* used chloramphenicol as standard drug.

All the compounds were inactive towards *P. aeruginosa* compared with ampicillin and chloramphenicol standard antibacterial drugs.

The compounds PD$_{II-C}$ and PD$_{II-G}$ were good active, compounds PD$_{II-A}$, PD$_{II-B}$, PD$_{II-D}$, PD$_{II-E}$ and PD$_{II-H}$ were highly active towards *S. aureus* with ampicillin used as standard drug. The compound PD$_{II-B}$ was moderately active towards *S. aureus* used chloramphenicol as standard drug.

The compound PD$_{II-B}$ was good active and compound PD$_{II-A}$ was highly active towards *S. pyogenus* used ampicillin as standard drug. Only one compound PD$_{II-A}$ was moderately active towards *S. pyogenus* used chloramphenicol as standard drug.

By visualizing the antifungal data it could be observed that compounds PD$_{II-B}$, PD$_{II-C}$, PD$_{II-E}$ and PD$_{II-H}$ were good active and compound PD$_{II-F}$ was highly active towards *C. albicans* used chloramphenicol as standard drug. All the compounds were inactive towards *C. albicans* used Nystatin as standard drug.

All the compounds were inactive towards *A. niger* and *A. Clavatus* in comparison of both standard antifungal drugs.

Looking to the structure activity relationship it can be concluded that remarkable inhibition was observed in compounds bearing Ar = 4-methoxy, 4-chloro, 4-bromo, 4-nitro, 3-nitro and phenyl substituents.
Studies of 1,3,4-oxadiazole derivatives

Section-III

Synthesis of
2-((4-acetyl-5-aryl-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-p-tolylquinazolin-4(3H)-one

Scheme - 2.3
Studies of 1,3,4-oxadiazole derivatives

**Experimental procedure**

### 2-Mercapto-3-p-tolylquinazolin-4(3H)-one (1)

A mixture of carbon disulphide (0.030 mole) and p-toluidine (0.012 mole) was added drop wise to a refluxing mixture of anthranilic acid (0.010 mole) and potassium hydroxide (0.012 mole) in methanol (15 mL). The mixture was refluxed on a water bath for about 10 h, and then poured over ice to get a solid product. Dissolved in 10% potassium hydroxide solution and filtered, con. HCl was added to the filtrate. The white precipitate obtained was filtered, washed with cold methanol (50%). The product is crystallized from methanol, to get compound (1). Yield-77% m.p. 271 °C (Found: C 67.12; H, 4.53; N 10.48%. Calc. for C_{15}H_{12}N_{2}O_{5}S : C, 67.14; H, 4.51; N, 10.44%)

### Ethyl 2-(4-oxo-3-p-tolyl-3,4-dihydroquinazolin-2-ylthio)acetate (2)

A mixture of compound (1) (0.1 mole) and ethyl chloro acetate (0.1 mole) in dry acetone (35 mL), in the presence of K_{2}CO_{3} (0.15 mole) was refluxed on a water bath for about 12 h. The reaction mixture was poured in ice to get a solid product, washed with methanol (50%). The product is crystallized from ethanol to get compound (2). Yield-80% m.p. 148 °C (Found: C, 64.38; H, 5.08; N, 7.93%. Calc. for C_{19}H_{18}N_{2}O_{3}S: C, 64.39; H, 5.12; N, 7.90%).

### 2-(4-Oxo-3-p-tolyl-3,4-dihydroquinazolin-2-ylthio)acetohydrazide (3)

A mixture of compound (2) (0.1 mole) and hydrazine hydrate (0.1 mole) was placed in a RBF and ethanol (40 mL) was added as a solvent. The reaction mixture was refluxed for 8 h, followed by cooling at room temperature and product is separated, filtered and washed with water, crystallized from ethanol to get compound (3). Yield-70% m.p.:160 °C (Found: C, 59.97; H, 4.76; N, 16.48% Calc. for C_{17}H_{16}N_{4}O_{2}S: C, 59.98; H, 4.74; N, 16.46%)

### (E)-N’-(4-methoxybenzylidene)-2-(4-oxo-3-p-tolyl-3,4-dihydroquinazolin-2-ylthio)acetohydrazide (4A)

A mixture of compound (3) (0.01 mole) and 4-methoxy acetophenone (0.01 mole) in ethanol in was refluxed. During the reflux 2-3 drops of acetic acid
Studies of 1,3,4-oxadiazole derivatives

was added and refluxed continued for 6 h. After completion of reaction the product was filtered and washed it with water and methanol (50%) mixture and the product was crystallized from methanol and chloroform (1:1 v/v) solution to get compound (4A). Yield 75% m.p. 223 °C (Found: C, 67.27; H, 4.70; N, 13.07%. Calc. For C₂₄H₂₀N₄O₂S: C, 64.85; H, 4.54; N, 12.60%).

2-((4-Acetyl-5-(4-methoxyphenyl)-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-p-tolylquinazolin-4(3H)-one (5A) (PD_{III-A})

A mixture of Schiff-base (4A) 2.15 g (0.005 mole) and acetic anhydride (10 mL) were taken into a 100 mL round bottom flask. The mixture was refluxed for 4 h on oil bath. The progress of the reaction was monitored with the aid of TLC. The excess of acetic anhydride was distilled off and remaining mixture was poured in the ice cold water to get main product. Product was separated by filtration and crystallized from chloroform - n-hexane to afford the title compound PD_{III-A}.

Other compounds of the series (PD_{III-B} to PD_{III-H}) were prepared by using a similar method and their analytical data are shown in Table - 2.9.
Studies of 1,3,4-oxadiazole derivatives

Analytical data of
2-((4-acetyl-5-(4-methoxyphenyl)-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-p-tolylquinazolin-4(3H)-one (PD_{III-A} - PD_{III-H})

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Ar</th>
<th>Molecular Formula</th>
<th>% Yield</th>
<th>M.P. °C</th>
<th>% C</th>
<th>% H</th>
<th>% N</th>
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<tr>
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<td>C_{28}H_{26}N_{4}O_{5}S</td>
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<td>283</td>
<td>65.35</td>
<td>65.33</td>
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<tr>
<td>PD_{III-B}</td>
<td>4-Cl-C_{6}H_{4}</td>
<td>C_{27}H_{23}N_{4}O_{5}S</td>
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<td>285</td>
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<td>2-OH-C_{6}H_{4}</td>
<td>C_{27}H_{24}N_{4}O_{5}S</td>
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<td>C_{27}H_{23}BrN_{4}O_{3}S</td>
<td>58</td>
<td>280</td>
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<td>4.11</td>
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</tr>
<tr>
<td>PD_{III-G}</td>
<td>4-CH_{3}-C_{6}H_{4}</td>
<td>C_{28}H_{28}N_{4}O_{5}S</td>
<td>68</td>
<td>289</td>
<td>67.45</td>
<td>67.43</td>
<td>5.26</td>
</tr>
<tr>
<td>PD_{III-H}</td>
<td>-C_{6}H_{5}</td>
<td>C_{27}H_{24}N_{4}O_{5}S</td>
<td>70</td>
<td>278</td>
<td>66.92</td>
<td>66.90</td>
<td>4.99</td>
</tr>
</tbody>
</table>

Table - 2.9
Studies of 1,3,4-oxadiazole derivatives

Figure 2.11 IR spectra of 2-((4-acetyl-5-(4-methoxyphenyl)-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-p-tolylquinazolin-4(3H)-one (PDIII-A)
Studies of 1,3,4-oxadiazole derivatives

Table-2.10 IR spectral data of 2-((4-acetyl-5-(4-methoxyphenyl)-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-p-tolylquinazolin-4(3H)-one(PDIII-A)

<table>
<thead>
<tr>
<th>Vibration mode</th>
<th>Frequency in cm$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed</td>
</tr>
<tr>
<td>C-H str. (asym.)</td>
<td>2923</td>
</tr>
<tr>
<td>C-H str. (sym.)</td>
<td>2854</td>
</tr>
<tr>
<td>C=O str. of amide</td>
<td>1680</td>
</tr>
<tr>
<td>C=O str. of -CH$_3$</td>
<td>1713</td>
</tr>
<tr>
<td>-CH$_3$ bending vib.</td>
<td>1396</td>
</tr>
<tr>
<td>C-H str. (aromatic).</td>
<td>3020</td>
</tr>
<tr>
<td>C=C str. Ring skelatal</td>
<td>1596</td>
</tr>
<tr>
<td>-CH$_2$ bending</td>
<td>1476</td>
</tr>
<tr>
<td>C-H inplane bending</td>
<td>1085</td>
</tr>
<tr>
<td>C-N str.</td>
<td>1276</td>
</tr>
<tr>
<td>C-O-C str.</td>
<td>1028</td>
</tr>
<tr>
<td>C-H o.o.p. bending (1,4-disub.)</td>
<td>811</td>
</tr>
</tbody>
</table>

The IR data of PD$_{III-A}$ clearly shows a C=O stretching of amide band at 1680 cm$^{-1}$, C-O absorption band at 1028 cm$^{-1}$ and C-N stretching band at 1276 cm$^{-1}$, which indicates ring closure of the 1,3,4-oxadiazole. The presence of stretching band at 811 cm$^{-1}$ indicate 1,4-disubstituted benzene ring is present in synthesized compound.
Studies of 1,3,4-oxadiazole derivatives

Figure 2.12  $^1$H NMR spectra of 2-((4-acetyl-5-(4-methoxyphenyl)-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-p-tolylquinazolin-4(3H)-one (PD$_{III}$-A)
Studies of 1,3,4-oxadiazole derivatives

Table-2.11 $^1$H NMR spectral data of 2-((4-acetyl-5-(4-methoxyphenyl)-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-p-tolylquinazolin-4(3H)-one (PD$_{\text{III-A}}$)

<table>
<thead>
<tr>
<th>Signal No.</th>
<th>Signal position (δppm)</th>
<th>Relative No. of protons</th>
<th>Multiplicity</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.65</td>
<td>6</td>
<td>s</td>
<td>$-\text{CH}_3$(g, j)</td>
</tr>
<tr>
<td>2</td>
<td>2.21</td>
<td>5</td>
<td>s</td>
<td>$-\text{CH}_2\text{-CH}_3$(h, i)</td>
</tr>
<tr>
<td>3</td>
<td>2.61</td>
<td>3</td>
<td>s</td>
<td>$-\text{CH}_3$(m)</td>
</tr>
<tr>
<td>4</td>
<td>7.45-7.61</td>
<td>5</td>
<td>m</td>
<td>Ar-H(e, e’, f, f’, d)</td>
</tr>
<tr>
<td>5</td>
<td>7.66-7.76</td>
<td>4</td>
<td>q</td>
<td>Ar-H(k, k’, l, l’)</td>
</tr>
<tr>
<td>6</td>
<td>7.95-8.06</td>
<td>2</td>
<td>t</td>
<td>Ar-H(b, c)</td>
</tr>
<tr>
<td>7</td>
<td>8.41-8.46</td>
<td>1</td>
<td>d</td>
<td>Ar-H(a)</td>
</tr>
</tbody>
</table>

It can be seen from the chemical structure of compound PD$_{\text{III-A}}$ that protons of the phenyl ring attached to the carbons C-e, C-e’, C-f, C-f’ and C-d appeared as a multiplet at 7.45-7.61 δppm. Chemical shift in the aromatic region with quartet and triplet at 7.66-7.76 δppm and 7.95-8.06 δppm corresponds to C-k, C-k’, C-l, C-l’ and C-b, C-c respectively. Chemical shift at 8.41-8.46 δppm correspond to C-a as a doublet. The protons, which were present in methylene (C-h) (present in oxadiazole nucleus) and methyl group of C-i (attached to phenyl ring), both appeared as a singlet at 2.21 δppm. The other two methyl group C-g and C-j are appeared as a singlet at 1.65 δppm, while the proton of methoxy group C-m appeared as a singlet at 2.61 δppm.
Studies of 1,3,4-oxadiazole derivatives

Figure 2.13  Mass spectral analysis of 2-((4-acetyl-5-(4-methoxyphenyl)-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-p-tolylquinazolin-4(3H)-one (PDIII-A)
Studies of 1,3,4-oxadiazole derivatives

Possible fragmentation of compound PD_{III-A}:
Studies of 1,3,4-oxadiazole derivatives

Thermal study of PD_{III-A}:

The thermal behaviour of PD_{III-A} is also characterized on the basis of TG and DTA methods. DTA and TG thermogram of PD_{III-A} is shown in Figure 2.14. It is evident from Figure 2.14 that PD_{III-A} is thermally stable up to about 155 °C and followed a two step degradation involving about 1.80% weight loss over the temperature range from 155 - 210 °C leaving 98.2% residue above 230 °C in first step while in second step it is thermally stable up to about 315 °C and degradation involving about 64.57% weight loss over the temperature range from 315 - 405 °C leaving 33.63% residue above 450 °C. The maximum weight loss is observed at about 350 °C. The characteristic temperatures for the assessment of the relative thermal stability of PD_{III-A} is initial decomposition temperature (T_o), temperature of 10% weight loss (T_{10}), temperature of maximum weight loss (T_{max}), temperature of final decomposition (T_f), % weight at the end of the reaction are 155 °C, 165 °C, 170 °C, 210 °C and 98.2% in the first step while in second step the values are 315 °C, 335 °C, 350 °C, 405 °C and 33.63% . The DTA technique provides much useful information about physicochemical changes occurring during the heating of the organic materials. DTA thermogram showed that reaction is endothermic.

Associated Kinetic parameters such as order of reaction (n), activation energy (E_a), frequency factor (A) and entropy change (ΔS^*) have been determined according to Anderson-Freeman method. Anderson-Freeman plots are shown in Figure 2.15 and 2.16. The determined least square values (R^2 = 0.965) of E_a, n, A and ΔS^* in the first step are 567596.8 kJmol^{-1}, 0.59, 4.63×10^{65} s^{-1} and 1008.93 JK^{-1} respectively while in second step least square values (R^2 = 0.982) of E_a, n, A and ΔS^* in the first step are 5651.86 kJmol^{-1}, 0.44, 8.68×10^{-4} s^{-1} and -309.65 JK^{-1}. Degeneration process is a complex process and involving a variety of reactions such as cross linking, rearrangement, branching, etc. Negative magnitude of ΔS^* confirmed that transition state is much in orderly state while positive value of ΔS^* suggested that intermediate step is less stable than reactant.
Figure-2.14  TGA and DTA thermograms of 2-((4-acetyl-5-(4-methoxyphenyl)-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-p-tolylquinazolin-4(3H)-one (PD_{III-A}) at the heating rate of 10 °C/min in an N\textsubscript{2} atmosphere
Studies of 1,3,4-oxadiazole derivatives

Figure 2.15  The Anderson-Freeman plot for thermal degradation of PDIII-A
(First step)

\[ y = 682.7x + 0.587 \]
\[ R^2 = 0.965 \]

Figure 2.16  The Anderson-Freeman plot for thermal degradation of PDIII-A
(Second step)

\[ y = 6.798x + 0.435 \]
\[ R^2 = 0.982 \]
Studies of 1,3,4-oxadiazole derivatives

Table-2.12 Antimicrobial activity of 2-((4-acetyl-5-aryl-5-methyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methylthio)-3-p-tolylquinazolin-4(3H)-one

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Ar</th>
<th>Minimal Bactericidal Concentrations (MBC) in µg/mL</th>
<th>Minimal Fungicidal Concentrations (MFC) in µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E. coli MTCC 443</td>
<td>P. aeruginosa MTCC 1688</td>
</tr>
<tr>
<td>PDIIIA</td>
<td>4-OCH₃-C₆H₄</td>
<td>100</td>
<td>62.5</td>
</tr>
<tr>
<td>PDIIIB</td>
<td>4-Cl-C₆H₄</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>PDIIIC</td>
<td>2-OH-C₆H₄</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>PDIIID</td>
<td>4-Br-C₆H₄</td>
<td>62.5</td>
<td>125</td>
</tr>
<tr>
<td>PDIIIE</td>
<td>4-NO₂-C₆H₄</td>
<td>125</td>
<td>200</td>
</tr>
<tr>
<td>PDIIIF</td>
<td>3-NO₂-C₆H₄</td>
<td>200</td>
<td>250</td>
</tr>
<tr>
<td>PDIIIG</td>
<td>4-CH₃-C₆H₄</td>
<td>250</td>
<td>125</td>
</tr>
<tr>
<td>PDIIIH</td>
<td>-C₆H₅</td>
<td>250</td>
<td>250</td>
</tr>
</tbody>
</table>

Antimicrobial activity:

By visualizing the antibacterial data it could be observed that compound PDIIIA was good active, compound PDIIIE was moderately active and compound PDIIID was highly active towards E. coli. used ampicillin as a standard drug. The compound PDIIID showed moderately active towards E. coli used chloramphenicol as a standard drug.

The compounds PDIIIA was moderately active against P. aeruginosa with chloramphenicol as standard drug. All the compounds were inactive towards P. aeruginosa with ampicillin standard antibacterial drugs.

The compound PDIIIC was good active, compounds PDIIIA, PDIIIB, PDIIID, PDIIIE, PDIIIF and PDIIIG were highly active towards S. aureus on compared with ampicillin used as a standard drug. The compound PDIIID was moderately active towards S. aureus used chloramphenicol as a standard drug.
The compounds PD_{III-B} and PD_{III-G} were good active, compound PD_{III-F} was moderately active towards *S. pyogenus* used ampicillin as a standard drug.

By visualizing the antifungal data it could be observed that compounds PD_{III-A} and PD_{III-D} were good active towards *C. albicans* used chloramphenicol as a standard drug. All the compounds were inactive towards *C. albicans* used Nystatin as a standard drug.

All the compounds were inactive towards *A. niger* and *A. Clavatus* in comparison of both standard antifungal drugs.

Looking to the structure activity relationship it can be concluded that remarkable inhibition was observed in compounds bearing Ar = 4-methoxy, 4-chloro, 4-bromo, 4-nitro, 3-nitro and 4-methyl substituents.
Studies of 1,3,4-oxadiazole derivatives

References:


Studies of 1,3,4-oxadiazole derivatives


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36. M. T. Khan, M. I. Choudhary, K. M. Khan and M. Rani, “Microbial transformation of (-)-isolongifolol and butyrylcholinesterase inhibitory
Studies of 1,3,4-oxadiazole derivatives


Studies of 1,3,4-oxadiazole derivatives


Studios of 1,3,4-oxadiazole derivatives


59. E. Haisenberg, Cellulose Chemie, 12, 159, (1931).


Studies of 1,3,4-oxadiazole derivatives


