T.C.
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DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

STUDIES ON NOVEL 3,5-DISUBSTITUTED-1,3,4-OXADIAZOLE-2-THIONE DERIVATIVES

MASTER OF SCIENCE THESIS

MARWA MOUSA BADER, B.Pharm.

İSTANBUL – 2017

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ADVISOR

Prof. Dr. Prof. Dr. Meriç Köksal Akkoç

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APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated 27.01.2017 and numbered 2017/62-12

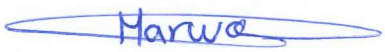
Prof. Dr. Bayram YILMAZ
Director of Institute of Health Sciences

DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

13.01.2017

Marwa Bader



To my lovely family

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TABLE OF CONTENTS

TABLES

FIGURES and SCHEMES

Figures

Schemes

ABBREVIATIONS

VEGF	Vascular endothelia growth factor
IC_{50}	Half maximum inhibitory concentration
SAR	Structure activity relationship
DCC	*N*,*N'*-Dicyclohexylcarbodiimide
$POCl_3$	Phosphoryl chloride
THF	Tetrahydrofurane
HMPA	Hexamethylphosphoramide
TCC	Trichloroisocyanuric acid
MW	Microwave
Nafion	Sulfonated tetrafluroethylene based fluoropolymer-copolymer
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
HATU	1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinum-3-oxidehexafluorophosphate
CDI	1,1'-Carbonyldiimidazole
CAN	Cerium ammonium nitrate
UV	Ultraviolet
IR	Infrared
^{1}H-NMR	Proton nuclear magnetic resonance
^{13}C-NMR	Carbon thirteen nuclear magnetic resonance
DNA	Deoxyribonucleic acid
Dump	Deoxyuridine monophosphate
Dtmp	Deoxythymidine monophosphate
MetAP	Methionine aminopeptidase
EGF	Epidermal growth factor
PTK	Protein tyrosine kinase
PI3K	Phosphoinositide 3-kinase
SMC	Structural maintenance of chromosomes
GSK-3	Glycogen synthase kinase 3
Ser/Thr	Serine/Threonine
MIC	Minimum inhibitory concentration

MDR	Multidrug resistant
COX	Cyclooxygenase
NSAID	Nonsteroidal anti-inflammtory drugs
CPE	Carrageenan-induced rat paw edema
HIV	Human immunodeficiency virus
DGAT-1	Diacylglycerol acyl transferase-1
TLC	Thin layer chromatography
$CDCl_3$	Chloroform
Ppm	Parts per million
HEPG2	Human liver cancer cell line
HELA	Cervical cell line cancer
SW 1116	Colon cancer cell line
BGC823	Gastric cancer cell line
UACC-62	Skin cancer cell line
NCI-460	Lung cancer cell line
RTCA	Real time cell analyzer

ABSTRACT

Bader M. (2017). Studies on Novel 3,5-Disubstituted-1,3,4-Oxadiazole-2-thione Derivatives. Yeditepe University Institute of Health Science, Department of Pharmaceutical Chemistry, Msc thesis, Istanbul.

In this study, eight compounds of 5-(2-hydroxyphenyl)-3-(4-substituted-piperidino/ piperazino)methyl-1,3,4-oxadiazol-2-(3*H*)thione derivative were synthesized. In the first step; salicylic acid was esterified with methanol in acidic medium. Then methyl salicylate was reacted with hydrazine hydrate to produce salicyl hydrazide. In the last step; carbon disulfide in basic media were reacted with salicyl hydrazide followed by acidifying with hydrochloric acid to obtain cyclization of 1,3,4-oxadiazol-2(3*H*)-thione ring. The obtained 5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2(3*H*)-thione was reacted with the appropriate piperidine/piperazine derivatives in the presence of formaldehyde to yield 5-(2-hydroxyphenyl)-3-(4-substitutedpiperidino/piperazino) methyl-1,3,4-oxadiazole-2(3*H*)-thione compounds.

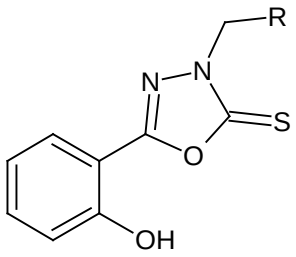
The physical properties and R_f values on thin layer chromatography of the synthesized compounds were determined. Structure elucidation of the compounds was done by IR, ^{1}H-NMR, ^{13}C-NMR spectra and elemental analysis. Anticancer activity screening was determined by using Xcelligence system.

According to endpoint cell index values, compound **5d** has the maximal cytotoxic activity, in addition compound **5b** and **5h** also have cytotoxic activity against MCF-7 cancer cell lines in high doses. Compound **5a**, **5c** and **5e** have antiproliferative effect, although **5e** may be cytotoxic in higher doses, compound **5g** may be antiproliverative in higher doses, compound **5f** has neither antiproliferative nor cytotoxic effect.

Among all novel synthesized 1,3,4-oxadiazole, compound **5d**, and **5b** possess best anticancer activity.

Key words: 1,3,4-Oxadiazole, Piperidine, Piperazine, Anticancer, *Mannich* base

Table 1. Formula of the synthesized compounds.



Compound	R
5a	3-Methylpiperidine
5b	4-Hydroxymethylpiperidine
5c	4-Phenylpiperidine
5d	4-Hydroxy-4-phenylpiperidine. HCl
5e	4-Acetyl-4-phenylpiperidine. HCl
5f	4-Cyano-4-phenylpiperidine. HCl
5g	4-Phenylpiperazine
5h	4-(2-Pyridyl)piperazine

ÖZET

Bader M. (2017). Yeni 3,5-Disübstitüe-1,3,4-Oksadiyazol-2-tiyon Türevleri Üzerine Çalışmalar. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Farmasötik Kimya Programı Bilim Uzmanlığı Tezi, İstanbul.

Bu çalışmada, 5-(2-hidroksifenil)-3-(4-sübstitutepiperidino)metil-1,3,4-oksadiyazol-2-(3*H*) tiyon türevi yedisi orjinal sekiz bileşik sentezlenmiştir. Başlangıç maddesi olarak kullanılan 5-(2-hidroksifenil)-3-(4-sübstitutepiperidino)metil-1,3,4-oksadiazol-2-(3*H*)tiyon için salisilik asit sülfürik asit varlığında metanolle esterleştirilmiş, takiben etanol içerisinde hidrazin hidratla hidrazonuna geçilmiştir. Potasyum hidroksit ve karbon disülfür ile reaksiyonun ardından hidroklorik asitle asitlendirme sonucu halka siklizasyonu sağlanmıştır. 5-(2-Hidroksifenil)-1,3,4-oksadiyazol-2-tiyonun uygun piperidin/piperazin türevleri ile *Mannich* reaksiyonu sonucu 5-(2-hidroksifenil)-3-(4-sübstitutepiperidino/piperazino)metil-1,3,4-oksadiazole-2-(3*H*)tiyon bileşik-leri elde edilmiştir.

Bileşiklerin fiziksel özellikleri ve ince tabaka kromatografisinde Rf değerleri belirlenmiştir. Yapıları IR, ^{1}H-NMR, ^{13}C-NMR spektroskopileri ve elementel analizlerle aydınlatılmıştır. Bileşiklerin antikanser aktiviteleri Xcelligence yöntemi ile belirlenmiştir.

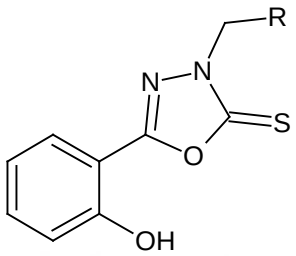
Aktivite sonuçlarına göre, MCF-7 kanser hücre hattında **5a** ve **5h** bileşikleri yüksek dozlarda sitotoksik etkiye sahipken maksimum sitotoksik etkinlik bileşik **5d** için görülmüştür.

Son nokta hücre indeksi değerine göre, **5a**, **5c** ve **5e** bileşiklerinin antiproliferatif etkili, uygulanan dozlardan daha yüksek dozlarda **5e** bileşiğinin sitotoksik bileşik **5g** nin de antiproliferatif olabileceği, bileşik **5f** nin ise ne antiproliferatif ne de sitotoksik etkiye sahip olmadığı belirlenmiştir.

Sonuç olarak, tüm sentezlenen 1,3,4-okzadiyazol türevi bileşikler içinde bileşik **5b** ve **5d** en iyi etki profilini göstermiştir.

Anahtar kelimeler: 1,3,4-Oksadiyazol, Piperazin, Piperidin, Antikanser, *Mannich* bazı

Table 1. Sentezi yapılan bileşiklerin genel formülleri.



Compound	R
5a	3-Metilpiperidin
5b	4-Hidroksimetilpiperidin
5c	4-Fenilpiperidin
5d	4-Hidroksi-4-fenilpiperidin. HCl
5e	4-Asetil-4-fenilpiperidin. HCl
5f	4-Siyano-4-fenilpiperidin. HCl
5g	4-Fenilpiperazin
5h	4-(2-Piridil)piperazin

1. INTRODUCTION and PURPOSE

The word cancer refers to a number of diseases in which abnormal cells multiply uncontrollably and spread. If the spread of these cells is not stopped or continued then the disease is likely to be fatal.

Cancer is the second most common cause of death in the world, according to World Health Organization (WHO) there are about 1.7 million new cases and 522 000 deaths from breast cancer each year [1]. Breast cancer in particular is the second most common cause of death for women [2]. However, the mortality rate has reduced by 36% over the last ten years for this specific type of cancer. Treatment of childhood cancers has improved dramatically since the 1970s with the survival rate going from 58% to 83% [3].

Cancer is caused by external factors such as diet, exercise, excessive alcohol consumption and smoking as well as internal factors such as a genetic predisposition towards certain types of cancers. A combination of these two factors is often responsible for the disease.

It is estimated that 20% of all cancers are preventable and have been caused by external factors such as smoking or alcohol abuse over a prolonged period of time [3].

Five million cases of skin cancer are also diagnosed each year, a large proportion of which are also likely to be preventable if the patients had taken prior precautions when exposing their skin to the sun [3].

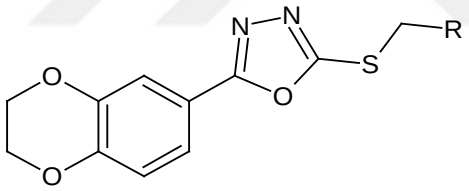
Cancers may also be caused by infections passed from person to person such as the HIV, Hepatitis C and B viruses. So it may also be argued that the prevention of the spread of these infections will reduce the development of cancer. Some preventative measures have been put in place to catch cancers in their early stages such as cervical, breast and colorectal cancer screening. In these cases early removal of pre-cancerous lesions can prevent the cancer developing further [3].

Treatments of cancer frequently include radiation therapy, chemotherapy, surgery, hormonal therapy, immunotherapy and targeted therapy or a combination of several different types of treatments [3].

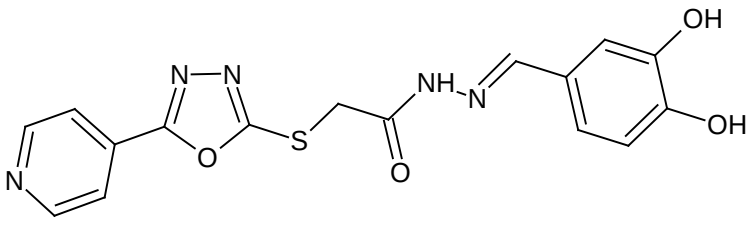
Staging of cancer depends on its extent at the time of diagnosis, usually based on the size of the main tumor and whether the cancer has spread to other areas of the body [3].

In the literature, it is seen that heterocyclic ring system plays a significant performance in the development of new series of drugs which encouraged their activities when combined with other ring systems [4-9]. For this reason researchers in the last ten decades focused on building up highly discriminatory anticancer drugs. The identification of cell-cycle regulators and apoptotic stimuli to fight with cancer cells provides an attractive strategy to discovery and development of effective anticancer agents [10].

This study aims to synthesize new 3,5-disubstituted-1,3,4-oxadiazole-2-thione derivatives as potent cytotoxic agents. In similar studies given in literature such 1,3,4-oxadiazole derivatives containing 1,4-benzodioxane moiety showed potential anticancer activity against various types of cancer cells, HEPG2, HELA, WS116 and BGC832, through their inhibitory activity against telomerase enzyme which is important enzyme in genomic stability during normal DNA replication [11-14].

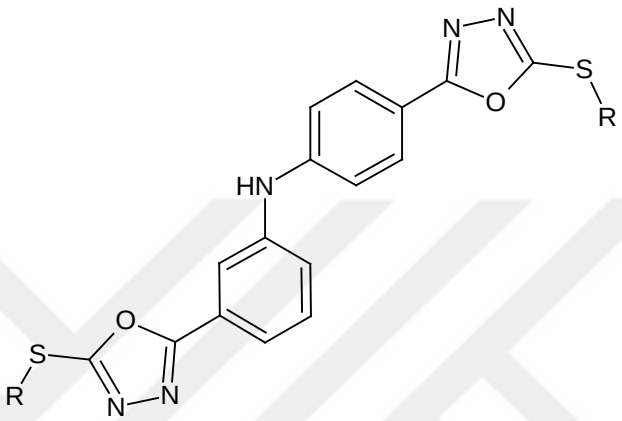


In the study by Zhange *et al*, of 1,3,4-oxadiazole derivatives carrying pyridine and acylhydrazone moeities in their skeleton were showed to have a potential activity against HEPG2, MCF7, SW1116, BGC823 cancer cell lines [15].



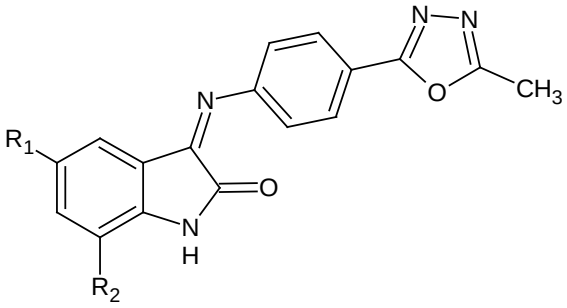
Similarly, in another study, 2,4"-bis(diphenylamine)oxadiazole derivatives are reported to play a significant role against epidermal growth factor receptors which are one of the main types of tyrosin kinase proteins [16]. These proteins are important in signaling pathway transduction during DNA replication, therefore inhibition of these

proteins participates in controlling cancer growth [17-20]. Furthermore vascular endothelial growth factor (VEGF) is another important type of tyrosin kinase enzymes which is responsible for angiogenesis process through three proteins named VEGF-1, VEGF-2, VEGF-3. The angiogenesis process is critical for tumor growth and spreading [21-24]. For this aim, a series of compounds containing 1,3,4-oxadiazole were synthesized to interrupt with downstream signaling pathways of VEGF, and activity results showed the inhibition effects of these compounds in cancerous cells.

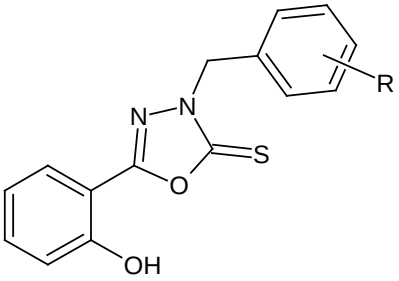


R= - H, -CH_2-C_2H_5, -C_3H_5

Many literature researchers focused on the modification of anticancer agents demonstrates, one of the most significant obstacles for oncologist and chemists as the resistance of different cancerous cells to chemotherapy treatment. In addtion, a dose of chemotherapeutic drug causes destructive effect on both cancerous and noncancerous cells. Aminoalkylation of aromatic substrates through the *Mannich* reaction is of appreciable importance for the synthesis and modification of biologically active compounds that possess selective anticancer activity, devoid of many unpleasant side effects of ordinary anticancer agents [25-28]. Literature denotes that derivatives which are synthesized via *Mannich* reaction possess potential anticancer activity [29-32]. As an example, 3-(3-chlorobenzyl)-5-(2-hydroxyphenyl)-1,3,4-oxadiazole-2(3*H*)thione and 3-(4-chlorobenzyl)-5-(2-hydroxyphenyl)1,3,4-oxadiazole-2-(3*H*)thione were synthesized by Aboraia *et al*. These compounds having electron withdrawing substitution on the aromatic ring attached to the 1,3,4-oxadiazole ring showed potential activity against different types of breast cancer cells [33].

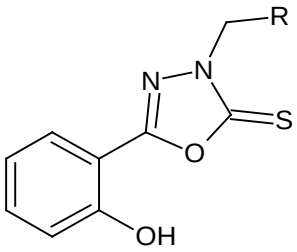


R = -Cl, -F, -Br
R_2 = -H



R = -Cl

Taking literature search into consideration, in this study, the resourceful efficacy of the *Mannich* bases in pharmaceutical chemistry convinced us to prepare and discover new and safer compounds having an effective cytotoxic activity against breast cancer cell lines, by design, synthesis, and structural identification of a novel series of 3,5-disubstituted-1,3,4-oxadiazole-2(3*H*)thione and study their anticancer activity. The modified pattern was performed in a manner with respect to examine SAR.

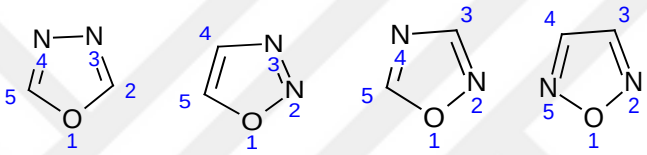


R= Alkyl, aryl substituted piperidine, piperazine

2. LITERATURE REVIEW

2.1. 1,3,4-Oxadiazoles

1,3,4-Oxadiazole is an aromatic heterocyclic compound consisting of an oxygen atom and two nitrogen atoms. These atoms exist in a five-membered ring with the molecular formula of $C_2H_2N_2O$. This ring is derived from furan by substituting of two methylene groups (=CH) with two pyridine type nitrogens (-N=) [34-35]. In addition, there are three well known isomers of this ring: 1,2,3-oxadiazole, 1,2,4-oxadiazole and 1,2,5-oxadiazole.

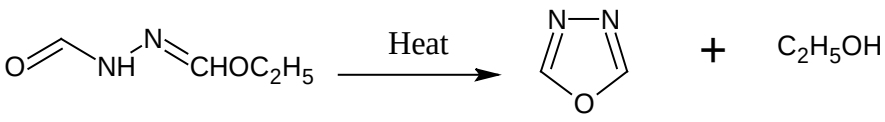


The derivatives of 1,2,3-oxadiazoles are called sidones. Sidones have high dipole moment and they exist as dipolar ionic structures. They are also known as oxadiazolium betaines [36].

1,2,4-Oxadiazoles are also known as azoxime, and they have an unstable ring system. Derivatives of 1,2,4-oxadiazole can be synthesized through the reaction of amidoxime with carboxylic acid or acid chloride in presence of DCC [36].

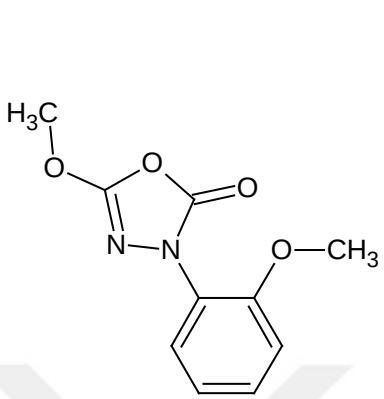
1,2,5-Oxadiazoles are known as furazans which can be synthesized via dehydration of 1,2-diketones.

Nonsubstituted 1,3,4-oxadiazole ring was prepared and synthesized through thermolysis of ethylformate, formally hydrazine, at atmospheric pressure [37].

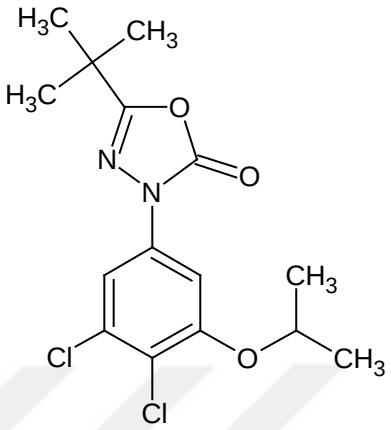


1,3,4-Oxadiazole is a very weak base due to the inductive effect of extra heteroatoms, and thermally stable. Through heterocyclic compounds, 1,3,4-oxadiazole has become crucial construction motif for adding extra properties to the new drugs [38]. By having this heterocyclic ring, the compounds have a broad biological activity spectrum which includes antibacterial [39-43], antifungal [44], analgesic [45], anti-

inflammatory [46], antiviral [47], anticancer [48-53], antihypertensive [54], anticonvulsant [55], and anti-diabetic properties [56]. Examples of commercially available 1,3,4-oxadiazole-2-ones are Metoxadiazone which acts as household insecticide, and Oxadiazon which is the first oxadiazole herbicide [57].

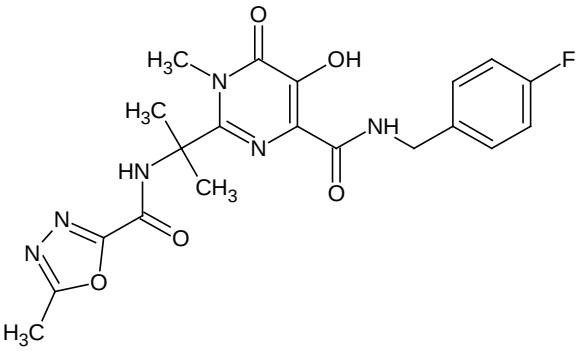


Metoxadiazone

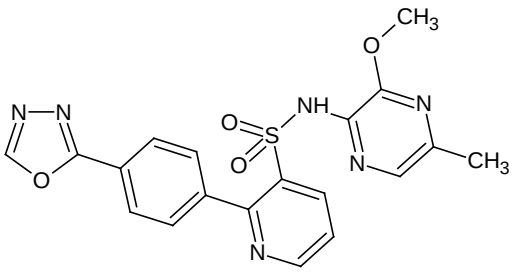


Oxadiazon

1,3,4-Oxadiazoles derivatives are usually used to act as bioisosteric replacements for amide and ester [35] which can contribute significant pharmacokinetic properties due to the presence of azole group, this group enhances the lipophilicity that has the ability to make the drug to reach the target site by transmembrane diffusion [58]. However, 1,3,4-oxadiazole ring is recited to enhance the pharmacological activity due to its ability to form hydrogen bonds with receptors [59]. In addition, this ring has the ability to undergo many chemical reactions which makes it play an important role for molecular planning. Some compounds having this ring are widely used nowadays as medicine such as Zibotentan an anticancer agent, and Raltegravir an antiretroviral drug [60].



Raltegravir

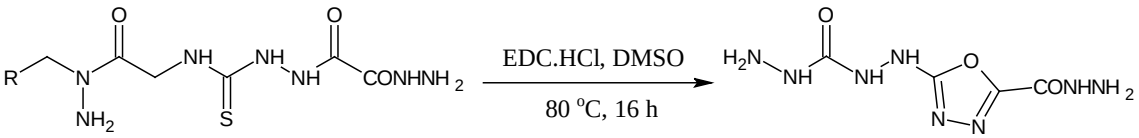


Zibotentan

2.1.1. Synthesis Methods of 1,3,4-Oxadiazole

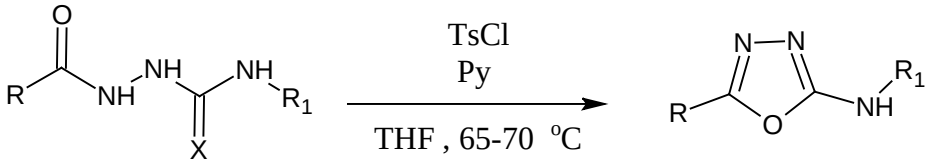
2.1.1.1. From Acylsemicarbazide and Acylthiosemicarbazides

Kilbur *et al*, reported the synthesis of 1,3,4-oxadiazole on solid support from acylthiosemicarbazide through treating Resin bond -1-acylthiosemicarbazide with assortment of dehydrating agents such as 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) at different temperatures resulting in releasing of substituted 1,3,4-oxadiazole from the solid support [61].



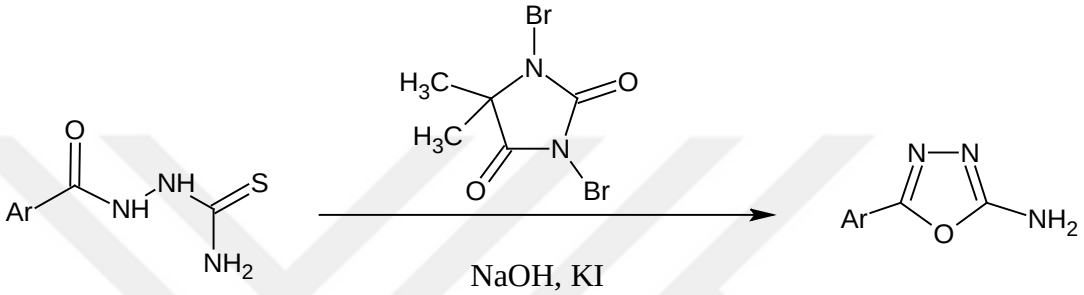
R = polymer support

In general, 5-substituted-2-amino-1,3,4-oxadiazoles are prepared by dehydration of semicarbazide or thiosemicarbazide derivatives. Dehydrating agents that are used for this aim ($POCl_3$ or any alternative reagent) have the ability to activate the carbonyl group. For this reason, Dolman and co-workers reported a new method of synthesis for 5-aryl(alkyl)-2-amino-1,3,4-oxadiazoles from acylsemicarbazides (X=O) and acylthio semicarbazides (X=S) by using tosyl chloride [62].



R, R_1 = alkyl, aryl

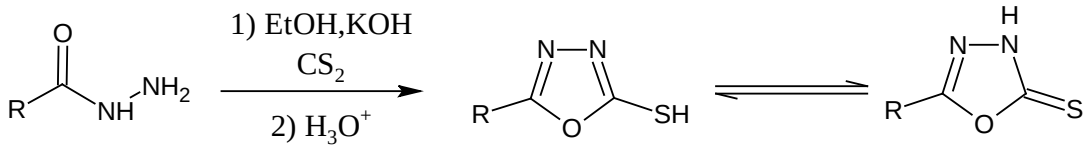
Alternatively, Rivera and co-workers synthesized 5-aryl-2-amino-1,3,4-oxadiazoles in high yields by cyclization of acylthiosemicarbazide using 1,3-dibromo-5,5-dimethylhydantoin which is an effective, cheap and safe cyclize agent [63].



Ar = Ph, 4-ClC_6H_4, $MeOC_6H_4$

2.1.1.2. From Mono and Diacylhydrazides

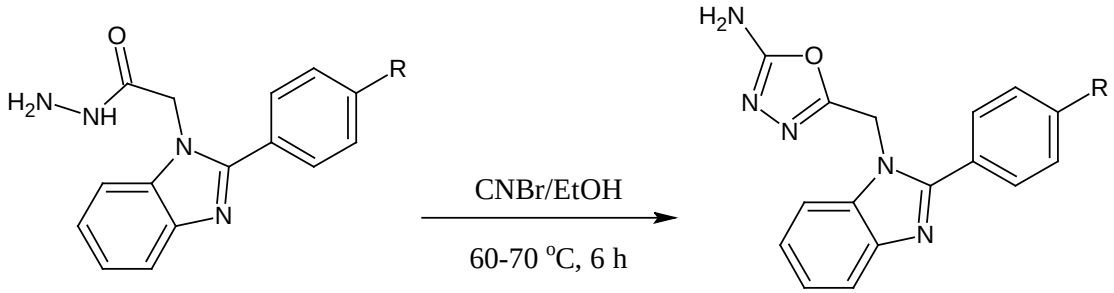
The main synthesis route for 5-substituted-1,3,4-oxadiazole-2-thiole/ones involves an initial reaction between an acylhydrazide with carbon disulfide in basic alcohol solution, followed by acidification of the reaction mixture with hydrochloric acid [64].



R= Furan, -H, $-CH_3$

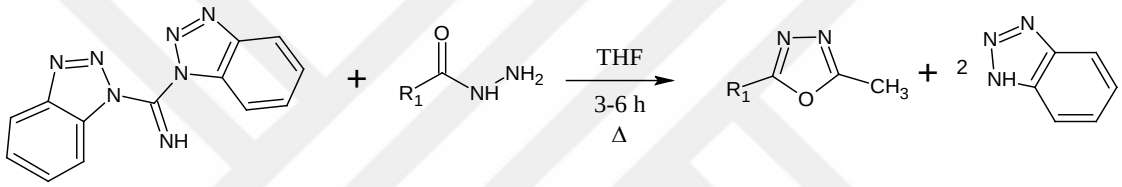
R_1= $-SCH_3$, $-CH_2CH(CH_3)_2$

The reaction between 2-[2-(4-substitutedphenyl)-1*H*-benzo[*d*]imidazole-1-yl] acetohydrazide and cyanogen bromide resulting in formation of a new series of 2-amino-1,3,4-oxadiazoles. This method was reported by Kerimov and co-workers [65].



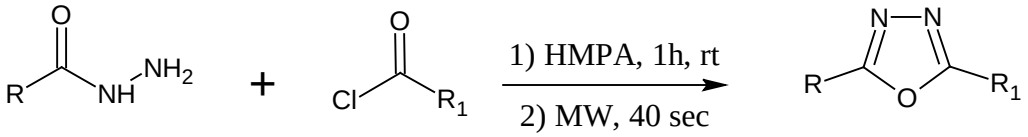
R= -H, -Cl, -OMe, -OCH_2Ph

Katritzky and co-workers were prepared 5-aryl-2-amino-1,3,4-oxadiazole compounds with high yields by using the reaction of di(benzotriazol-1-yl)methanimine and arylhydrazides [66].



R_1= Ph, 4-t-BuC_6H_4,
4-$NH_2C_6H_4$, 4-OHC_6H_4,
4-$NO_2C_6H_4$, 3-$NO_2C_6H_4$,
4-ClC_6H_4, 2-ClC_6H_4, 4-Pyridyl
-OC_2H_5

2,5-Disubstituted-1,3,4-oxadiazoles prepared by condensation of monoarylhydrazide by using acid chlorides in hexamethylphosphoramide solvent under microwave heating. This method undergoes without need to acid as catalyst or dehydrating agent [67].

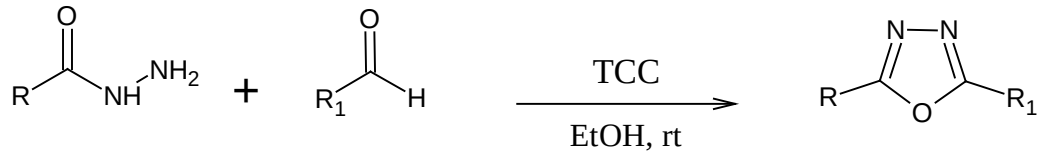


R = Ph, 4-NO_2Ph

4-MeOph- 4-Pyridyl

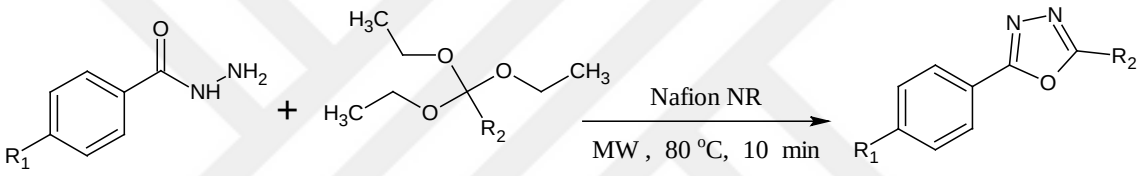
R_1= Ph, 2-furyl,
-CH_2Ph, -CH_3,
2-propyl,
2-thienyl,
4-NO_2Ph,
4-MeOP

2,5-Disubstituted 1,3,4-oxadiazoles were synthesized by an efficient method developed by Pore and co-workers by using trichloroisocyanuric acid (TCCA) at ambient temperature from acylhydrazide [68].



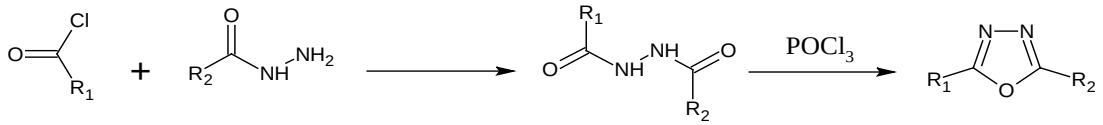
R = Ph, 4-ClC_6H_4 R_1 = Ph, 4-ClC_6H_4

1,3,4-Oxadiazoles was synthesized by condensation of benzohydrazide and triethylorthoalkanates under microwave irradiation in the presence of catalyzing agent Nafion NR50 (solid supported) and phosphorus pentasulfide in alumina ($P4S10/Al_2O_3$) [69].



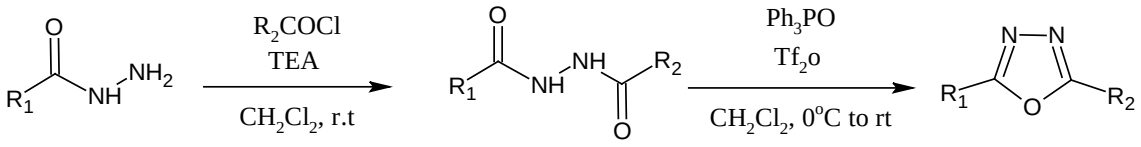
R_1= -H, -F, -OMe
R_2= -H, -Et, Ph

Isloor and co-workers synthesized 2,5-diaryl-1,3,4-oxadiazoles through the reaction of diaroylhydrazines with phosphorus oxychloride. Diaroylhydrazines were synthesized firstly from the reaction of aromatic acyl hydrazines and aromatic acid chlorides [70-71].



R_1, R_2: Aryl

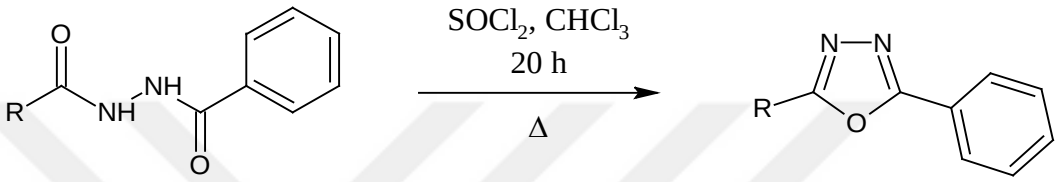
Bostrom and co-workers synthesized 2,5-disubstituted-1,3,4-oxadiazole derivatives by cyclodehydration of diacylhydrazine in the presence of triphenyl phosphine oxide and triflic anhydride. The yield of compounds were 26-96% [35].



R_1= Ph, 3-Pyridyl, p-chlorphenyl

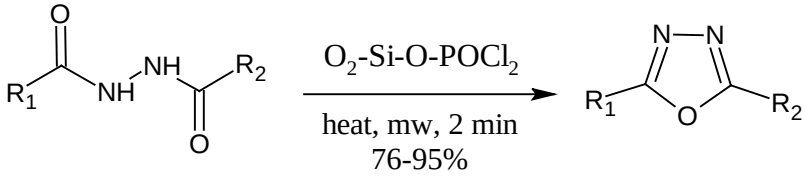
R_2= Ph, Et, p-tolyl,
p-chlorophenyl

In literature thionyl chloride is another agent used for dehydration of diacylhydrazines [72]



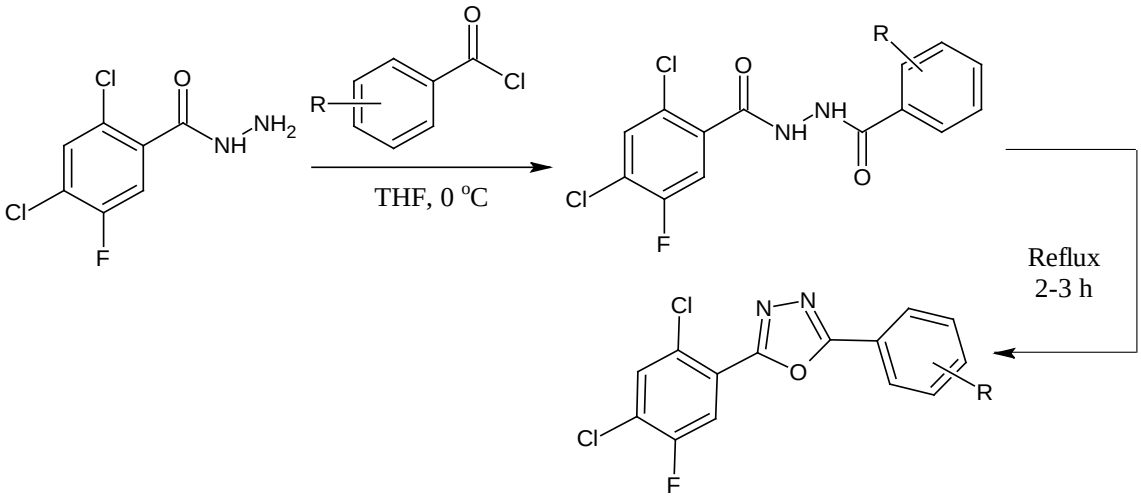
R= substituted pyrolidine

Li and co-workers used silica-supported dichlorophosphate method for synthesizing alkyl, aryl, heterocyclic substituted of 1,3,4-oxadiazole under microwave conditions without using any solvent [73] .



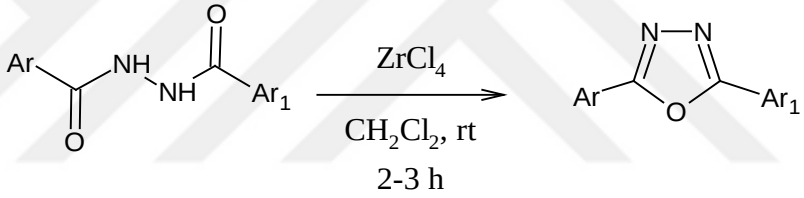
R_1,R_2= aryl, alkyl, hetrocycle

Zheng and co-workers have reported the synthesis of 5-(2,4-dichloro-5-flurophenyl)-2-aryl-1,3,4-oxadiazole compounds in good yields by refluxing the diacylhydrazine with phosphorus oxychloride [74].



R= 2,3,4,5-tetrafluoro, 2,4,5-trifluoro, 2,6-difluoro

In another study, 1,3,4-oxadiazole ring was prepared by an easy, and shorter method from diacylhydrazines using inexpensive $ZrCl_4$ as catalyst, this method was applied by Sharma and co-workers [75].

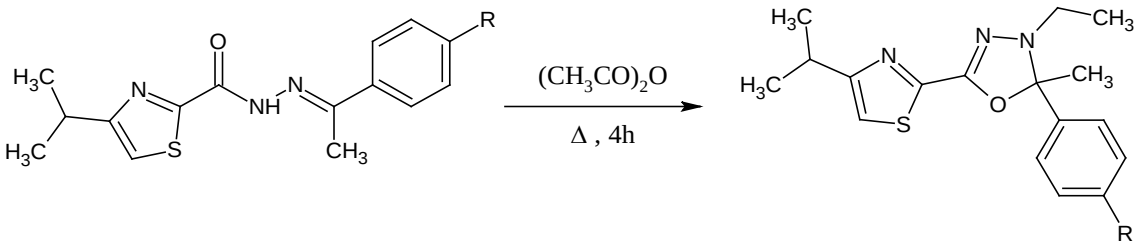


Ar = C_6H_5, p-$NO_2C_6H_4$, p-FC_6H_4

Ar_1 = C_6H_5, p-ClC_6H_4, p-FC_6H_4

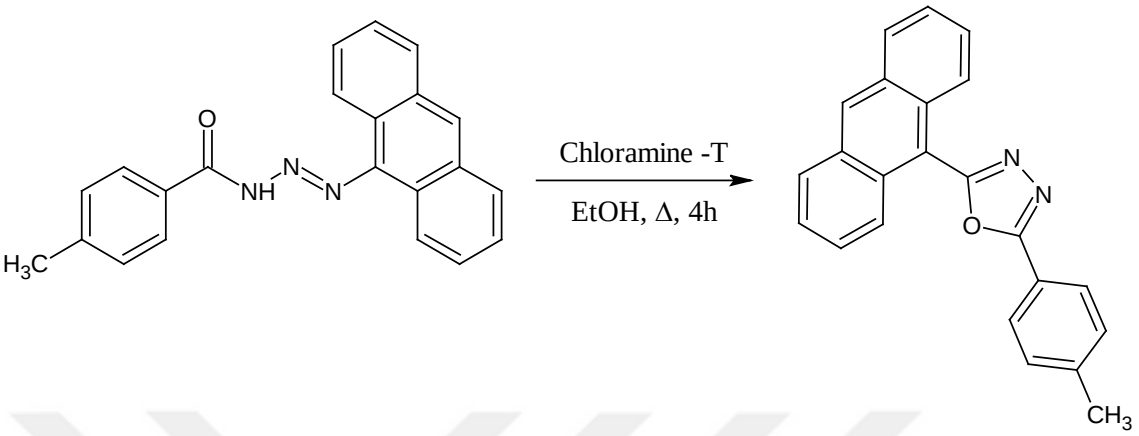
2.1.1.3. From *N*-Acylhydrazone:

1,3,4-Oxadiazole have been prepared by the reaction of *N*-acylhydrazones with acetic anhydride under reflux conditions [76].

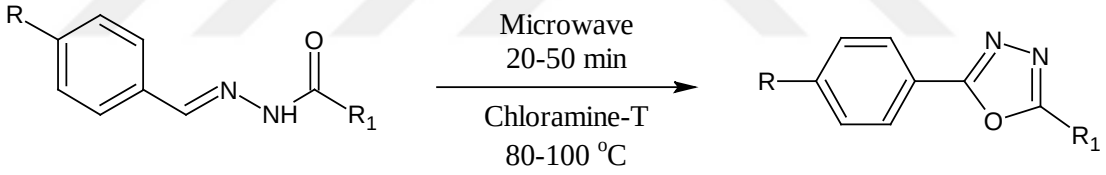


R= -Cl, -NO_2, -Br, -OH

Oxidative cyclization of *N*-acylhydrazones using chloramine-T was one of the method that has been used by Li and He to give 2-(anthracen-9-yl)-1,3,4-oxadiazole in high yield [77].

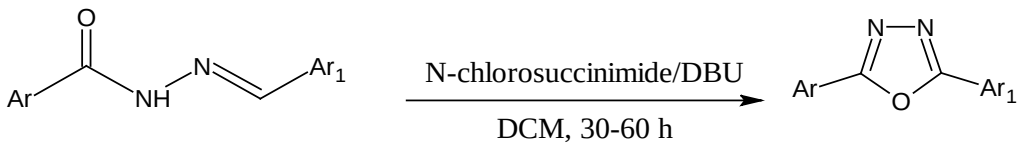


Similary Goknar and co-workers also synthesize 1,3,4-disubstituted oxadiazoles through the oxidative cyclization of *N*-acylhydrazones under miceowave irradiation using the same reagent [78].



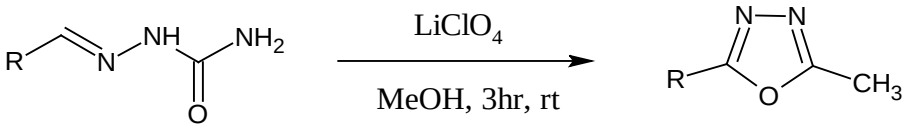
R= 2-N-methyl-N-ethoxy pyridine
R_1= Phenyl, 2-fluorophenyl,
1-chlorophenyl, 2-furanyl,
2-thiophenyl

Pardeshi and co-workers synthesized various structures of 2,5-disubstituted 1,3,4-oxadiazoles by using N-chlorosuccinimide in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to cyclize *N*-acylhydrazones [79].



Ar = Ph,4-$NO_2C_6H_4$,4-$OCH_3C_6H_4$, 4-$CH_3C_6H_4$
Ar_1= Ph, 4-$OCH_3C_6H_4$, 4ClC_6H_4, 4-$CH_3C_6H_4$

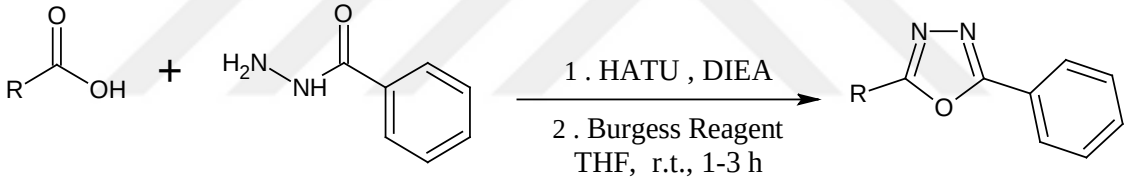
In addition electrocyclization of *N*-aclhydrazone to their corresponding 1,3,4-oxadiazoles has also been used as another method for the preparation of this moiety [80-81].



R= C_6H_5, 4-Cl-C_6H_4,
4-MeOH-C_6H_4, 3-NO_2-C_6H_4,
4-OH-C_6H_4, CH_3

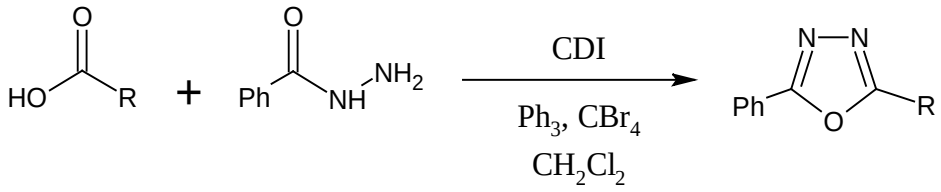
2.1.1.4. From Carboxylic Acid

The synthesis of 1,3,4-oxadiazoles from carboxylic acids and hydrazides using 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium-3-oxihexafluoro-phosphate (HATU) as coupling agent and methyl *N*-(trimethylammoniumsulfonyl) carbamate (Burgess reagent) as dehydrating agent is one of the most important method which was developed by Li and Dickson [82] .



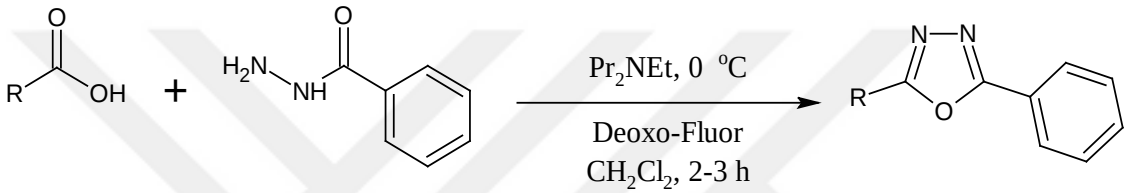
R= Substitued benzyl,
5-bromo-2-methylthiophenyl,
butyl

Rajapakse and co-workers synthesized 2,5-disubstituted-1,3,4-oxadiazoles from benzohydrazide and carboxylic acid by using the coupling agent 1,1'-carbonyldiimidazole (CDI), triphenylphosphyne as dehydrating agent and tetrabromomethane CBr_4 which is a convenient one pot method for obtaining a good yield of 1,3,4-oxadiazole [83].



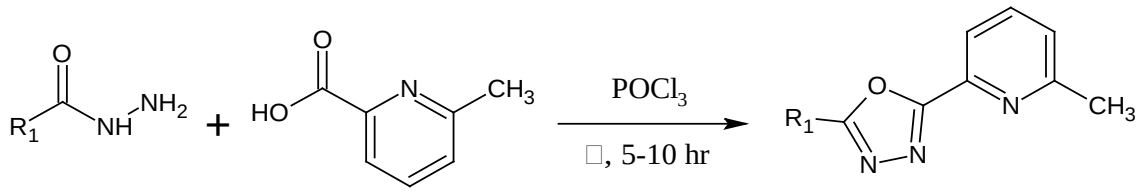
R = 2,2-dimethylethanyl
1-phenylpyrrolidinyl

Another one–pot direct synthesis of 1,3,4-oxadiazoles in good yield was described by Kanagani and co-workers by using different carboxylic acids, benzohydrazide, and Deoxo-Fluor reagent [84].



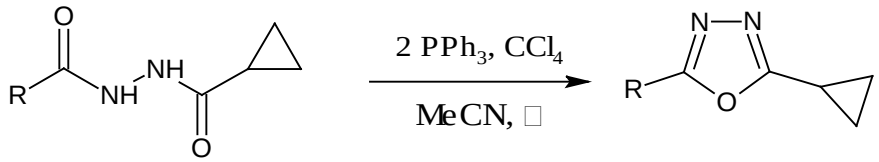
Carboxylic acid = palmitic acid, linoleic acid, benzoic acid

Dooddahosuru and co-workers reported a modification for the synthesis of 2,5-disubstituted-1,3,4-oxadiazole through the reaction of substituted carboxylic acid with hydrazine in the presence of $POCl_3$ as cyclizing agent [85] .

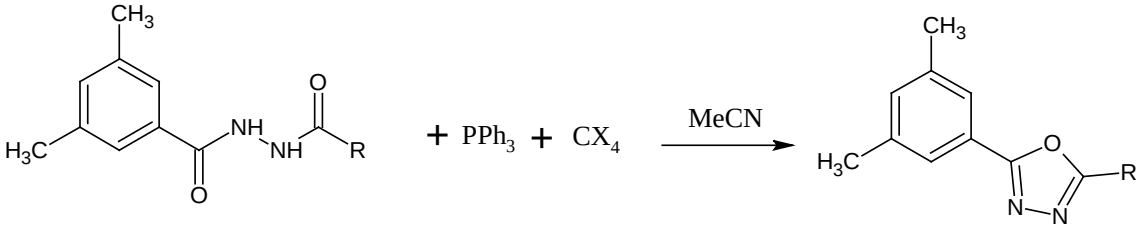


R_1= 4-(1-cyano-1-mehylethyl)phenyl,
pyridine, 4-bromophenyl

Yang and Shi reported the effect of halogens by using PPh_3/CX_4 (X = Cl, Br, I) as dehydration agents in a Robinson-Gabriel type reaction of cyclopropane-carboxylic acid *N'*-substituted-hydrazides which leads to formation of 1,3,4-oxadiazoles. However, using CBr_4 or CI_4 instead of CCI_4 in Robinson-Gabriel reaction results in opening of cyclopropane ring after dehydration to give the corresponding 2-(3-halopropyl)-5-substituted-1,3,4-oxadiazoles in good yields [86].

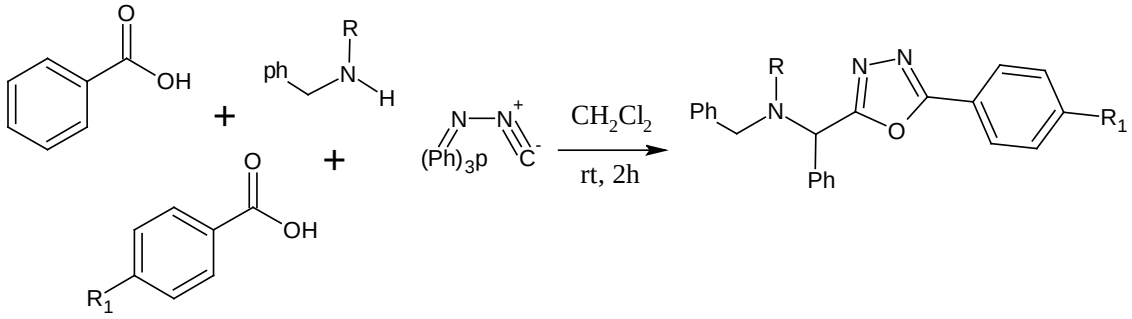


R= 3,5-$Me_2C_6H_3$, C_6H_5,
$-CH_2-C_6H_5$



R= cyclopropyl, halopropyl

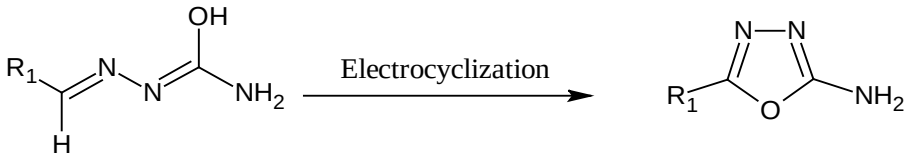
In different studies, there are some methods are reported for synthesis of 2,5-disubstituted 1,3,4-oxadiazoles by condensation procedure in CH_2Cl_2 at room temperature involving the presence of (*N*-isocyanimino)triphenylphosphorane, a secondary amine, a carboxylic acid, and an aromatic aldehyde, as starting materials [87-92].



R = $-CH_3$, $-CH_2Ph$,
R_1 = -H, -F, -Br, -Cl, -I

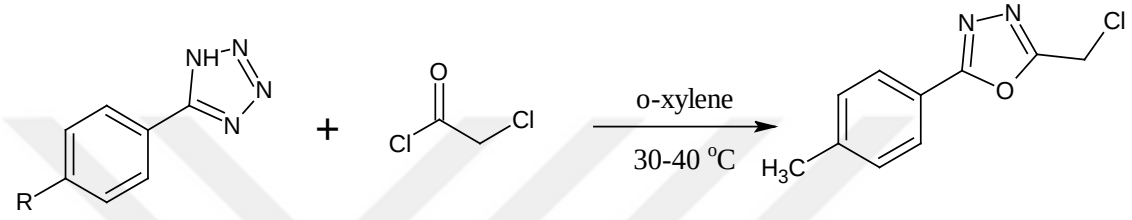
2.1.1.5. From Others

Sharma *et al*, have reported the synthesis of 5-substituted-2-aminooxadiazoles through an ecofriendly synthetic method called electroorganic cyclization which does not require harmful oxidizing chemicals and can be performed at room temperature [81].



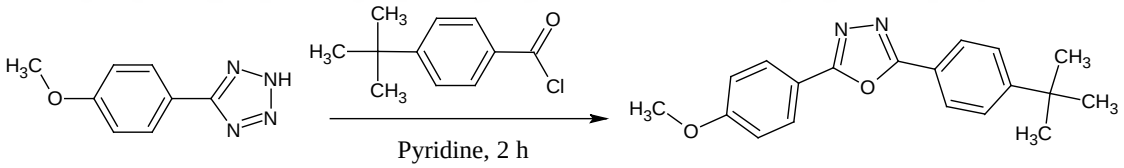
R_1 = C_6H_5, 4-Cl-C_6H_4, 4-OH-C_6H_4

The Huisgen reaction is widely used for synthesis of various 2,5-disubstituted-1,3,4-oxadiazole. In this reaction tetrazole and chloroacetyl chloride gives disubstituted oxadiazole [93].

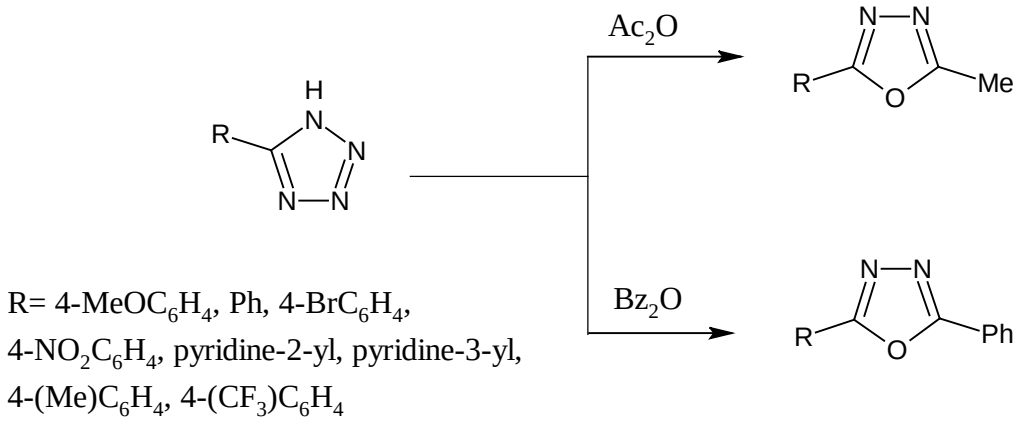


R = -H, -Cl, -NO_2, -OEt

Instead of chloroacetylchloride, 4-*tert*-butylbenzoyl chloride was used to synthesize 2-(4-tert-butylphenyl)-5-(4-methoxyphenyl)1,3,4-oxadiazole [94].



Similarly, Efimova and co-workers, also processed the Huisgein reaction with acid anhydrides instead of acid chlorides, through acylation of a series of 5-aryl(hetaryl)tetrazoles with acetic and benzoic anhydrides under microwave irradiation conditions [95, 96].



2.2. Spectral Properties

2.2.1. UV Spectra

2,5-Diaryl-1,3,4-oxadiazole derivatives have maximum absorption at 274-310 nm [41,97]. 2-Substituted-5-substitutedphenyl-1,3,4-oxadiazoles have two maximum absorption bands at 276-298 nm [98], and 2-amino-5-aryl-1,3,4-oxadiazole derivatives have two absorption bands at 270 and 330 nm [99].

2.2.2. IR Spectra

1,3,4-Oxadiazoles have C=N stretching bands at 1656-1600 cm^{-1} and C=S stretching bands at 1438-1419 cm^{-1}, and C-O-C stretching bands at 1267-1093 cm^{-1} [33, 100-101].

2.2.3. ^{1}H-NMR Spectra

Hydrogen atom at fifth carbon of 2-aryl-1,3,4-oxadiazole derivatives have a singlet peak at 9.2-9.3 ppm [102], methylene protons at aryloxymethyl moiety of 2-aryloxymethyl-5-alkyl-1,3,4-oxadiazole derivatives give peak at 4.6-5.4 ppm as singlet [103-104], protons at amino group of 2-substitutedamino-1,3,4-oxadiazoles show up at 9.55-10.60 ppm [105-107] and protons of acyl group that is attached to amine group give a peak at 11.05-12.40 ppm [98].

2.2.4. ^{13}C-NMR Spectra

Carbon atoms at the second and fifth positions of 2,5-disubstituted-1,3,4-oxadiazole have a peak at 158-157.9 and 175.2-174.8 ppm respectively [33, 108].

2.3. Biological Activity

2.3.1. Cancer

Cancer is the Latin word for crab. The ancients used this word to mean malignancy, doubtless because of the crab-like tenacity a malignant tumor sometimes seems to show in grasping the tissues it invades. It is an abnormal growth of cells which tend to proliferate in an uncontrolled way and, in some cases to spread [109].

Cancer can involve any tissue of the body and have many forms in each body area. Most cancers are named for the type of cell or organ in which they start. The maintenance of mammalian cells to be healthy is based on an accurate balance between growth promoting signals and growth inhibitory signals. If there is any deregulation of this precise balance normal cells will be converted to abnormal or cancer cells. In addition there are many macromolecules established and characterized that included in the systemization of cell signaling pathways which take a place either as tumor promoters or tumor suppressors [110].

2.3.2. Important Principles on the Cell and Cancer

Cell cycle or cell-division cycle is a process consisting of stages of events included G1 and G2 phase, an S phase and an M phase which includes two steps: division of nucleus (Mitosis) and division of cytoplasm (Cytokinesis).

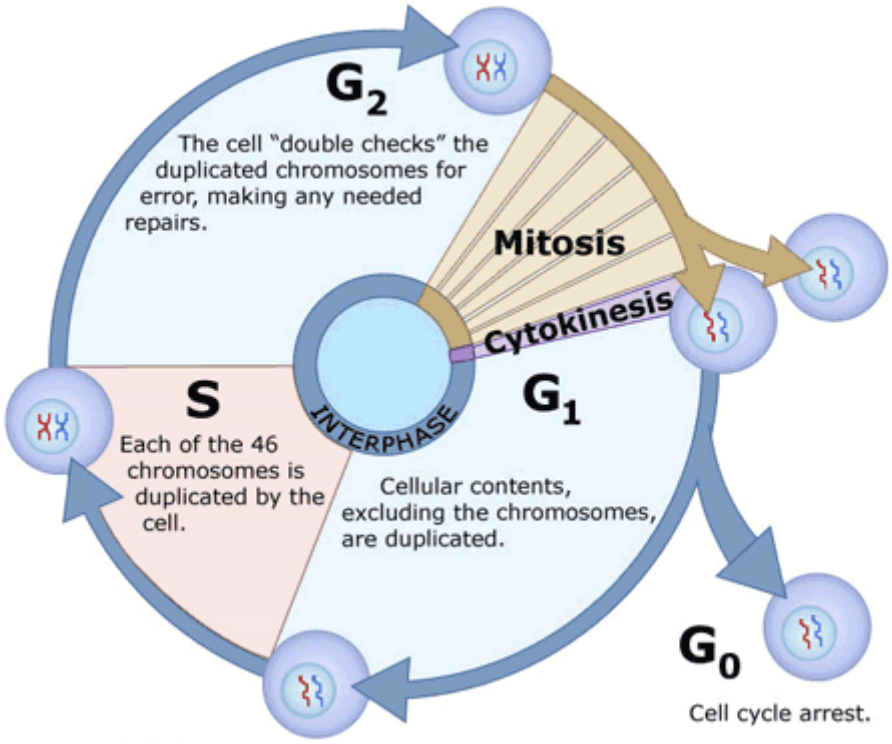


Figure 2.1. Cell cycle process

G1 First growth phase. Metabolic changes prepare the cell for division. At a certain point - the restriction point - the cell is committed to division and moves into the S phase.

S phase. DNA replication. Each chromosome now consists of two sister chromatids.

G2 Second growth phase. Metabolic changes assemble the cytoplasmic materials necessary for mitosis and cytokinesis.

Mitotic phase. A nuclear division (mitosis) followed by a cell division (cytokinesis).

The period between mitotic divisions - that is, G1, S and G2 - is known as interphase

In mitosis phase two daughter cells with the same genetic materials as a parent cell are produced through a process included replication of chromosomes in such a way as in S phase. The replicated chromosomes are attached to special cytoskeletal machine called mitotic spindle that aligns them and then segregates the sister chromatids to produce the genetic materials, this separation of the genetic materials is followed by breakdown of cell cytoplasm to produce two daughter cells [111].

Mitosis is divided into five stages named: prophase, prometaphase, metaphase, anaphase, and telophase [111].

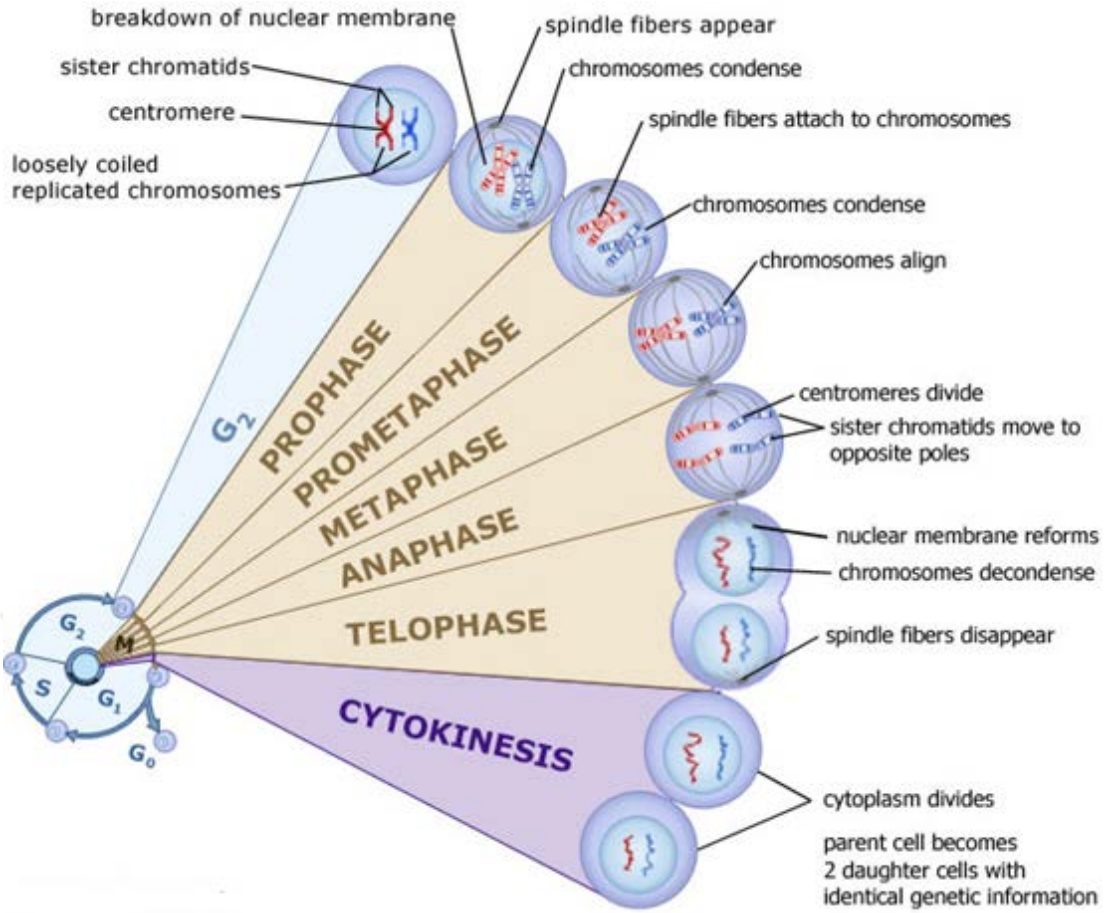


Figure 2.2. Phases of mitosis

In prophase, a structure known as centrosome duplicated to form two daughter centrosomes that move to the opposite poles of the cell. In addition, these centrosomes organize the production of microtubules that are the fiver constitute of mitotic spindle. In this phase, the replicated chromosome becomes visible and consists of two identical chromatids held tightly together by centromere [111].

The chromosomes in the prometaphase, led by their centromeres, move to the equatorial plane in the med-line of the cell. This region of mitotic spindle is known as the metaphase plate. Kinetochores are proteins that assemble on each side of chromosome through which the spindle fiber of mitotic spindle attached to it. Each chromosome consists of two kinetochore which faces on opposite directions.

In metaphase, the chromosomes align themselves along the metaphase plate region of the mitotic spindle by using the property of continuous growing and shrinking of microtubules [111].

In anaphase, synchronous separation of daughter chromosomes occurs. The centromers divided, and the sister chromatids of each chromosome moved toward the poles of the cell by the spindle fibers which attached to the kinetochore regions. Telophase is the last stage of mitosis in which vesicles of nuclear membrane reassembles and cluster around the chromosomes and fuse to reform an envelope around them. The chromosome uncoils and become diffuse, and the spindle fibers disappear [111].

Cytokinesis is the final cellular division stage to form two new cells. The cell then enters interphase-the interval period between mitotic divisions [111].

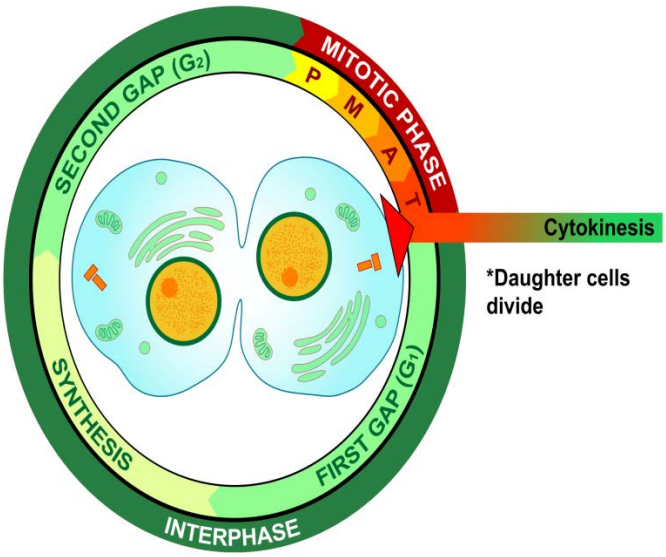


Figure 2.3. Cytokinesis

2.4. Cancer Treatment

There are lots of methods which play a significant role in diagnosis, determining, and treatment of cancer, these methods involve: surgery, radiation chemotherapy and immunotherapy.

Surgery is the oldest way for treating cancer. However, stills one of the most effective ways to treat cancer, as it has the ability to diagnose cancer, find out where it is located, whether it has spread, and whether it is affecting the functions of other organs in the body [112].

Surgery may be used to relieve pain or to restore physical function of patient if tumor presses on the spinal cord, or a nerve, blocks the bowel or intestines. Furthermore

surgery can stop the bleeding that occurs as a side effect of some drugs used to treat cancer, or to stop bleeding which happened related to the pressure from the cancer in areas with a high concentration of blood vessel, such as uterus, or due to the passes of food and waste products through esophagus, stomach, and bowel which can easily bleed. Another importance of surgery in case of patients having cancer is the ability for inserting a feeding tube that delivers medications or chemotherapy [112].

There are different types of chemotherapy drugs that are used in a variety of ways to treat different types of cancer by destroying cancer cells, shrinking existing tumors, and preventing cancer cells from multiplying, in order to improve the patient's quality of live by minimizing the pain and the pressure which produced by cancer leading to patient's live for long time [113].

The administration of ionized energy is referred to radiation process which plays a significant role in controlling the growth of cancer cells by applying a high energy waves X-rays, gamma rays or electron beams that damage the genetic material in cancer cells by breaking a piece of the DNA resulting in no more growing or splitting of cancer cells [114].

Discoveries in the last two decades of cancer research have shown that cancer is mediated by somatic aberration in the host genome, leading many researchers to pursue genetic manipulation as a form of therapy for the disease. While many innovative genetic treatments for cancer are still undergoing clinical trials, it is anticipated that they will play an important role in the future of cancer therapy when combined with other existing and emerging treatments. Currently most approaches to gene therapy treatment of cancer focus on tackling one or more critical gene defects known as monogenic gene therapy. Determination of the molecular nature of the disease specific to the patient as well as their immune status is necessary in selecting the appropriate type of gene therapy for the patient [115].

Immunotherapy has become an important element in cancer regression or progression. There are four varieties of immunotherapy in cancer treatment. Sensitizing the body's immune system to tumor specific antigens is known as active immunotherapy while passive immunotherapy uses humanized antibodies to target tumor antigens without directly activating the immune system [115].

Adoptive immunotherapy uses either T cells or dendritic cells which have been manipulated to enhance their reaction to tumor antigens [115].

Immune enhancement therapy aims to either block inhibitory molecules or to improve co-stimulatory molecules. Any of the approaches listed above may be used either as the sole form of cancer treatment for a patient or in combination with other forms of treatment [115].

2.4.1. General Classes of Drugs Used for Cancer Treatment

There are many types of drugs that are in common use for cancer treatment, each type of them having a certain mechanism of action to inhibit the growth of cancer cells [116].

The most commonly used drugs that are in clinical use are:

1-Alkylating Agents

2-Antimetabolies

3- Natural Products

4-Plant Products, Microorganism Products

5-Miscellaneous

6- Hormones and Antagonists

2.4.2. 1,3,4-Oxadiazole as Enzyme Inhibitors

Telomerase enzyme is an important enzyme of genomic stability, during DNA replication the length of this enzyme becomes short while in cancerous cells telomerase enzyme activated which restore and stabilize the telomere length leading to continuous cell division and preventing replicative senescence by adding hexameric repeats (TTAGGG) [12-14].

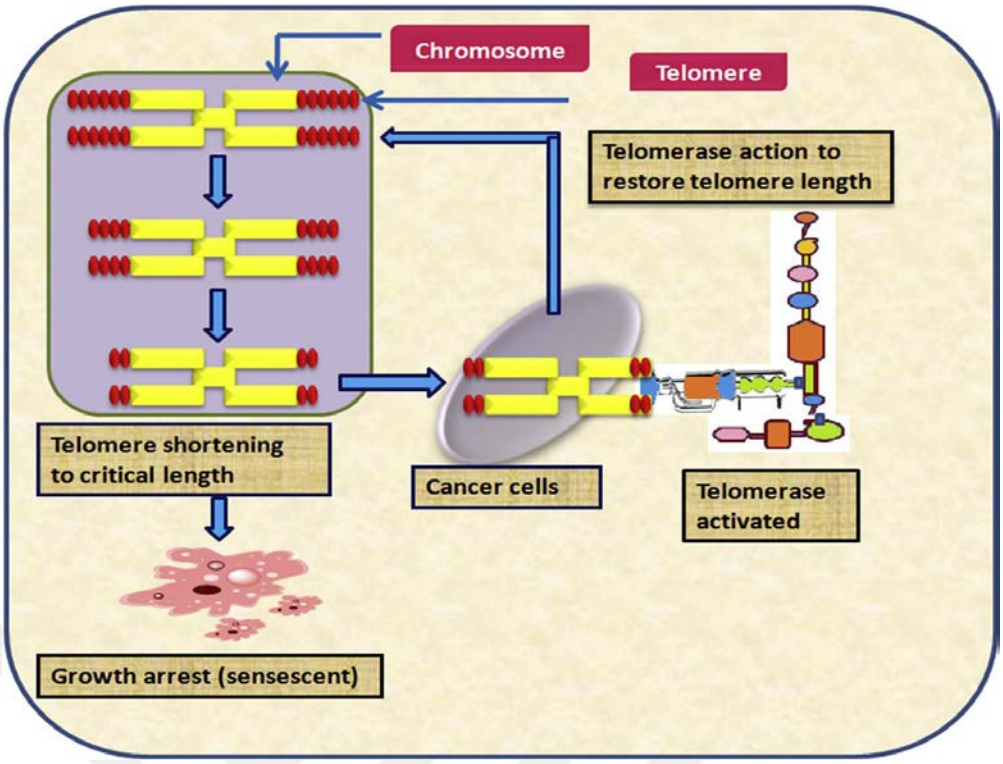
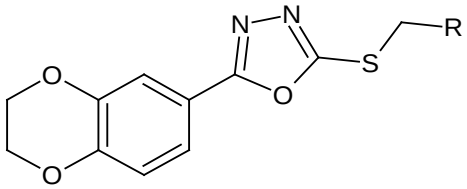


Figure 2.4. Role of telomeres and telomerase enzyme in cancer development

Zhang *et al*, have reported the synthesis of novel series of 1,3,4-oxadiazole derivatives containing 1,4-benzodioxan moiety and examine their inhibitory activity against telomerase enzyme. Tumor extending can be interrupted through telomerase shortening, and the inhibition of this enzyme by these derivatives was screened by using TRAP-PCR-ELISA assay in which theses derivatives show a great anticancer activity against the four cell lines (HEPG2, HELA, ,3and BGC823) compared with the known anticancer agent, 5-fluorouracil [11].



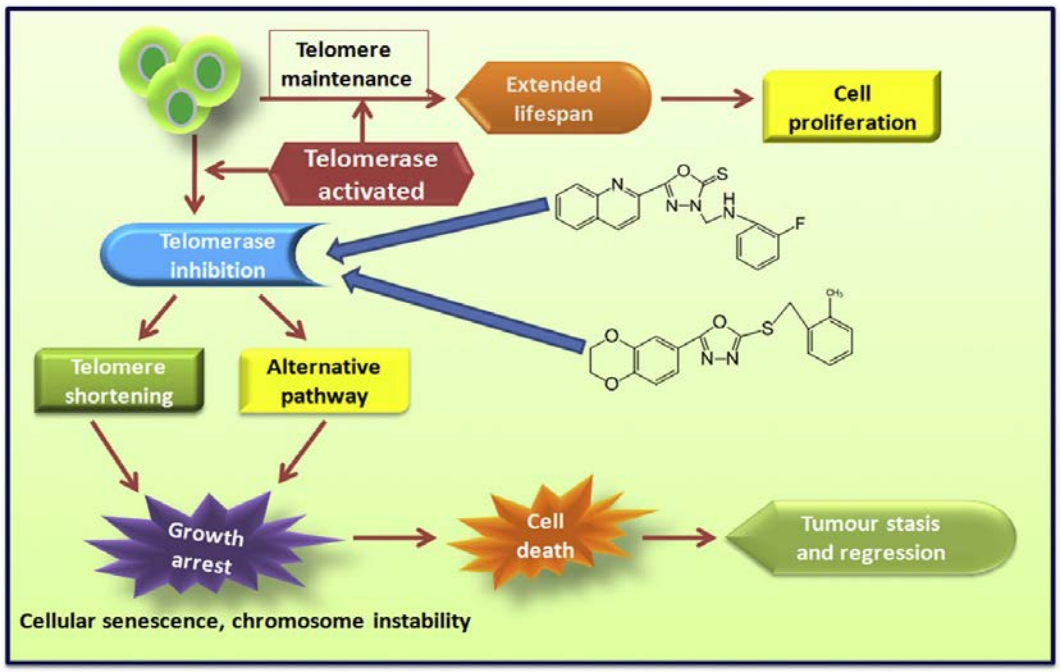
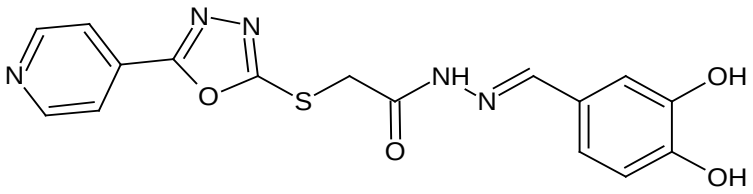


Figure 2.5. A dual role of telomere shortening and telomerase activation in tumor initiation and progression

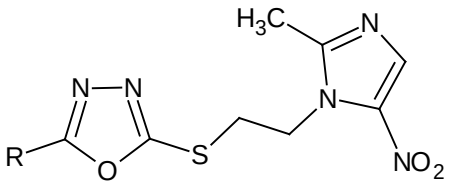
In another study by Zhange *et al*, new derivatives of 1,3,4-oxadiazole containing pyridine and acylhydrazone moieties were reported, these compounds were also investigated by using TRAP-PCR-ELISA assay and they show a higher value of activity against MCF7, HEPG2, SW1116, BGC823 and exhibited a potential inhibitory effect against telomerase enzyme [15].



Thymidylate synthase is another enzyme which plays an important role in the replication, transcription and repair of DNA by catalyzing the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) and forms one of the important nucleic acids of DNA thymine [117].

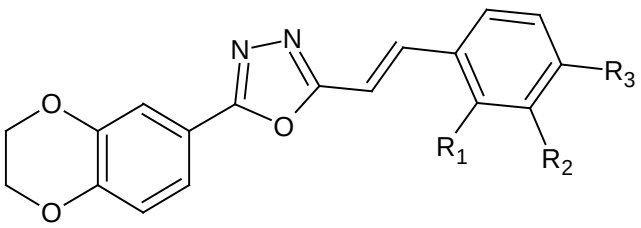
Du *et al*, have reported the synthesis of a new series of 1,3,4-oxadiazole derivatives containing thioether in their structure, these derivatives act as anticancer

agent against this enzyme, their anti-proliferative activity were enhanced in the presence of electron withdrawing group rather than *di* or *tri*-substituted electron donating group [118].



R = Ar, Ar_{Het}

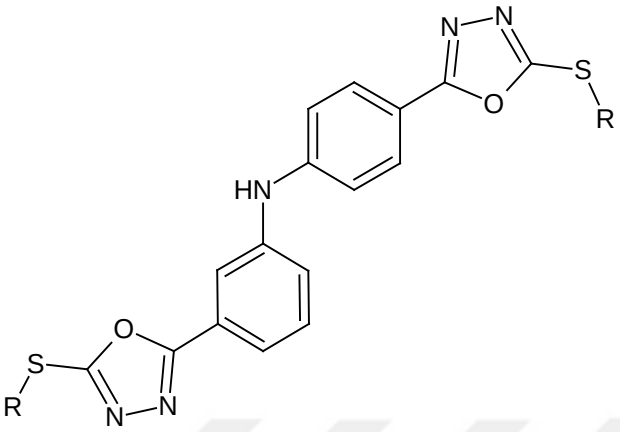
Methionine aminopeptidase enzyme is a bi-functional protein plays an important role in protein synthesis and regulation of post translational process of DNA. This enzyme is classified into two classes MetAP1 and MetAP2, inhibition of MetAP enzymes generates an effective therapeutic role for the treatment of different types of diseases such as: cancer, rheumatoid arthritis, malarial and fungal infection [119]. Sun *et al*. have reported the synthesis of a series of 1,3,4-oxadiazole derivatives carrying 1,4-benzodioxan moiety which has the ability to inhibit methionine aminopeptidase (MetAP) type two enzyme [120].



R= -F, -Cl, -Br, -NO_2, -CH_3, -CH_3O

Epidermal growth factor receptors (EGFR) are one of the main types of protein tyrosin kinase (PTK) which plays an important role in signaling transduction pathway, therefore development of compounds acting against these kinases taking an important place in controlling the activation of many intracellular signaling pathways such as PI3K, Ras, and SFC which are responsible for cell growth, proliferation, cell survival and gene expression [17-20].

Seri *et al*, have reported the synthesis of 2,4"-bis diphenylamine oxadiazole derivatives having the affinity to exhibit antiproliferative effect against EGFR dependent tumor cell line, which over express EGFR [16] .



R= -H, -CH_2-C_2H_5, C_3H_5

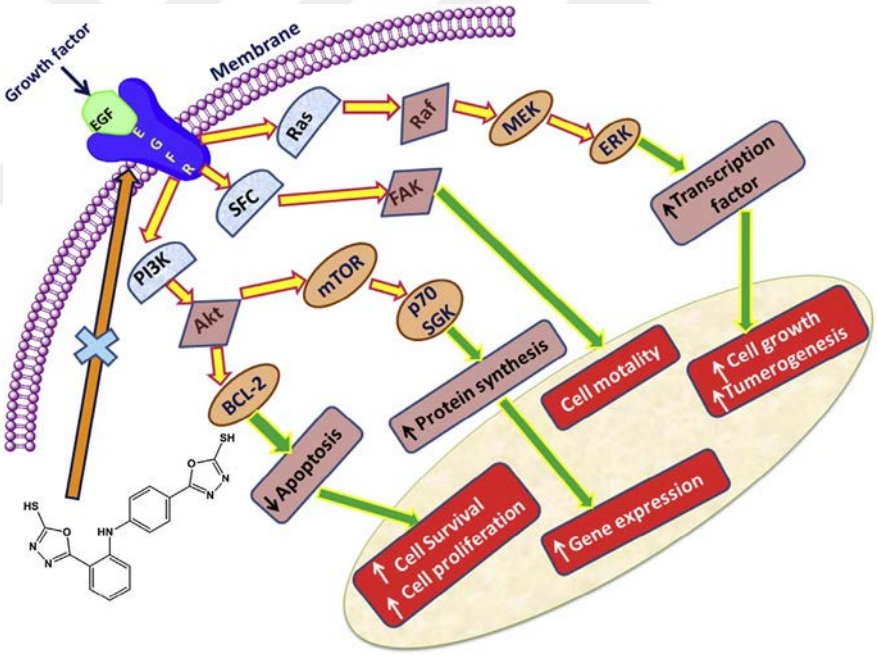
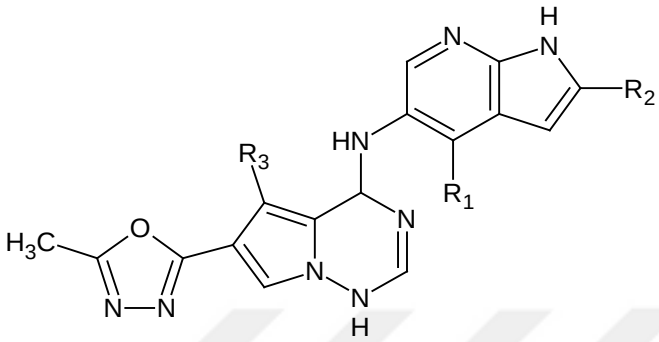


Figure 2.6. Role of epidermal growth factor in cell cancer

Vascular endothelial growth factor (VEGF) is a pro-angiogenic cytokine which responsible for angiogenesis process and this process is mediated through a family of three proteins named VEGF-1, VEGF-2, and VEGF-3 which are present on endothelial cells. The angiogenesis process is critical for tumor growth and spreading [21-24]. VEGF-2 was targeted by a series of compounds containing 1,3,4-oxadiazole as these compounds have the ability to interrupt the downstream signaling pathways of VEGF-2,

therefore inhibition of proliferation of cancerous cells [121]. Ruel *et al*, have reported the synthesis of *N*-(2,4-dimethyl-1*H*-pyrrolo[2,3-b]pyridine-5-yl)-5-methyl-6-(5-methyl-1,3,4-oxadiazol-2-yl)pyrrolo[2,1,f][1,2,4]triazin-4-amine (BMS-645737) derivatives in which substitution at R_1, R_2, and R_3 position produced compounds showed potential anticancer activity by inhibition of VEGF-2 [122].



BMS-645737

R_1 = -H, -CH_3, R_2 = -H, -F, R_3= -i-Pr

Glycogen Synthase Kinase-3 (GSK3) is a key regulator of numerous signaling pathways, involving receptor tyrosine kinase, G-protein coupled receptors and is involved in regulating many cellular processes, ranging from metabolism of glycogen to cell cycle regulation and proliferation [123-125]. Over express of this enzyme leads to increasing the number of cancer cells.

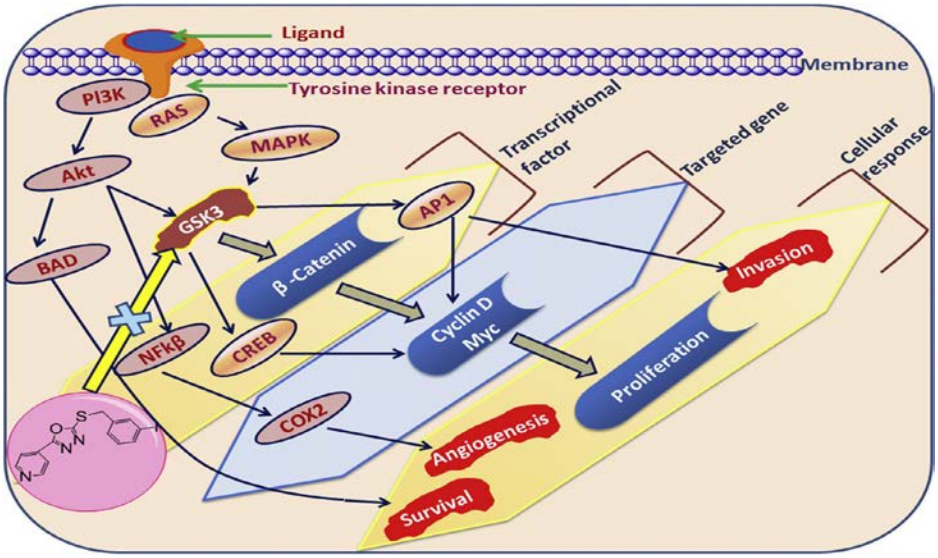
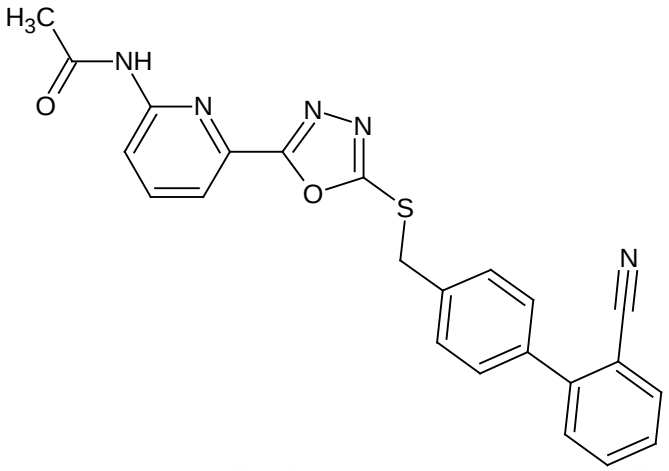


Figure 2.7. Glycogen synthase kinase-3 modulates multiple signaling pathways involved in carcinogenesis.

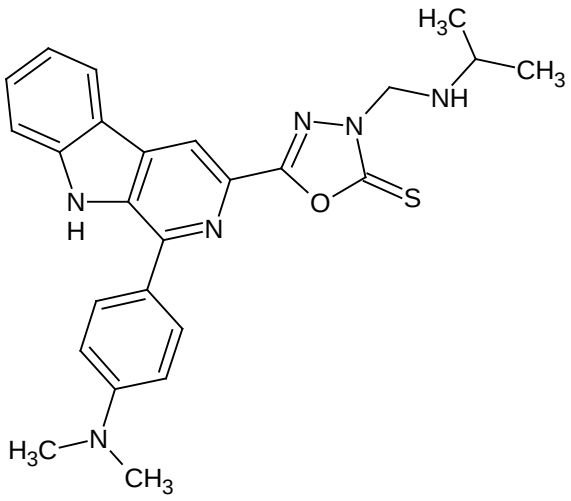
Compounds containing maleimide derivatives such as acetamide group attached to oxadiazole show selective potency against GSK-3 [126].



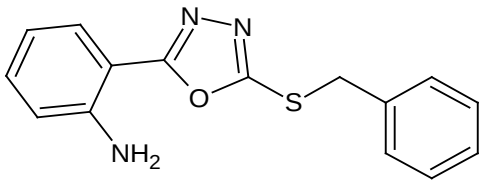
2.5. Biological Properties of 1,3,4-Oxadiazole

2.5.1. Anticancer Activity

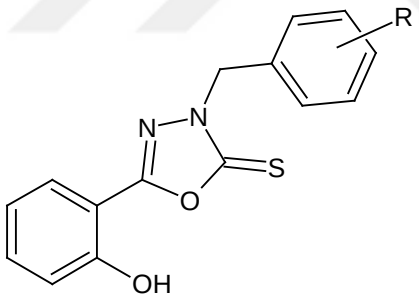
In many literature scientists focused on the preparation of new series of compounds having 1,3,4-oxadiazole ring as their main core, and screened their ability to act against various types of cancer cells such as; breast, lung, colon, and liver cells, also their potency to act as enzyme inhibitors was examined.
A new series of *Mannich* bases were examined for their antitumor activity by Savariz and coworkers. Among the tested compounds, compound given showed a potent activity against melanoma (UACC-62), and lung (NCI-460) cell lines with effective GI_{50} values of 0.88 and 1.01 mmol/L respectively [127].



Li and co- workers designed and synthesized a series of 2-(benzylthio)-5-aryloxdiazole derivatives and screened their ability to act as inhibitors for epidermal growth factor receptors. 2-[5-(Benzylsulfanyl)-1,3,4-oxadiazol-2-yl]aniline showed potential biological activity with an effective IC_{50} values against MCF-7 and EGFR 1.09 and 1.51 µM respectively [128].



Aboraia and co-workers synthesized a series of 5-(2-hydroxyphenyl)-3-substituted-2,3-dihydro-1,3,4-oxadiazole-2-thione derivatives and thirteen of these compounds were selected and evaluated by National Cancer Institute using 5-fluorouracil and cyclophosmaide as a reference. Compounds were exhibited a promising anticancer activity with less toxicity against different types of cancer cells [33,129-131].

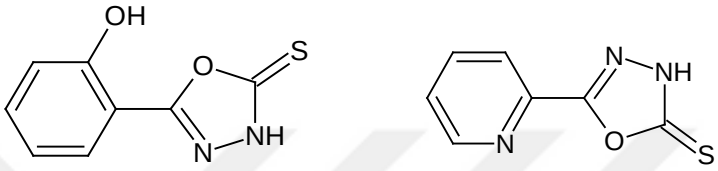


R = -Cl

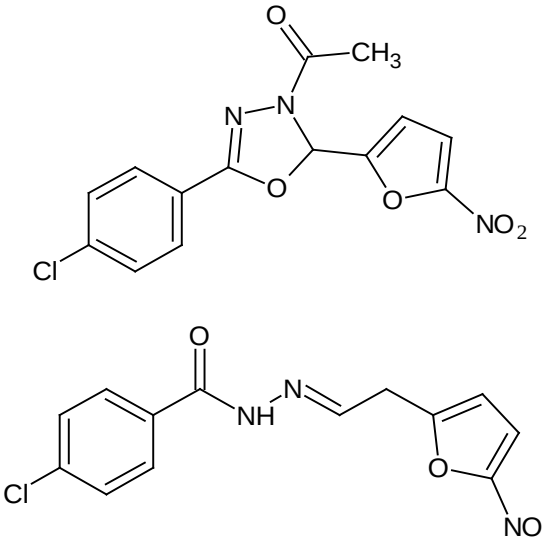
2.5.2. Antimicrobial Activity

In several literature, researchers reported the synthesis of new series of safer compounds having a potential antimicrobial activity against Gram positive and Gram negative bacteria. These compounds were in vitro investigated to diminish the growth of these bacteria at concentration that can be achieved with acceptable risks of toxicity.

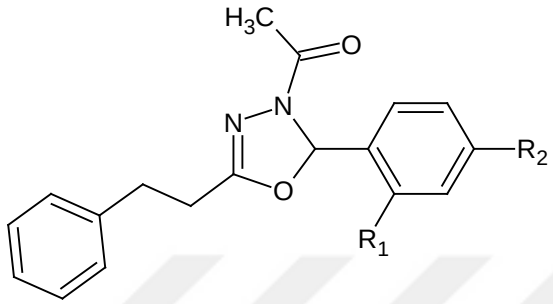
Othman *et al*, synthesized derivatives of 1,3,4-oxadiazole starting from salicylic acid and piconilic acid these compounds were screened against *E.coli, S.aureus, P.aeruginosa, E. faecalis*, for their antimicrobial activity using ampicilline, gentamycine and cephalosporine as references. According to the results 5-(2-hydroxyphenyl)1,3,4-oxadiazole-2-thione showed a moderate to slightly activity, whereas 5-(2-pyridyl)-1,3,4-oxadiazole-2-thione has relatively a lower inhibition effect on *E.coli* and *S.aureus*, but exhibited more effect on *P.aeruginosa* [132].



Oliveira and co-workers reported synthesis of antistaphylococcal activity of 1,3,4-oxadiazolines against several strains of *Staphylococcus aureus* having a multidrug resistant MDR to methicillin and aminoglycoside. Among the tested compounds 1-[5-(4-chlorophenyl)-2-(5-nitrofuran-2-yl)-1,3,4-oxadiazol-3(2*H*)-yl]-ethanone and 4-chloro-*N*'-[(*E*)-2-(5-nitrofuran-2-yl)ethenyl]benzohydrazide were showed activity against this strain of bacteria at 4 to 32 µg/mL, and this concentration making these compounds 2-8 times more active than the standard drug chloramphenicol [133].

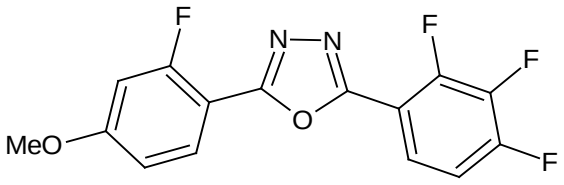


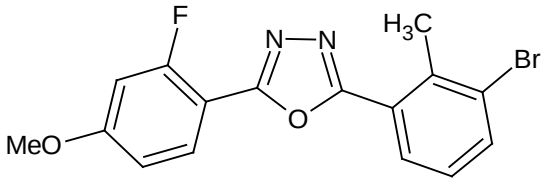
In another hand, the presence of acetyl group in the third position of 2,5-disubstituted oxadiazole compounds were examined against two strains of bacteria, *S. aureus* and *P.aeruginosa*, and against two species of fungi *C. albicans* and *A. flavus* by using disk diffusion method. Fluconazole and ampicillin were used as standards; all of the synthesized compounds were equally potent to fluconazole and ampicillin standards [134].



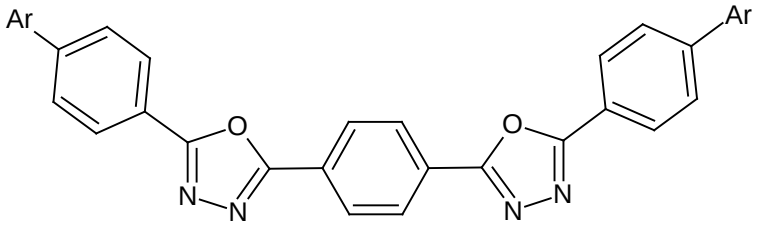
R_1= -H, R_2= -Cl

In derivatization of 2,5-disubstitutedaryl-1,3,4-oxadiazole, it is observed that compounds carrying electron withdrawing groups such as : 2-(2-fluoro-4-methoxyphenyl)-5-(2,3,4-trifluorophenyl)-1,3,4-oxadiazole 2-(3-bromo-2-methylphenyl)-5-(2-fluoro-4-methoxyphenyl)-1,3,4-oxadiazole, and had a potential antimicrobial activity against *E. coli* and *P. aerugenosa* than furacin [135].



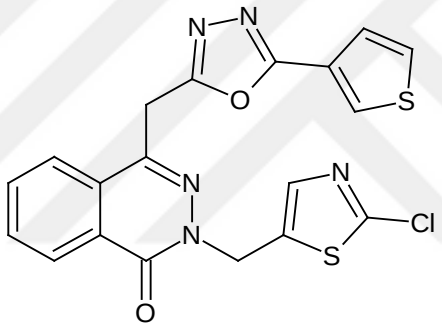


Vikrant *et al*, synthesized and screened a novel series of compounds carrying 1,3,4-oxadiazole moiety for their antibacterial activity by using the well-diffusion method. According to SAR data, compounds having electron withdrawing group (-NO_2, -Cl) exhibited potential antibacterial activity against *E. coli* and *B. cereus* than the compounds containing parallel polar groups (-OH, -NH_2) [136].



Ar = 4-Cl-C_6H_4, 4-NO_2-C_6H_4

In other studies, researchers examined phthalazine-oxadiazoles substituted at C_2 position with heterocyclic moieties, Ajjanna and coworkers investigated that phthalazine compounds with a 2-chloro-1,3-thiazol-5-ylmethyl substitutionand an oxadiazole with thiophene substitution was the most active compound against four human pathogenic bacteria [137].



Ramaprasad *et al*, designed and synthesized a series of biphenyl-1,3,4-oxadiazoles. Synthesized compounds were investigated for their antibacterial activities and among the tested compounds, the fluoro substituted compound has the highest activity against Gram-positive and Gram-negative bacteria [138].

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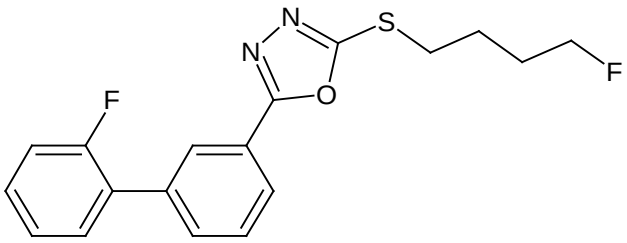
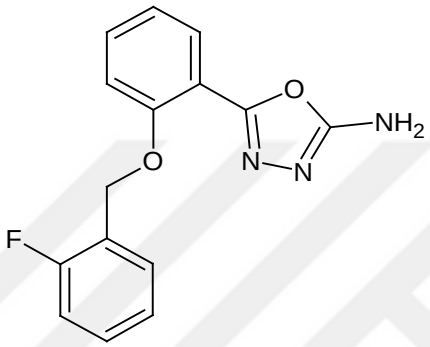


2.5.3. Anticonvulsant Activity

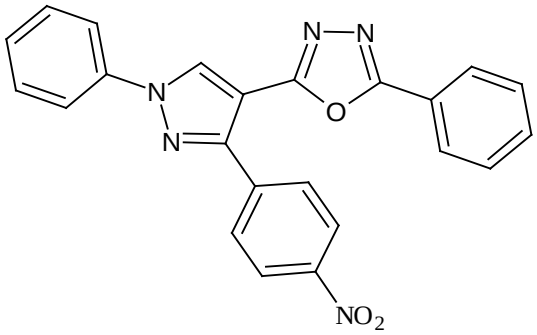
Zarghi *et al*, were synthesized and screened a new series of 2-substituted-5-[2-(2-halobenzyloxy)phenyl]-1,3,4-oxadiazoles for their anticonvulsant activity to protect mice against convulsion effect produced by a lethal dose of pentylenetetrazole and electroshock as two routine models. The best anticonvulsant activity of synthesized compounds was observed through the presence of amino group on the second position of oxadiazole ring and fluoro substituent on *ortho* position of benzyloxy group [139].



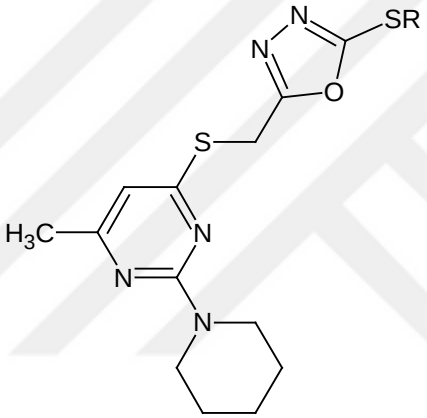
2.5.4. Anti-inflammatory Activity

A series of novel compounds carried 1,3,4-oxadiazole ring system were examined for their anti-inflammatory activity. The anti-inflammatory activity mechanism of 1,3,4-oxadiazole have been recognized by their ability to inhibit cyclooxygenase enzymes which are responsible for synthesis of prostaglandins, a well-known mediator for pain, swelling, and inflammation [140].

In many studies, some of 2,5-disubstituted-1,3,4-oxadiazoles show selectivity towards inhibition of (COX2) than (COX1), especially when an electron withdrawing group present in the structure without producing side effects such as gastric ulceration, whereas most of NSAID such as aspirin produce this side effect [141-144].

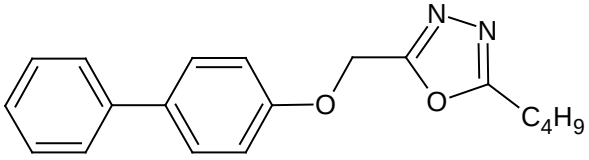


Burbuliene and co-workers investigated the anti-inflammatory activity of 5-[(2-disubstituteddiamino-6-methyl-pyrimidin-4-yl)sulphanylmethyl]-3*H*-1,3,4-oxadiazol-2-thione derivatives and found that some of these compounds have more potent anti-inflammatory activity than ibuprofen [145].

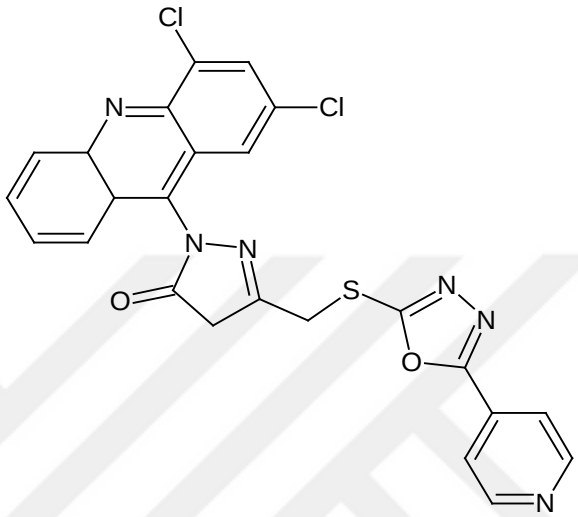


R= Alkyl derivatives

Kumar *et al*, synthesized a new series of compounds containing 1,3,4-oxadiazole structure and examined them in order to obtain new compounds with potential anti-inflammatory activity. All of the synthesized compounds were tested for their anti-inflammatory activity by the carrageenan induced rat paw edema test method. Out of all tested compounds, the substitution of oxadiazole ring with butyl group showed the more potent anti-inflammatory activity (81.18%) than the reference drug (79.54), low ulcerogenic potential, and protective action on lipid peroxidation [146].



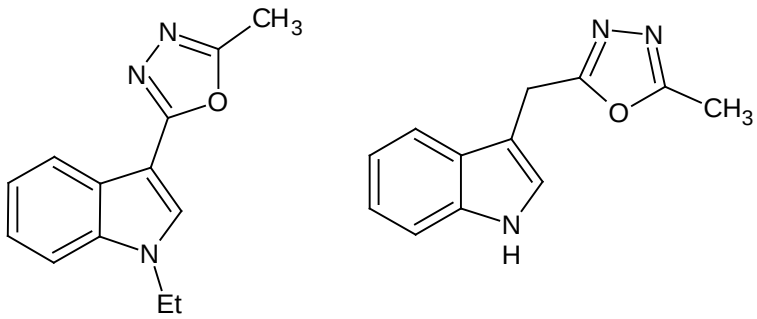
Chandra and co-workers designed compounds containing 1,3,4-oxadiazole moieties derived from acridines and screened for their anti-inflammatory activity by using albino rats of the Charles-Foster strain of either sex ,which was substituted with 2,4-dichloro at acridinyl pyrazoline ring exhibited potent anti-inflammatory activity around 26.4, 40.8, and 60.5% at three different concentrations against carrageenan induced oedema [147].



In another literature, replacing the carboxylic acid moiety that present in the (NSAID) diclofenac produced a remarkable anti-inflammatory activity in carrageenan-induced rat pew edema (CPE) test .However, the gastro intestinal damage increased [148].

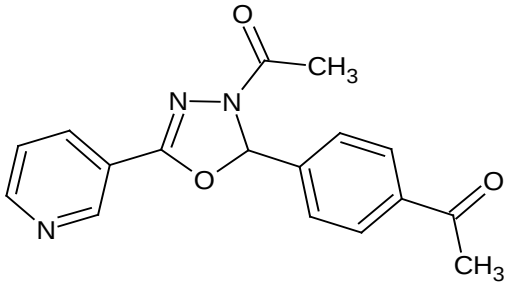
2.5.5. Antifungal Activity

Zhang *et al*, reported the principle of combination of active structural units by modification of indole ring system through synthesizing a three series of indole-based 1,3,4-oxadiazole derivatives to act as an alternative of naturally occurring antifungal compound primprinine. According to this study, the antifungal activity of synthesized compounds was improved by substituting an ethyl group onto the indole ring nitrogen, or by adding a methylene bridge between the indole and oxadiazole ring [57].



2.5.6. Antihypertensive Activity

In many literature, researchers focused on the synthesis of compounds having the ability to act against hypertension. Oxadiazole derivatives show selective potential activity to act as L-type calcium channel blockers compared to ordinary used drug diltiazem [149-150]. Bankar *et al*, synthesized and examined a series of oxadiazoles for their ability to have an effect on the vascular smooth muscles and calcium influx in rat aorta. 4-[3-Acetyl-5-(pyridine-3-yl)-2,3-dihydro-1,3,4-oxadiazole-2-yl]phenylacetate(NOX-1) in this study showed an activity to induce vasorelaxant effect and have antagonist effect on L-type calcium channels [151].

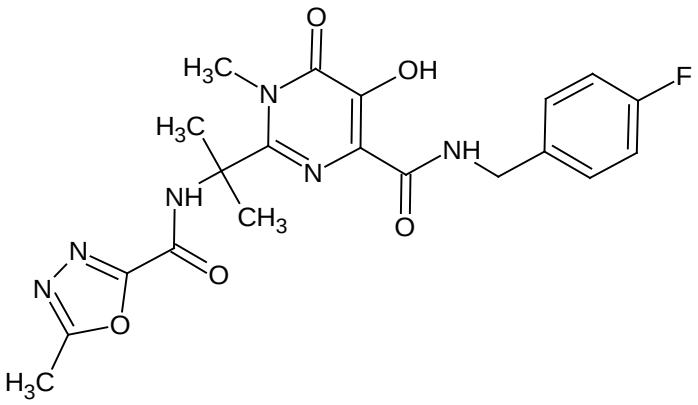


2.5.7. Antiviral Activity

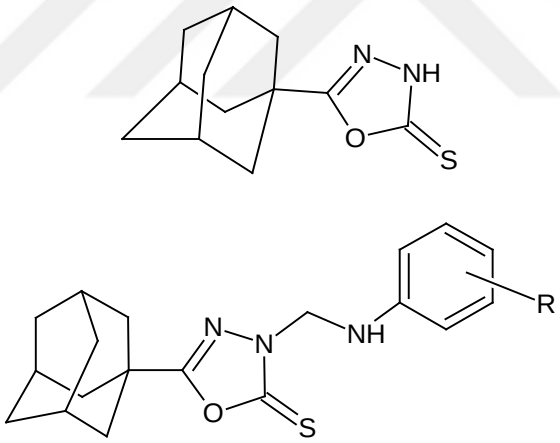
There are lots of drugs which are in clinical use against various types of viruses such as adamantine derivatives that have been widely used as antiviral agent acting against Influenza A, and HIV viruses [152-154].

Raltegravir is one of the most common drugs known as integrase inhibitor that is used for treatment of human immunodeficiency virus (HIV)-1 infection; 1,3,4-oxadiazole containing drug was approved by US Food and Drug Administration in 16th

of October 2007 to be administered in combination with other antiretroviral agents for treatment of adult patients having an evidence of viral replication [155].

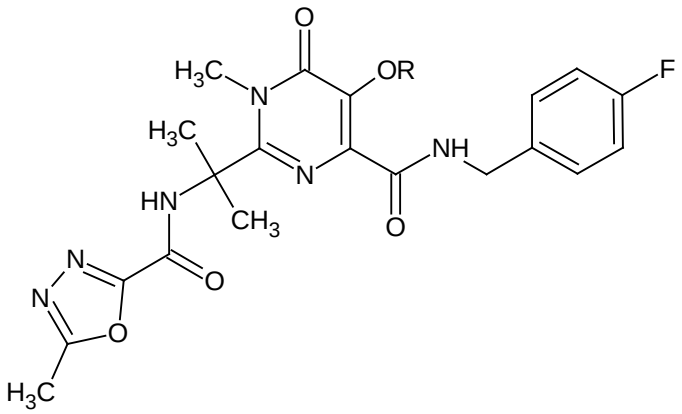


El-Emam and coworkers synthesized a novel series of compounds having 1,3,4-oxadiazole moiety derived from adamantone-1-carbohydrazide, 5-(1-adamantyl)-1,3,4-oxadiazoline-2-thione showed a potential antiviral activity against HIV-1 using the XXT assay on MT-4 cells, by producing 100%, 43%, and 37% reduction of viral replication [156].



R= -H, 2-F, 4-Cl, 2-Cl, 2-Br, 4-Br, 3-NO_2, 4-NO_2, 4-OCH_3, 2-CN,2-CF_3

Many studies have been focused on developing raltegravir derivatives to be more effective agents against (HIV) virus. For this reason Wang and co-workers prepared and systemically evaluated a series of raltegravir for their anit-HIV activities. The compound given seemed to be one of the most potent compound in this study having potential anti-HIV activity [157].



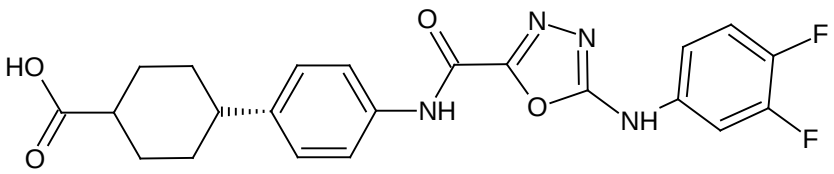
R= $-CH_2-C_6H_5$

The 5-hydroxyl group modification of raltegravir derivatives enhanced the HIV activity, which indicated that the hydroxyl group may be indispensable for raltegravir.

2.5.8. Enzyme Inhibitors

Nowadays treatments of other diseases especially as obesity, and diabetes, which constitute metabolic syndrome become very significant [158].

McCoull and co-workers focused on finding the suitable drugs to act as inhibitors for diacylglycerol acyl transferase-1 enzyme (DGAT-1) based on the optimization of oxadiazole amide inhibitors, the clinical candidate **53(AZD3988)** demonstrates excellent DGAT-1 potency [159].



53 (AZD3988)

3. MATERIALS and METHODS

3.1. Chemistry

3.1.1. Materials

In this work, the used salicylic acid, 3-(3-methylpiperidine), 3-(4-hydroxymethylpiperidine), 3-(4-phenylpiperidine), 3-(4-hydroxy-4-phenylpiperidine), 3-(4-acetyl-4-phenylpiperidine), 3-(4-cyanophenylpiperidine), 3-(4-phenylpiperazine), 3-(4-(2-pyridyl)piperazine were purchased from Sigma-Aldrich.

3.1.2. Methods of Synthesis

3.1.2.1. Synthesis of Methyl Salicylate

A solution of 2-hydroxybenzoic acid (salicylic acid) (1g), methanol (10 ml), and catalytic amount of conc (H_2SO_4) are refluxed for 3 hour. The reaction mixture is cooled and the solid formed is filtered to get ester, after cooling the solution at room temperature, 3 ml of (CH_2Cl_2) dichloromethane is added to the reaction mixture then suspension is shaked. The layer is allowed to separate and the organic layer is collected which contains the product and the process is repeated for three times, 3 ml of an aqueous sodium bicarbonate ($NaHCO_3$) is added to combine CH_2Cl_2. After shaking and separating, the organic layer is transferred to another vial, dried with anhydrous sodium sulfate, the CH_2Cl_2 is evaporated with a gentle stream of compressed air [160].

3.1.2.2. Synthesis Method of Salicyloyl Hydrazide

The mixture of methyl ester of salicylic acid (0.1 mol) and hydrazine hydrate (0.2 mol) is refluxed in absolute alcohol (50 ml) for 8 hour. The excess solvent is distilled off under reduced pressure and the concentrated solution is quenched into ice cold water. The solid separated is filtered, washed and dried; the crude product is purified by recrystallization from ethanol [161].

3.1.2.3. Synthesis of 5-(2-hydroxyphenyl)-1, 3, 4-oxadiazole-2-thione

A mixture of salicylic acid hydrazide (0.05 mol, 7.5 g), potassium hydroxide (0.05 mol, 3 g), carbon disulfide (10 ml, 0.17 mol), and ethanol (70 ml) is heated under reflux with stirring until the evolution of hydrogen sulfide ceased (~ 12 h). Ethanol is distilled off under reduced pressure and the residue is dissolved in water and then acidified with dilute hydrochloric acid (10%). The resulting precipitate is filtered, washed with water, dried, and recrystallized from ethanol [162].

3.1.2.4. Synthesis of 5-(2-hydroxyphenyl)-3-substituted-1,3,4-oxadiazole-2-thione

Formalin 40% (1.5 ml, 0.02 mol) is added to a stirred solution of 5-(2-hydroxy phenyl)1,3,4-oxadiazole-2-thione (4 g, 0.02 mol) in absolute ethanol (40 ml). An ethanolic solution (10 ml) of the appropriate amine (0.02 mol) is added portion wise to the reaction mixture, stirred for 3 hours at room temperature, and left overnight in a refrigerator. The precipitate formed is filtered, washed with cold ethanol, dried, and crystallized from the suitable solvent [33]. If necessary HCl for oily compounds were prepared.

3.2. Analytical Methods

3.2.1. Melting Point Determination

Melting points (ºC) of the compounds were determined by using Metttler Toledo FP62 capillary melting point apparatus

3.2.2. Controls by Thin Layer Chromatography

Materials:

Plates: TLC aluminium sheets 60 × 60 cm Silica gel 60 F_{254} (Merck)**.**

Solvent systems: Three different solvent systems were prepared to be used in chromatographic controls of compounds.

S-1: Benzene-Methanol	(80:20)
S-2: Benzen-Ethanol	(40:10)
S-3: Toluene-Ethyl acetate-Diethylamine	(75:25:10)

Dragging condition: Solvent systems were poured to chambers and waited for 24 hours to saturation.

Synthesized compounds and their starting materials dissolved in suitable solvents were applied to thin layer chromatography (TLC) plates and waited to drag 10 cm at room temperature. Rf values of compounds were determined.

Stain determination Stains of the synthesized compounds and their starting materials were determined by UV light (254/365 nm).

3.2.3. Spectrometric Analyses

3.2.3.1. Infrared Spectra

Infrared spectra were recorded on a Perkin-Elmer Spectrum One series FT-IR apparatus (Version 5.0.1), using potassium bromide pellets, the frequencies were expressed cm^{-1}.

3.2.3.2. ^{1}H-NMR Spectra

The ^{1}H-NMR spectra were recorded with a Varian Mercury-400 FT-NMR spectrometer (Varian Inc., Palo Alto, CA, USA), using tetramethylsilan (TMS) as the internal reference, with dimethylsulfuoxide (DMSO) as solvent, the chemical shifts were reported in parts per million (ppm).

3.2.3.3. ^{13}C-NMR Spectra

The ^{13}C-NMR spectra of compounds were recorded with a Varian Mercury-400 FT-NMR spectrometer (Varian Inc., Palo Alto, CA, USA).

3.2.3.4. Elemental Analyses

Elemental analyses were performed on LECO 932 CHNS (Leco-932, St. Joseph, MI, USA) instrument.

3.3. Biological Activity

3.3.1. Xcelligence System

Xcelligence system consists of microelectronic biosensor system which provides cellular analysis. The structuring of the Xcelligence system uses microtiter plates containing microelectrode to monitor the viability of cultured cells through the use of electrical impedance.

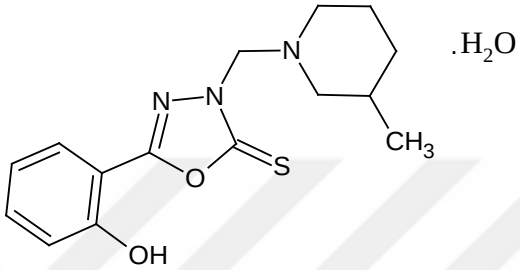
The RTCA SP station and the E-plate are both inserted into a standard cell culture incubator which controls the temperature, humidity and CO_2 levels throughout the experiment. The control unit of the RTCA receives the data measured by the Analyzer and utilizes RTCA for the analysis and real time display of each experiment. The Analyzer can automatically select wells for measurement and transfer the data to the computer. Cell index values based on the measurement are available for the software user to seen on display.

The human breast adenocarcinoma cells (MCF7) was purchased from ATCC (HTB-22). For cell culture, Dulbecco's Modified Eagle's Medium, supplemented with 10% (v/v) heat inactivated fetal bovine serum and 100 U/ml penicillin and 100 µg/ml streptomycine was used. Media and supplements were obtained from Sigma. Cells were cultivated at 37 °C, under humidified conditions with 5% CO_2 and were routinely tested for any contamination. 5000cells/well were seeded to E-plates and followed up for around 80 h. All the tested compounds were dissolved in dimethylsulfoxide (DMSO) and added to the incubation media, resulting in the final concentrations of the tested compounds and 1% (v/v) DMSO [163].

4. RESULTS

4.1. Chemical data

5-(2-Hydroxyphenyl)-3-[(3-methylpiperidin-1-yl)methyl]-1,3,4-oxadiazole-2(3*H*)-thione (Compound 5a)



5-(2-Hydroxyphenyl)-1,3,4-oxadiazole-2(3*H*)-thione (1.28 mmol, 250 mg), 3-methylpiperidine (1.05 mmol, 130 mg) and formaldehyde (1.05 mmol, 30 μml) in ethanol were reacted according to synthesis method at 3.1.2.4. The yield is (31%).

This compound forms white, opaque, powder, and has a melting point more than 300 °C. It is soluable in ethanol, methanol and dimethylsulfoxide at room temperature. Rf values in TLC at S-1, S-2 and S-3 solvent systems are 0.77, 0.63 and 0.74, respectively.

UV (MeOH, λ_{max}), 207 (log ε: 4.93), 318 (log ε: 4.65).

FT-IR (KBr, cm^{-1}) , 3473 (OH), 2950 (C-H aromatic), 2811 (C-H aliphatic), 1625 (C=N), 1572 (C=C),1490 (C=S), 1235 (C-N), 1150 (C-O).

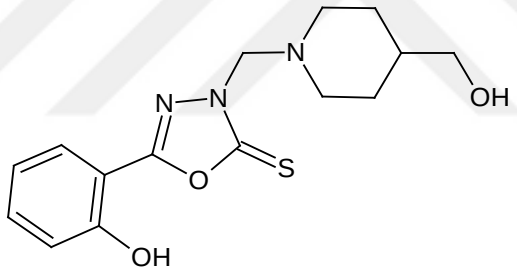
^{1}HNMR (DMSO, ppm); 10.54 (bs, 1H, OH), 7.62 (dd, 1H, 2-hydroxyphenyl H_3, *J*= 8 Hz, *J'*= 2), 7.4-7.40 (m, 1H, 2-hydroxyphenyl H_4), 7.02 (d, 1H, 2-hydroxyphenyl H_6, *J*= 8 Hz), 6.96 (t, 1H, 2-hydroxyphenyl H_5, *J*= 7.6 Hz), 5.04 (s, 2H, N-CH_2-N), 2.96 (d, 2H, piperidine H_2, *J*= 10.8 Hz), 2.39 (t, 2H, piperidine H_6, *J*= 12 Hz), 2.08(t, 1H, piperidine H_3, *J*= 10.8 Hz), 1.59-1.40 (m, 4H, H_4+H_5), 0.80 (d, 3H, -CH_3, *J*= 6.4 Hz).

^{13}C-NMR (DMSO, pm); 177.01 (oxadiazole C=S), 157.98 (oxadiazole C_5), 156.28 (2-hydroxyphenyl C_2), 133.40 (2-hydroxyphenyl C_1), 128.79 (2-hydroxyphenyl C_3), 119.40 (2-hydroxyphenyl C_6), 116.94 (2-hydroxyphenyl C_4), 108.99 (2-hydroxyphenyl C_5), 70.49 (N-CH_2-N), 49.96 (piperidine, C_2+C_6), 40.05 (piperidine, C_4), 37.46 (piperidine, C_3+C_5).

Elemental analysis of $C_{15}H_{19}N_3O_2S.H_2O$ (MW : 323.39)

	% C	% H	% N	% S
Calculated	55.71	6.54	12.99	9.91
Found	55.77	5.83	13.01	9.90

5-(2-Hydroxyphenyl)-3-[(4-hydroxymethylpiperidin-1-yl)methyl]-1,3,4-oxadiazole-2(3*H*)-thione (Compound 5b)



5-(2-Hydroxyphenyl)-1,3,4-oxadiazole-2(3*H*)-thione (1.28 mmol, 250 mg), 4-hydroxymethylpiperidine (1.05 mmol, 3 mg) and formaldehyde (1.05 mmol, 30 µml) in ethanol were reacted according to synthesis method at 3.1.2.4. The yield is (72%).

This compound forms white, opaque, powder, and has a melting point more of 169.1 °C. It is soluable in ethanol and methanol in hot medium and dimethylsulfoxide at room temperature. Rf values in TLC at S-1, S-2 and S-3 solvent systems are 0.84, 0.59, and 0.67, respectively.

UV (MeOH, λ_{max}) ; 206 (log ε: 5.46), 318 (log ε: 5.30).

FT-IR (KBr, cm^{-1} , 3469 (OH), 2938 (C-H aromatic), 2810 (C-H aliphatic), 1610(C=N), 1573 (C=C), 1436 (C=S), 1254 (C-N), 1115 (C-O).

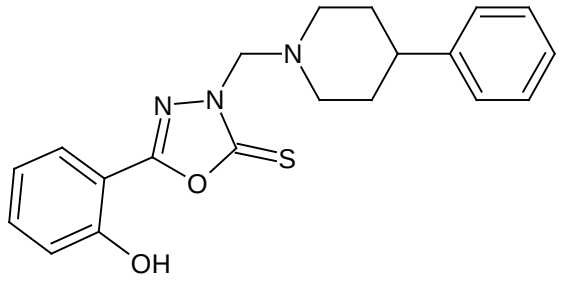
^{1}HNMR (DMSO, ppm); 10.55 (bs, 1H, OH), 7.62 (dd, 1H, 2-hydroxyphenyl H_3, *J*= 7.8 Hz , *J'*= 1.6), 7.43-7.39 (m, 1H, 2-hydroxyphenyl H_4), 7.02 (d, 1H, 2-hydroxyphenyl H_6, *J*= 8.4 Hz), 6.98-6.94 (m, 1H, 2-hydroxyphenyl H_5), 5.00 (s, 2H, N-CH_2-N), 4.39 (t, 1H, OH), 3.21 (t, 2H, piperidine H_2, *J*= 5.6 Hz), 3.02 (d, 2H, -CH_2OH, *J*= 11.2 Hz), 2.42 (t, 2H, piperidine H_6, *J*= 11.6 Hz), 1.61 (d, 2H, piperidine H_3, *J*= 11.2 Hz), 1.26-1.21 (m, 1H, piperidine H_4), 1.14-1.01 (m, 2H, piperidine H_5).

^{13}C-NMR (DMSO, pm); 177.03 (oxadiazole C=S), 157.35 (oxadiazole C_5), 156.35 (2-hydroxyphenyl C_2), 133.51 (2-hydroxyphenyl C_1), 128.39 (2-hydroxyphenyl C_3), 119.45 (2-hydroxyphenyl C_6), 117.02 (2-hydroxyphenyl C_4), 109.04 (2-hydroxyphenyl C_5), 70.56 (N-CH_2-N), 50.03 (piperidine, C_2+C_6), 37.53 (piperidine, C_4), 28.60 (piperidine, C_3+C_5).

Elemental analysis of $C_{15}H_{19}N_3O_3S$ (MW : 321.39)

	% C	% H	% N	% S
Calculated	56.06	5.96	13.07	9.98
Found	55.98	6.29	13.02	9.90

5-(2-Hydroxyphenyl)-3-[(4-phenylpiperidin-1-yl)methyl]-1,3,4-oxadiazole-2(3*H*)-thione (Compound 5c)



5-(2-Hydroxyphenyl)-1,3,4-oxadiazole-2(3*H*)-thione (1.05 mmol, 2 mg), 4-phenylpiperidine (1.05 mmol, 169 mg) and formaldehyde (1.05 mmol, 30 µml) in ethanol were reacted according to synthesis method at 3.1.2.4. The yield is (43%).

This compound forms white powder, and has a melting point of 166.2 °C. It is soluble in ethanol, methanol, and dimethylsulfoxide at room temperature. Rf values in TLC at S-1, S-2 and S-3 solvent systems are 0.86, 0.57, and 0.91, respectively.

UV (MeOH, λ_{max}) ; 207 (log ε: 5.23), 318 (log ε: 4.96).

FT-IR (KBr, cm^{-1}); 3369 (OH), 2959 (C-H aromatic), 2817 (C-H aliphatic), 1625 (C=N), 1592 (C=C), 1489 (C=S), 1253 (C-N), 1121 (C-O).

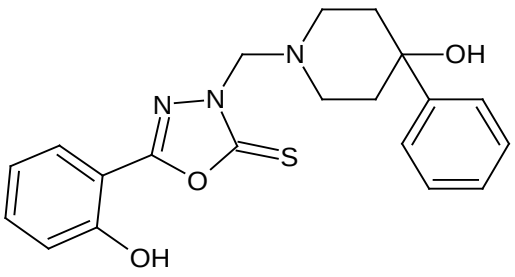
^{1}H NMR (DMSO, ppm); 10.52 (bs, 1H, OH), 7.66 (dd, 1H, 2-hydroxyphenyl H_3, *J*= 8 Hz, *J'*=2), 7.46-7.41 (m, 1H, 2-hydroxyphenyl H_4), 7.25-7.13 (m, 5H, phenyl), 7.04 (dd, 1H, 2-hydroxyphenyl H_6, *J*= 8.4 Hz, *J'*= 0.8 Hz), 6.99-6.95 (m, 1H, 2-hydroxyphenyl H_5), 5.07 (s, 2H, N-CH_2-N), 3.14 (d, 2H, piperidine H_2, *J*= 12 Hz), 2.60 (t, 2H, piperidine H_3, *J*= 10.4 Hz), 2.46-2.40 (m, 1H, piperidine H_4), 1.73 (d, 2H, piperidine H_6, *J*= 10.8 Hz), 1.67-1.58 (m, 2H, piperidine H_5).

^{13}C-NMR (DMSO, ppm); 176.96 (oxadiazole C=S), 157.85 (oxadiazole C_5), 156.28 (2-hydroxyphenyl C_2), 145.98 (phenyl C_1), 133.57 (2-hydroxyphenyl C_1), 129.02 (2-hydroxyphenyl C_3), 128.22 (phenyl C_3+C_5), 126.57 (phenyl C_2+C_6), 125.92 (phenyl C_4), 119.41 (2-hydroxyphenyl C_6), 117.00 (2-hydroxyphenyl C_4), 108.96 (2-hydroxyphenyl C_5), 70.33 (N-CH_2-N), 50.57 (piperidine $C_{2+}C_6$), 40.87 (piperidine C_4), 32.84 (piperidine C_3+C_5).

Elemental analysis of $C_{20}H_{21}N_3O_2S$. (MW: 367.46)

	% C	% H	% N	% S
Calculated	65.37	5.76	11.44	8.73
Found	64.64	5.49	11.44	8.73

5-(2-Hydroxyphenyl)-3-[(4-hydroxy-4-phenylpiperidin-1-yl)methyl]-1,3,4-oxadiazole-2(3*H*)-thione (Compound 5d)



. HCl

5-(2-Hydroxyphenyl)-1,3,4-oxadiazole-2(3*H*)-thione (1.54 mmol, 3 mg), 4-hydroxy-4-phenylpiperidine (1.54 mmol, 273 mg) and formaldehyde (1.05 mmol, 30 μml) in ethanol were reacted according to synthesis method at 3.1.2.4. The yield is (42%).

This compound forms white, opaque, powder, and has a melting point of 251.1 °C. It is soluable in ethanol, methanol and dimethylsulfoxide at room temperature. Rf values in TLC at S-1, S-2 and S-3 solvent systems are 0.85, 0,57, and 0.73, respectively.

UV (MeOH, λ_{max}) ; 270 (log ε: 4.57).

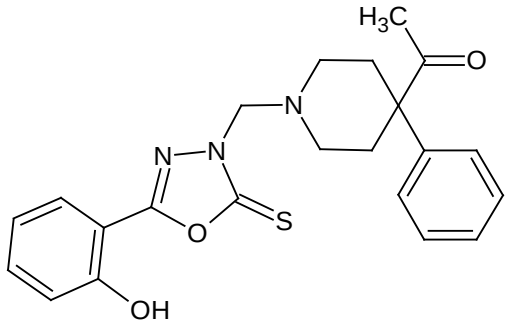
FT-IR (KBr, cm^{-1}); 3335 (OH), 2954 (C-H aromatic), 2829(C-H aliphatic), 1625 (C=N), 1591 (C=C), 1489 (C=S), 1252 (C-N), 1145 (C-O).

^{1}H NMR (DMSO, ppm); 9.08 (bs, 2H, OH), 7.46 (d, 1H, 2-hydroxyphenyl H_3, *J*=2), (m, 1H, 2-hydroxyphenyl H_4), 7.48-7.24 (m, 5H, aromatic Hs), 5.45 (s, 2H, N-CH_2-N), 3.22 (d, 2H, piperidine H_2, *J*=7.6 Hz), 2.50 (t, 2H, piperidine H_3, *J*= 3.8 Hz), 2.32-2.20 (m, 1H, piperidine H_4), 1.74 (d, 2H, piperidine H_6, *J*= 6.4 Hz).

Elemental analysis of $C_{20}H_{21}N_3O_3S$. HCl (MW:420.46)

	% C	% H	% N	% S
Calculated	62.64	5.52	10.96	8.36
Found	62.71	5.78	10.45	8.41

5-(2-Hydroxyphenyl)-3-[(4-acetyl-4-phenylpiperidin-1-yl)methyl]-1,3,4-oxadiazole-2(3*H*)-thione (Compound 5e)



. HCl

5-(2-Hydroxyphenyl)-1,3,4-oxadiazole-2(3*H*)-thione (1.54 mmol, 3 mg), 4-acetyl-4-phenylpiperidine (1.54 mmol, 369 mg) and formaldehyde (1.05 mmol, 30 µml) in ethanol were reacted according to synthesis method at 3.1.2.4. The yield is (53%).

This compound forms white, opaque, powder, and has a melting point of 154.7°C. It is soluble in ethanol, methanol and dimethylsulfoxide at room temperature. Rf values in TLC at S-1, S-2 and S-3 solvent systems are 0.89, 0.64, and 0.60, respectively.

UV (MeOH, λ_{max}) ; 315 (log ε: 5.35), 293 (log ε: 5.24), 252 (log ε:5.05).

FT-IR (KBr, cm^{-1}); 3372 (OH), 2963 (C-H aromatic), 2830 (C-H aliphatic), 1799 (C=O), 1630 (C=N), 1596 (C=C), 1490 (C=S), 1259(C-N), 1151 (C-O).

^{1}H NMR (DMSO, ppm); 10.56 (bs, 1H, OH), 7.61 (dd, 1H, 2-hydroxyphenyl H_3, *J*= 8 Hz, *J'*= 1. Hz 6), 7.56-7.43 (m, 1H, 2-hydroxyphenyl H_4), 7.47-7.24 (m, 5H, phenyl), 7.06 (d, 1H, 2-hydroxyphenyl H_6, *J*= 8 Hz), 6.99-6.96 (m, 1H, 2-hydroxyphenyl H_5), 5.00 (s, 2H, N-CH_2-N), 2.94-2.91 (m, 2H, piperidine H_2), 2.64 (t, 2H, piperidine H_3, *J*= 9.6 Hz), 2.43 (d, 2H, piperidine H_6, *J*= 14 Hz), 1.99-1.93 (m, 2H, piperidine H_5), 1.84 (s, 3H, CH_3)

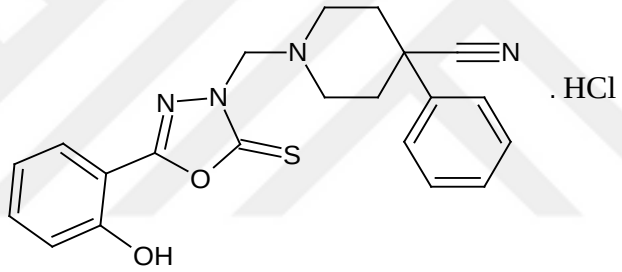
^{13}C-NMR (DMSO, ppm); 208.91 (C=O), 176.92 (oxadiazole C=S), 157.83 (oxadiazole C_5), 156.36 (2-hydroxyphenyl C_2), 141.26 (phenyl C_1), 133.63 (2-hydroxyphenyl C_1), 129.02 (2-hydroxyphenyl C_3), 128.75 (phenyl C_3+C_5), 126.97 (phenyl C_2+C_6), 126.22 (phenyl C_4), 119.40 (2-hydroxyphenyl C_6), 117.03 (2-

hydroxyphenyl C_4), 108.86 (2-hydroxyphenyl C_5), 69.98 (N-CH_2-N), 53.50 (piperidine, $C_{2+}C_6$), 47.40 (piperidine C_4), 32.11 (piperidine C_3+C_5), 25.47 (CH_3).

Elemental analysis of $C_{22}H_{23}N_3O_3S$.HCl (MW: 446.501)

	% C	% H	% N	% S
Calculated	64.53	5.66	10.26	7.83
Found	64.26	5.83	10.45	7.95

5-(2-Hydroxyphenyl)-3-[(4-cyano-4-phenylpiperidin-1-yl)methyl]-1,3,4-oxadiazole-2(3*H*)-thione (Compound 5f)



5-(2-Hydroxyphenyl)-1,3,4-oxadiazole-2(3*H*)-thione (1.54 mmol, 3 mg), 4-cyano-4-phenylpiperidine (1.54 mmol, 343 mg) and formaldehyde (1.05 mmol, 30 µml) in ethanol were reacted according to synthesis method at 3.1.2.4. The yield is (52%).

This compound forms white, opaque, powder, and has a melting point of 183.8 °C. It is soluble in ethanol, methanol, and dimethylsulfoxide at room temperature. Rf values in TLC at S-1, S-2 and S-3 solvent systems are 0.92, 0.67, and 0.67 ,respectively.

UV (MeOH, λ_{max}) ; 208(log ε: 5.36), 315 (log ε: 5.08), 293 (log ε:4.92).

FT-IR (KBr, cm^{-1}); 3363 (OH), 2952 (C-H aromatic), 2832 (C-H aliphatic), 2240 (C≡N), 1625 (C=N), 1597 (C=C), 1490 (C=S), 1253 (C-N), 1122 (C-O).

^{1}H NMR (DMSO, ppm); 10.61 (bs, 1H, OH), 7.65 (dd, 1H, 2-hydroxyphenyl H_3, *J*= 7.6 Hz , *J'*=1.6 Hz), 7.54-7.35 (m, 5H, phenyl) 7.09 (d, 1H, 2-hydroxyphenyl

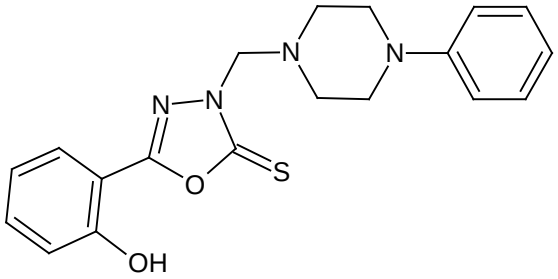
H_6, *J*= 8.4 Hz), 7.00 (t, 1H, 2-hydroxyphenyl H_5, *J*= 7.6 Hz), 5.13 (s, 2H, N-CH_2-N), 3.25 (d, 2H, piperidine H_2, *J*= 12.4 Hz), 2.85-2.83 (m, 2H, piperidine H_3), 2.15 (d, 2H, piperidine H_6, *J*= 12.8 Hz), 2.06-1.99 (m, 2H, piperidine H_5).

^{13}C-NMR (DMSO, ppm); 176.99 (oxadiazole C=S), 157.98 (oxadiazole C_5), 156.46 (2-hydroxyphenyl C_2), 139.92 (phenyl C_1), 133.74 (2-hydroxyphenyl C_1), 129.07 (C=N), 128.93 (phenyl C_3+C_5), 128.00 (2-hydroxyphenyl C_3), 125.52 (phenyl C_2+C_6), 121.60 (phenyl C_4), 119.43 (2-hydroxyphenyl C_6), 117.06 (2-hydroxyphenyl C_4), 108.81 (2-hydroxyphenyl C_5), 69.62 (N-CH_2-N), 47.52 (piperidine C_2+C_6), 41.29 (piperidine C_4), 35.12 (piperidine C_3+C_5).

Elemental analysis of $C_{20}H_{21}N_3O_2S$. HCl (MW: 429.47)

	% C	% H	% N	% S
Calculated	64.27	5.14	14.28	8.17
Found	63.86	4.86	14.37	8.28

5-(2-Hydroxyphenyl)-3-[(4-phenylpiperazin-1-yl)methyl]-1,3,4-oxadiazole-2(3*H*)-thione (Compound 5g) [33]

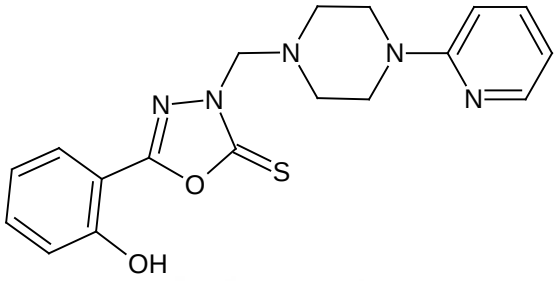


5-(2-Hydroxyphenyl)-1,3,4-oxadiazole-2(3*H*)-thione (2 mmol, 3 mg), 4-(phenylpiprazine (2 mmol, 613 mg), and formaldehyde (1.05 mmol, 30 µl), in ethanol were reacted according to synthesis method at 3.1.2.4. The yield is (8.31%). Rf values in TLC at S-1, S-2 and S-3 solvent systems are 0.52, 0.65, and 0.17, respectively.

This compound forms white, opaque, powder, and has a melting point of 172 °C. It is soluble in ethanol, methanol, and dimethylsulfoxide at room temperature.

FT-IR (KBr, cm^{-1}); 3550-3240 (OH), 1616 (C=N), 1595 (C=C), 1438 (C=S), 1249-1186 (C-O-C).

5-(2-Hydroxyphenyl)-3-[(4-(2-pyridyl)piperazin-1-yl))methyl]-1,3,4-oxadiazole-2(3*H*)-thione (Compound 5h)



5-(2-Hydroxyphenyl)-1,3,4-oxadiazole-2(3*H*)-thione (1.54 mmol, 3 mg), 4-(2-pyridylpiprazine (1.54 mmol, 153.8 mg), and formaldehyde (1.05 mmol, 30 μml), in ethanol were reacted according to synthesis method at 3.1.2.4. The yield is (27%).

This compound forms white, opaque, powder, and has a melting point of 147.4 °C. It is soluble in ethanol, methanol, and dimethylsulfoxide at room temperature. Rf values in TLC at S-1, S-2 and S-3 solvent systems are 0.82, 0.56, and 0.59, respectively.

UV (MeOH, λ_{max}) ; 206 (log ε: 4.93), 243 (log ε: 4.80), (log ε: 4.66).

FT-IR (KBr, cm^{-1}); 3326 (OH), 2942 (C-H aromatic), 2848 (C-H aliphatic), 1625 (C=N), 1597 (C=C), 1489 (C=S), 1256 (C-N), 1169 (C-O).

^{1}H NMR (DMSO, ppm); 10.51 (bs, 1H, OH), 8.05 (dd, 1H, 2-hydroxyphenyl H_3, *J*= 4 Hz , *J'*=2 Hz), 7.63 (dd, 1H, 2-hydroxyphenyl H_6, *J*= 8 Hz, *J'*= 1.6 Hz), 7.50-7.46 (m, 1H, 2-hydroxyphenyl H_4), 7.44-7.38 (m, 1H, 2-hydroxyphenyl H_5), 7.01 (d, 1H, 2-pyridyl H_3, *J*= 8 Hz), 6.,95 (t, 1H, 2-pyridyl H_5, *J*= 7.2 Hz), 6.79 (d, 1H, 2-pyridyl H_6, *J*= 8.8 Hz), 6.60-6.57 (m, 1H, 2-pyridyl H_4) , 5.08 (s, 2H, N-CH_2-N), 3.48 (t, 4H, piperazine H_3+H_5, *J*= 4.8 Hz), 2.82 (t, 4H, piperizine H_2+H_6, *J*= 4.8 Hz).

^{13}C-NMR (DMSO, ppm); 176.98 (oxadiazole C=S), 158.77 (oxadiazole C_5),157.84 (pyridine C_3), 156.35 (2-hydroxyphenyl C_2), 147.41 (pyridine C_3), 137.38 (pyridine C_6), 133.61 (2-hydroxyphenyl C_1), 129.10 (2-hydroxyphenyl C_3), 119.46 (2-

hydroxyphenyl C_6), 117.00 (2-hydroxyphenyl C_4), 112.92 (pyridine C_5), 108.92 (2-hydroxyphenyl C_5), 107.06 (pyridine C_4), 69.64 (N-CH_2-N), 49.38 (piperazine, $C_{3+}C_5$), 44.46 (piperazine C_2+C_6).

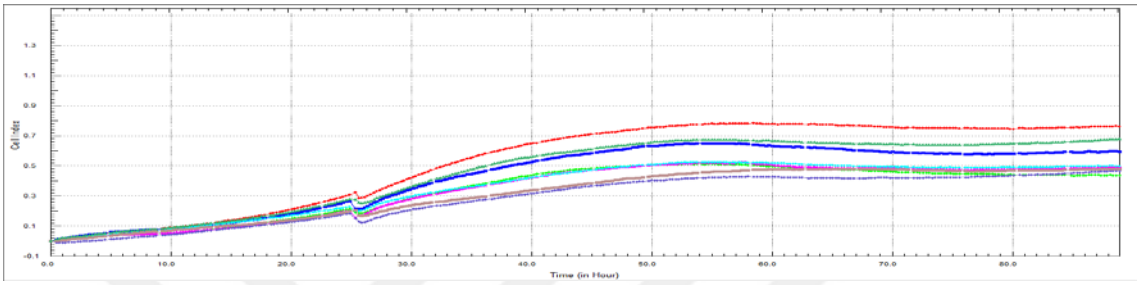
Elemental analysis of $C_{20}H_{21}N_3O_2S$ (MW: 367.46)

	% C	% H	% N	% S
Calculated	58.52	5.18	18.96	8.68
Found	58.80	5.34	18.86	8.72

4.2. Biological Data

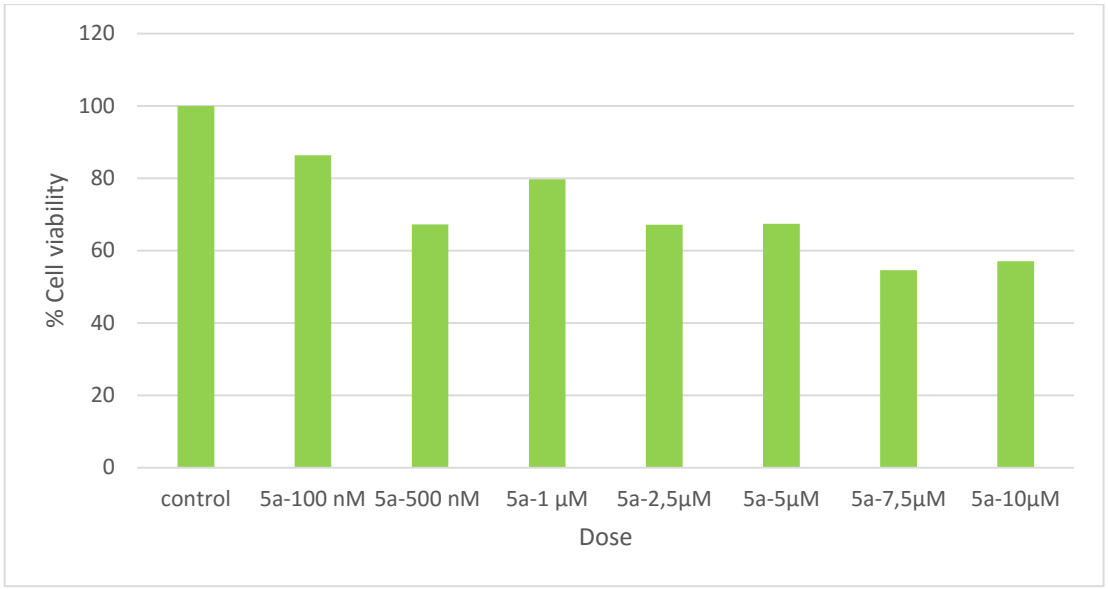
Cell viability profile of synthesized compounds on MCF-7 cell line obtained by Xcelligince system given below.

5-(2-Hydroxyphenyl)-3-[(3-methylpiperidine-1-yl)methyl]-1,3,4-oxadiazole-2(3*H*)-thione (Compound 5a)

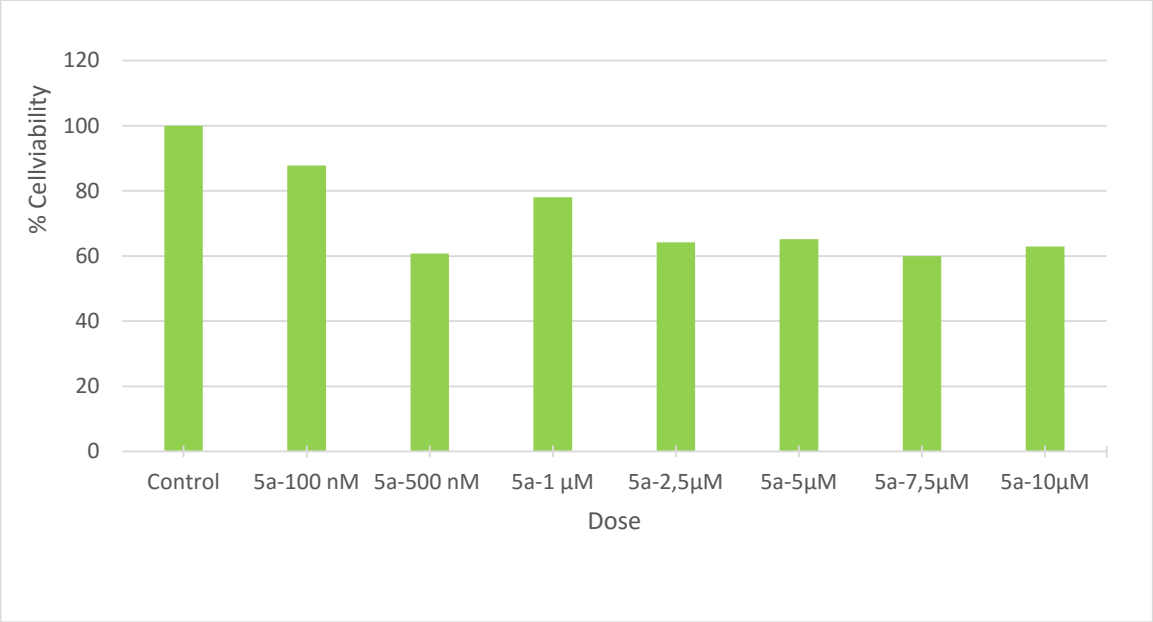


Cell index of compound **5a** in RTCA cytotoxicity assay

		IC_{50} (mM)	
Compound	**n**	**24 hrs**	**48 hrs**
5a	3	7.4	250

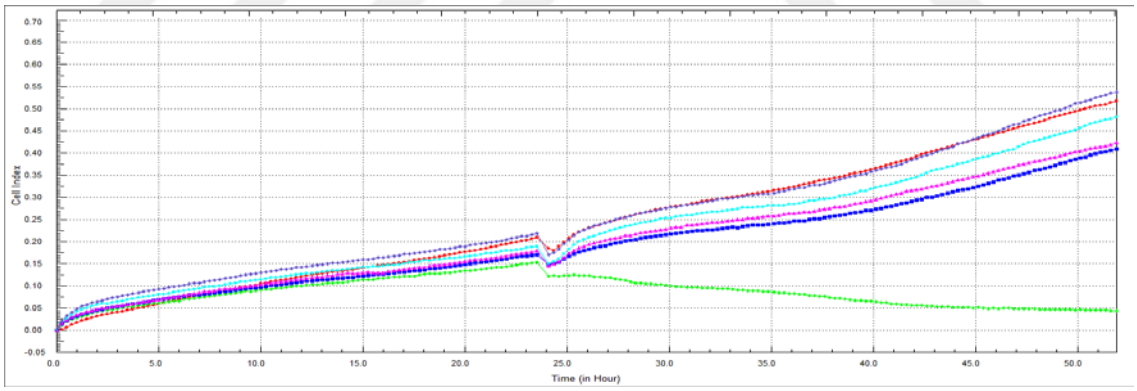


Endpoint cell index values (24 h incubation with compound **5a**)



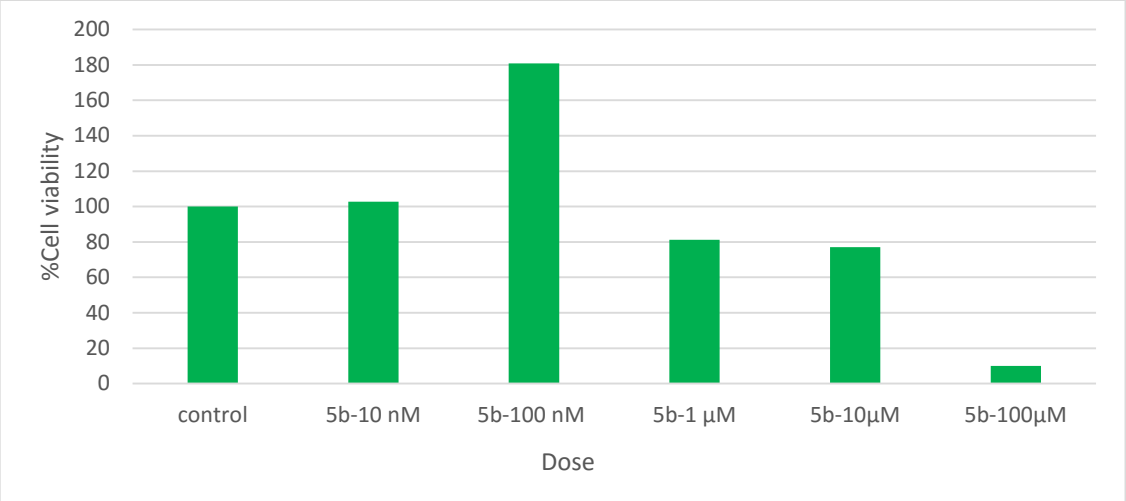
Endpoint cell index values (48 h incubation with compound **5a**)

5-(2-Hydroxyphenyl)-3-[(4-hydroxymethylpiperidine-1-yl)methyl]-1,3,4-oxadiazole-2(3*H*)-thione (Compound 5b)

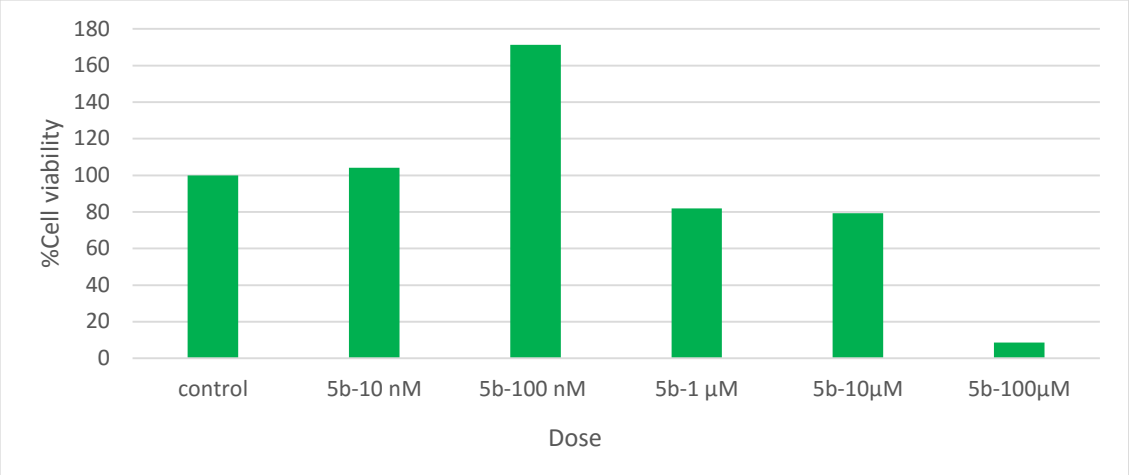


Cell index of compound **5b** in RTCA cytotoxicity

		IC_{50} (mM)	
Compound	**n**	**24 hrs**	**48 hrs**
5b	3	73.8	89.3

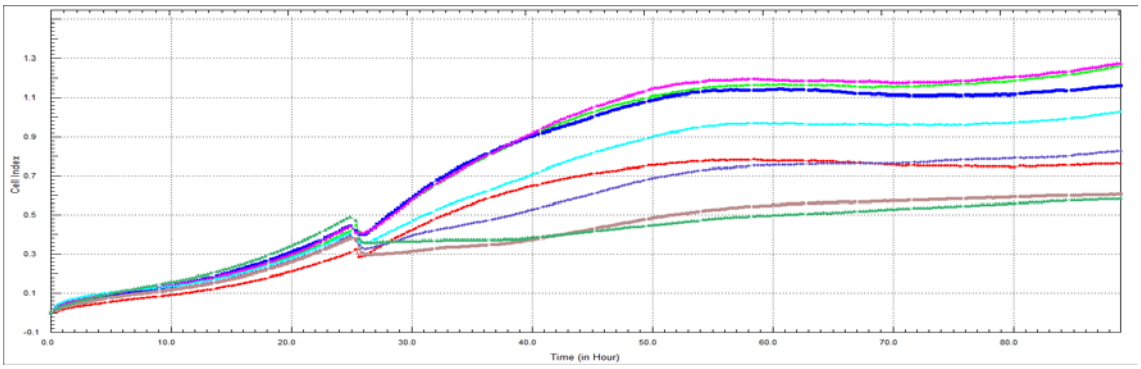


Endpoint cell index values (24 h incubation with compound **5b**)



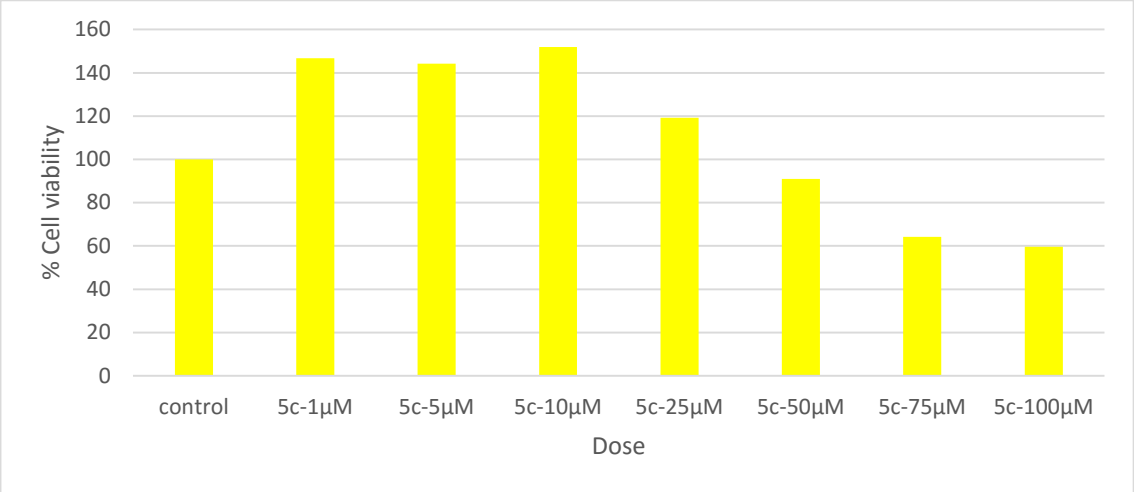
Endpoint cell index values (48 h incubation with compound **5b**)

5-(2-Hydroxyphenyl)-3-[(4-phenylpiperidine-1-yl)methyl]-1,3,4-oxadiazole-2(3*H*)-thione (Compound 5c)

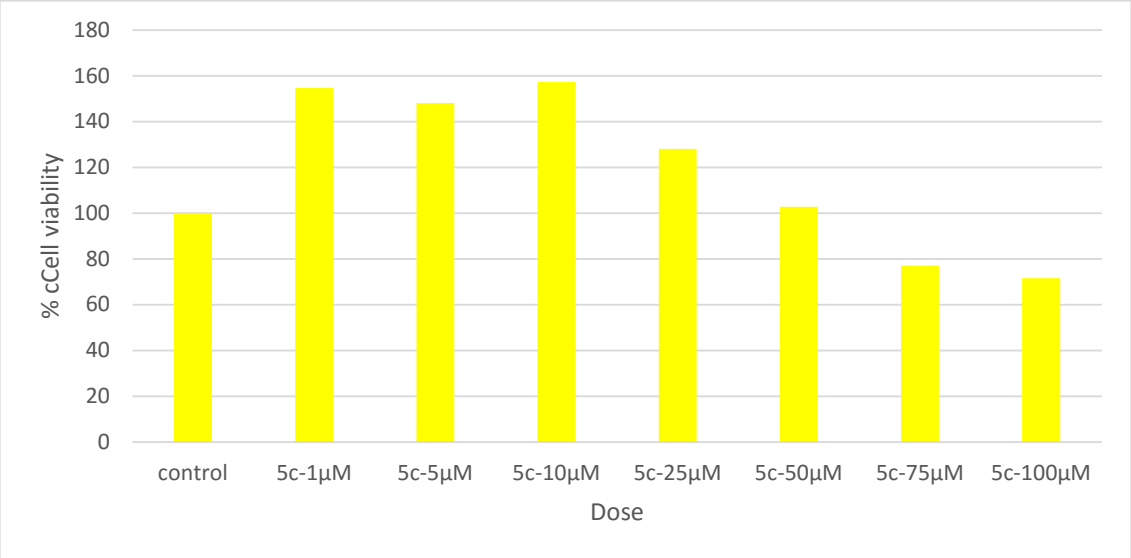


Cell index of compound **5c** in RTCA cytotoxicity assay

Compound	n	IC_{50} (mM)	
		24 hrs	48 hrs
5c	3	0.14	0.17

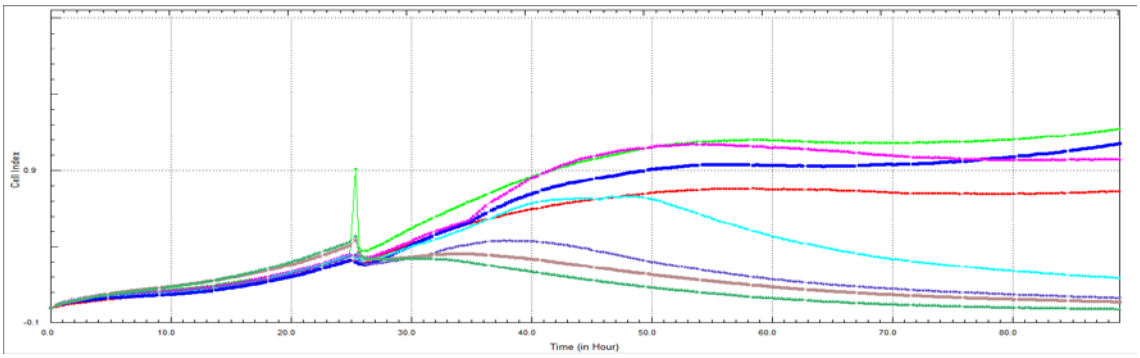


Endpoint cell index values (24 h incubation with compound **5c**)



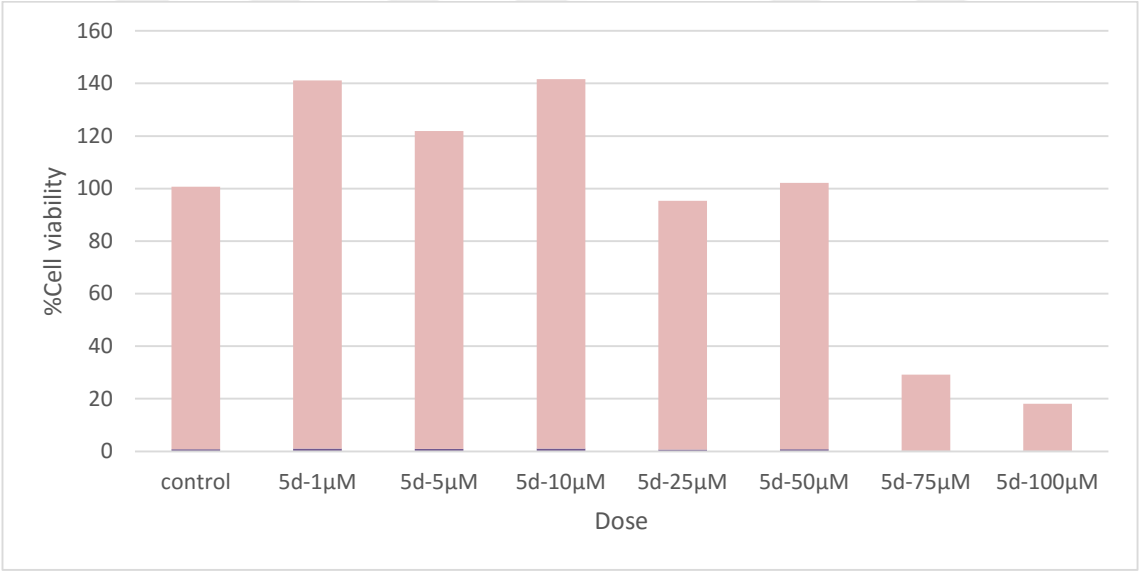
Endpoint cell index values (48 h incubation with compound **5c**)

5-(2-Hydroxyphenyl)-3-[(4-hydroxy-4-phenylpiperidine-1-yl)methyl]-1,3,4-oxadiazole-2(3*H*)-thione (Compound 5d)

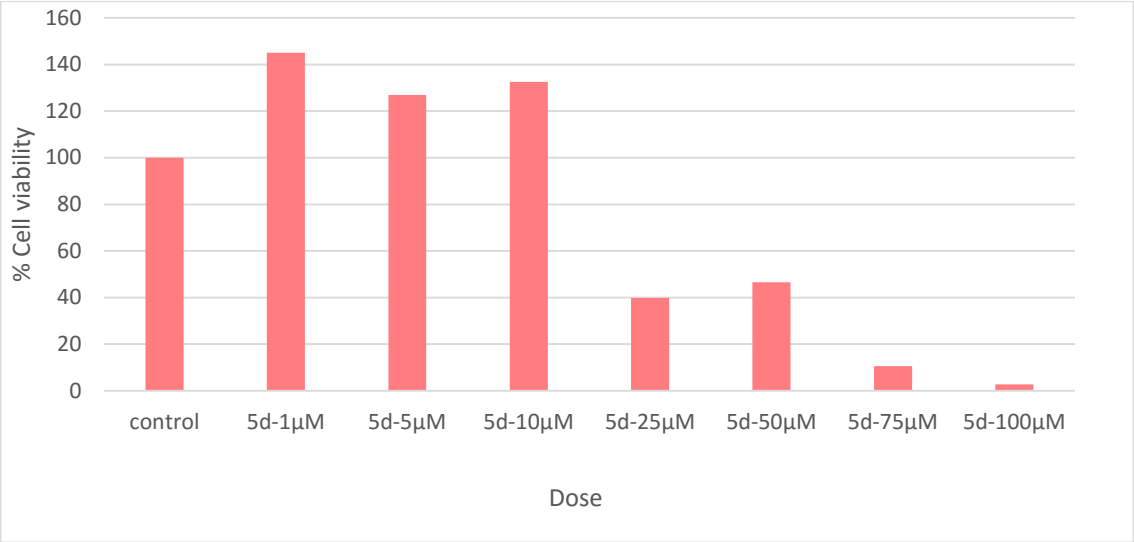


Cell index of compound **5d** in RTCA cytotoxicity assay

		IC_{50} (mM)	
Compound	**n**	**24 hrs**	**48 hrs**
5d	3	0.13	0.03

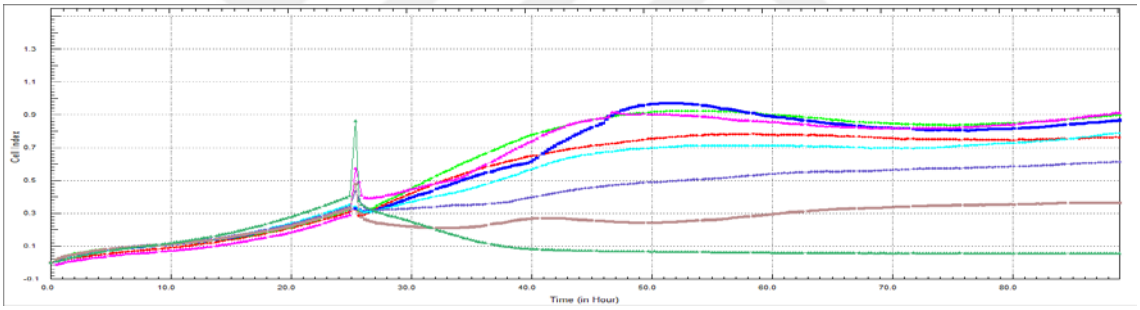


Endpoint cell index values (24 h incubation with compound **5d**)



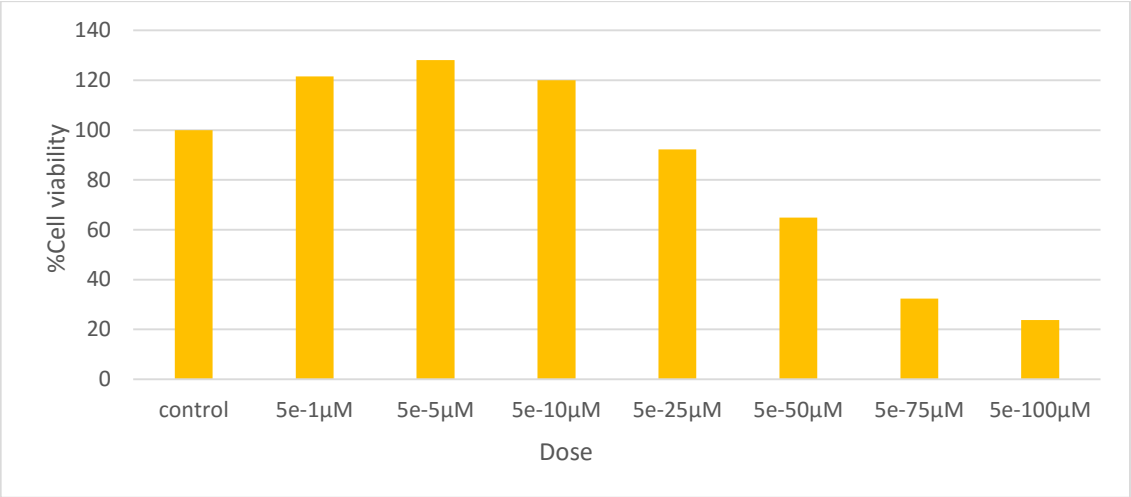
Endpoint cell index values (48 h incubation with compound **5d**)

5-(2-Hydroxyphenyl)-3-[(4-acetyl-4-phenylpiperidine-1-yl)methyl]-1,3,4-oxadiazole-2(3*H*)-thione (Compound 5e)

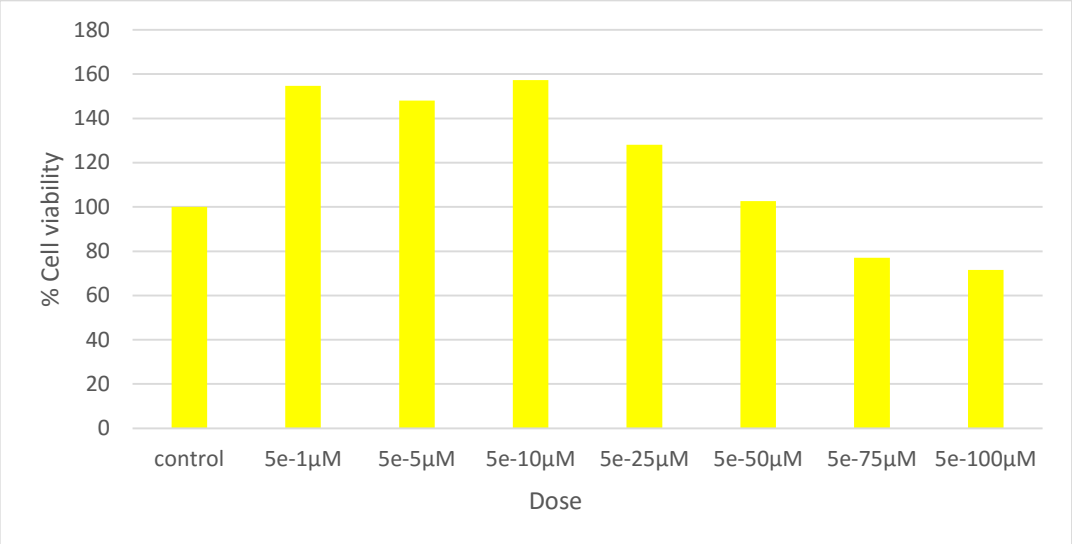


Cell index of compound **5e** in RTCA cytotoxicity assay

		IC_{50} (mM)	
Compound	**n**	**24 hrs**	**48 hrs**
5e	3	0.53	1400

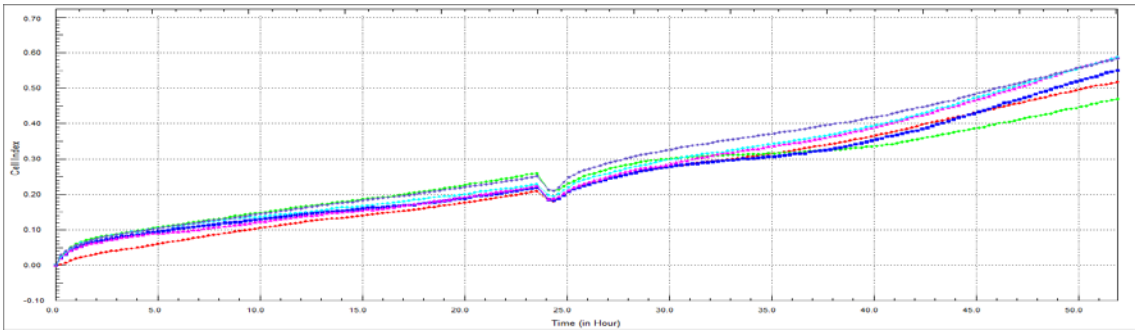


Endpoint cell index values (24 h incubation with compound **5e**)



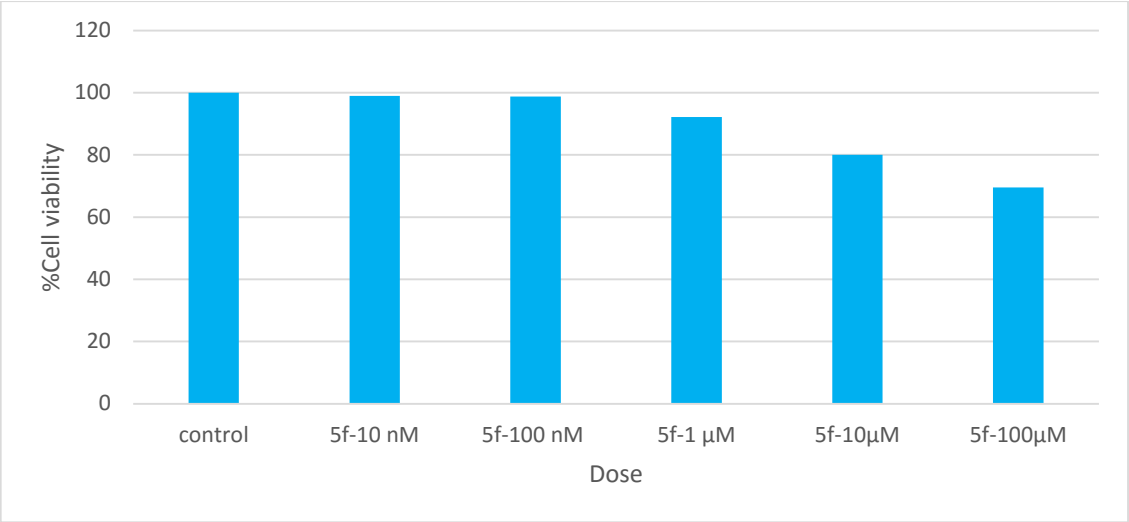
Endpoint cell index values (48 h incubation with compound **5e**)

5-(2-Hydroxyphenyl)-3-[(4-cyano-4-phenylpiperidine-1-yl)methyl]-1,3,4-oxadiazole-2(3*H*)-thione (Compound 5f)

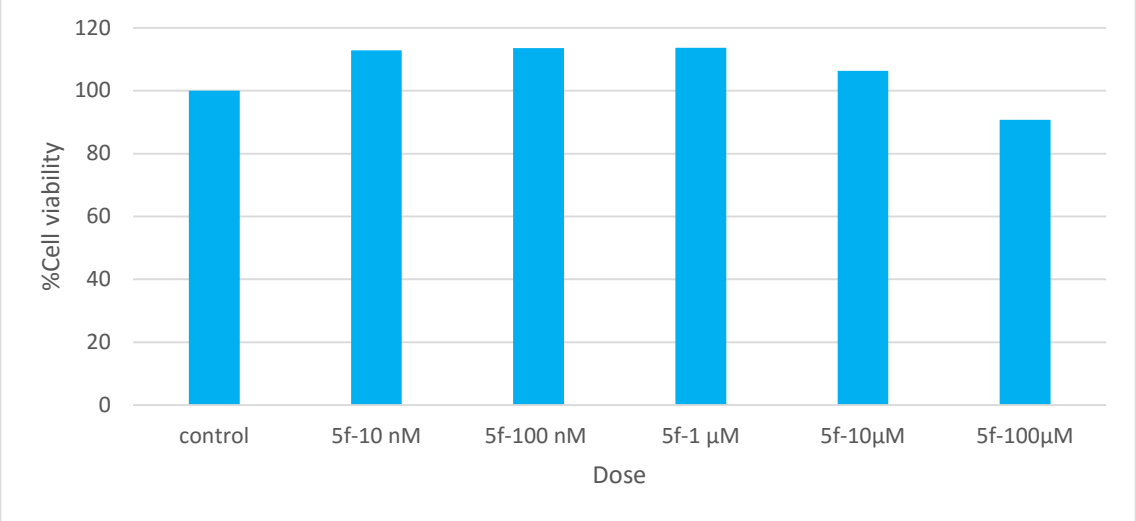


Cell index of compond **5f** in RTCA cytotoxicity assay

		IC_{50} (µM)	
Compound	**n**	**24 hrs**	**48 hrs**
5f	3	22	32

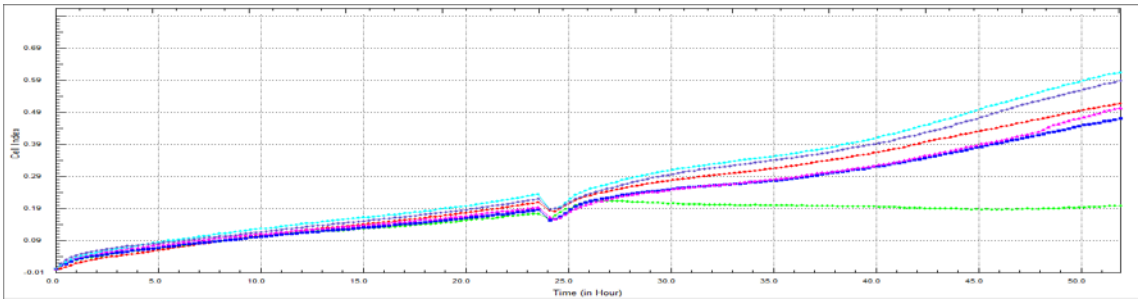


Endpoint cell index values (24 h incubation with compound **5f**)



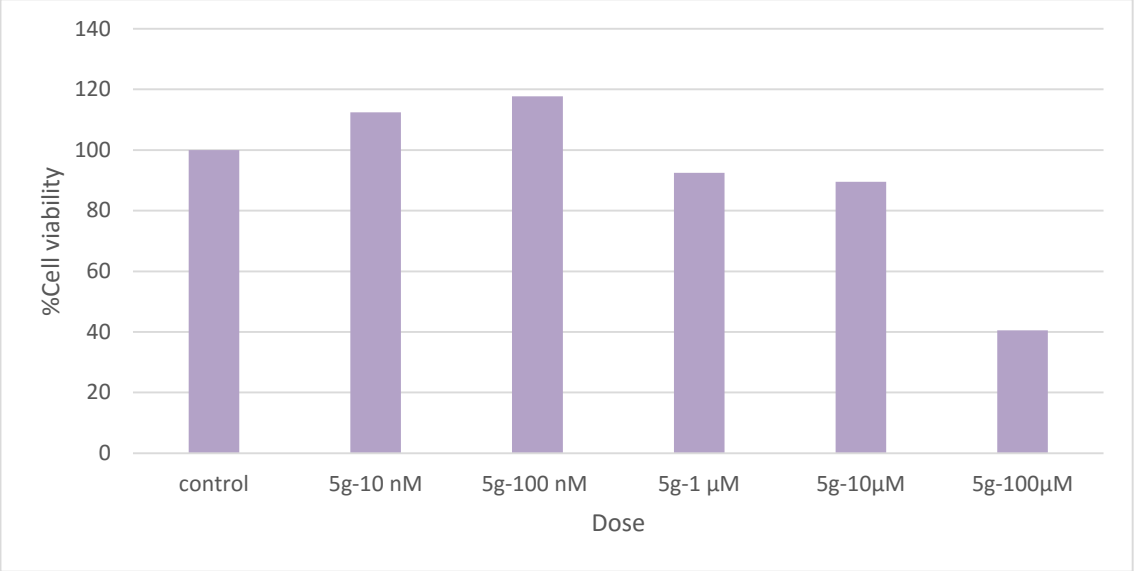
Endpoint cell index values (48 h incubation with compound **5f**)

5-(2-Hydroxyphenyl)-3-[(4-phenylpiperazine-1-yl)methyl]-1,3,4-oxadiazole-2(3*H*)-thione (Compound 5g)

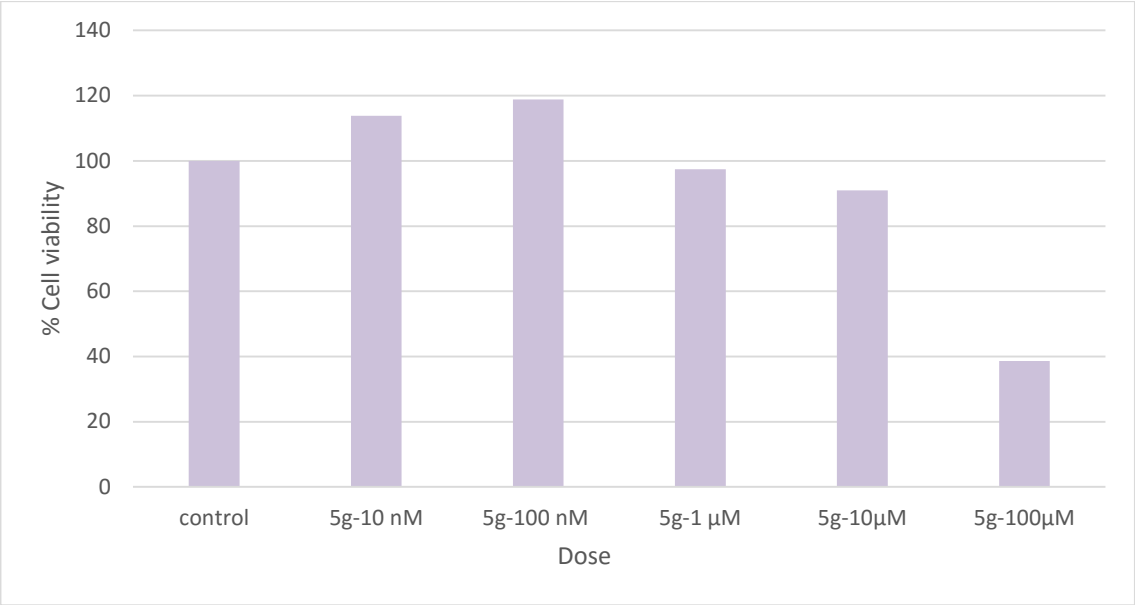


Cell index of compond **5g** in RTCA cytotoxicity assay

		IC_{50} (µM)	
Compound	**n**	**24 hrs**	**48 hrs**
5g	3	30	32.8

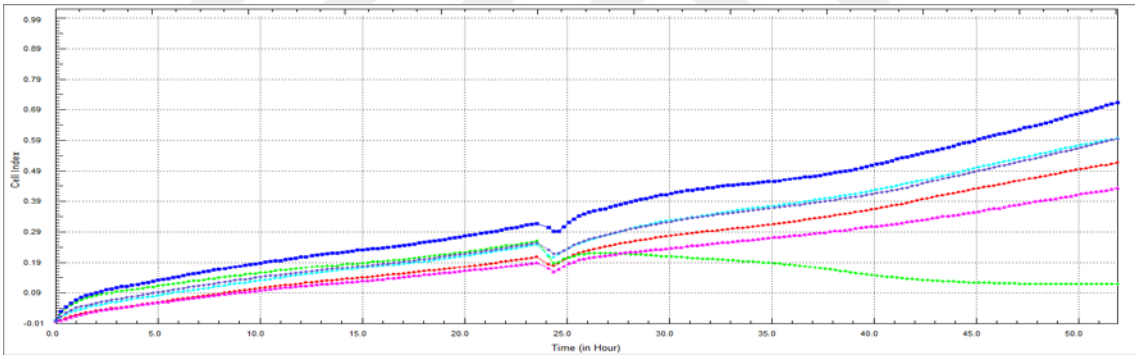


Endpoint cell index values (24 h incubation with compound **5g**)



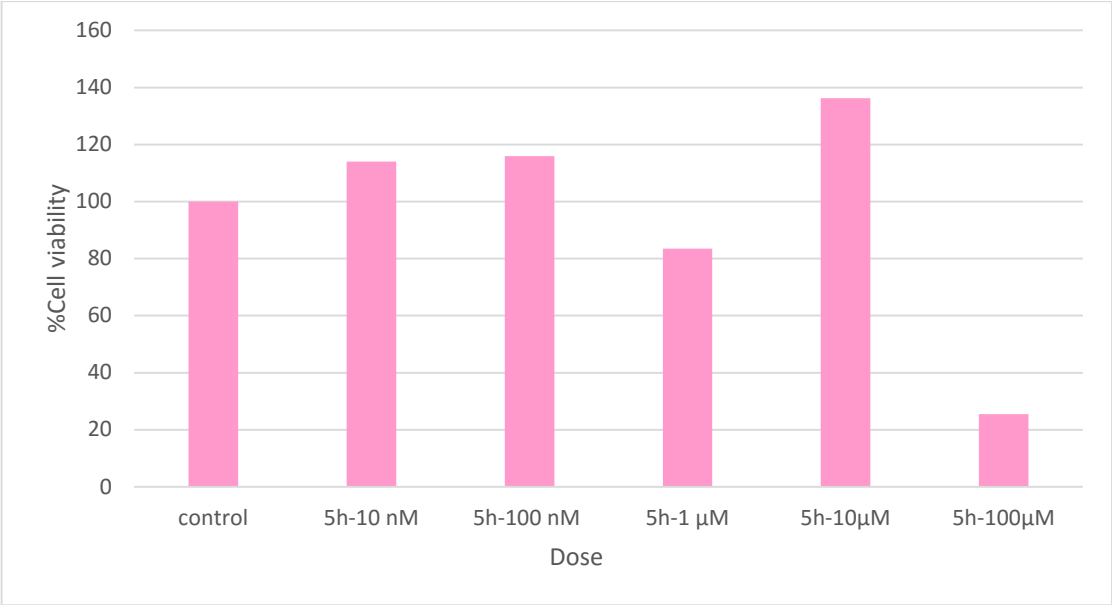
Endpoint cell index values (48 h incubation with compound **5g**)

5-(2-Hydroxyphenyl)-3-[(4-(2-pyridyl)piperazine-1-yl))methyl]-1,3,4-oxadiazole-2(3*H*)-thione (Compound 5h)

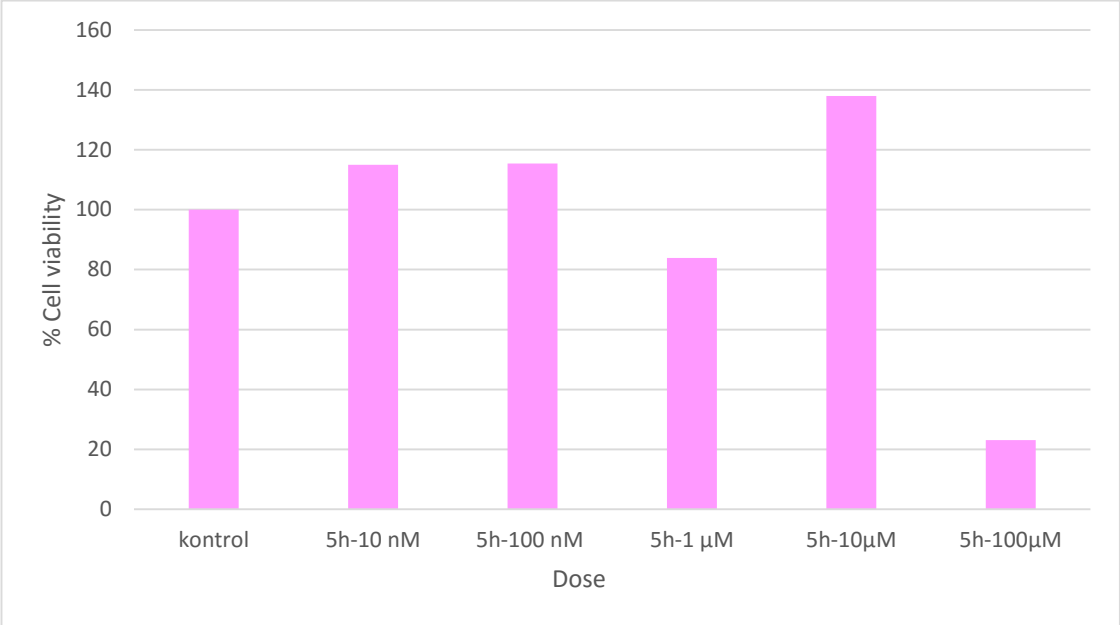


Cell index of compound **5h** in RTCA cytotoxicity assay

		IC_{50} (mM)	
Compound	**n**	**24 hrs**	**48 hrs**
5h	3	190	1500



Endpoint cell index values (24 h incubation with compound **5h**)

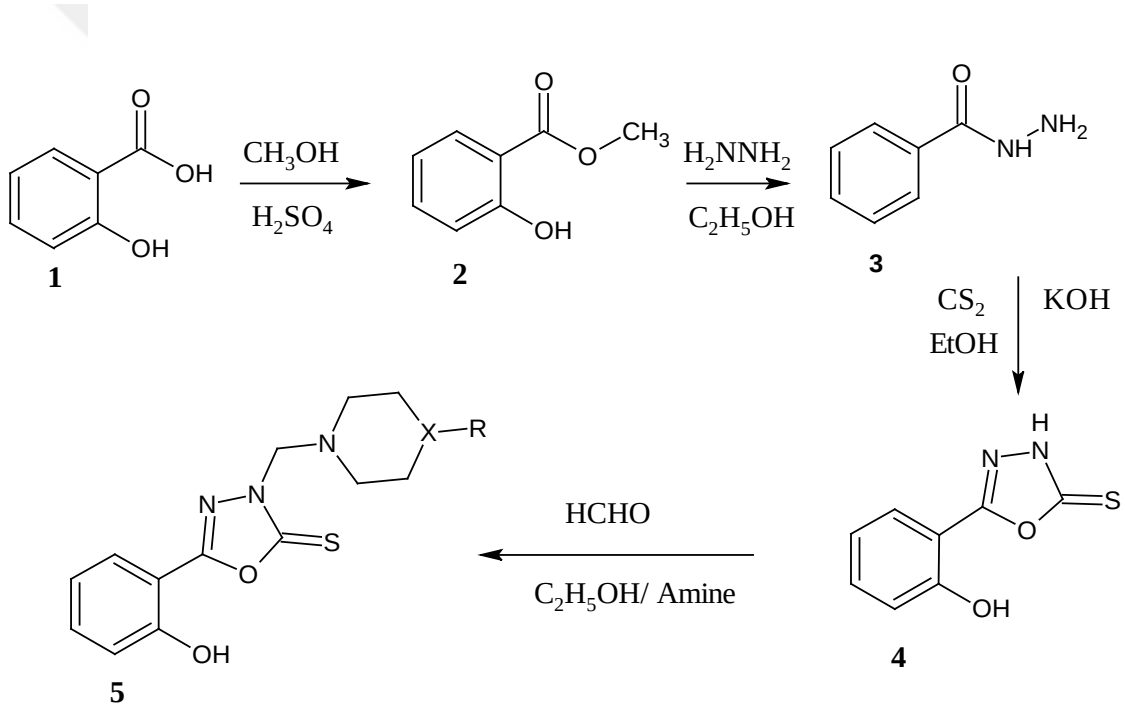


Endpoint cell index values (48 h incubation with compound **5h**)

5- DISSCUSION and CONCLUSION

In this study, different 3,5-disubstituted 1,3,4-oxadiazol-2-thione derivatives have been synthesized to evaluate the anticancer activity of them against MCF-7 cancer cell lines. The structure of the synthesized compounds was estimated by UV, IR, ^{1}HNMR, ^{13}CNMR, and elemental analysis.

The target compounds present in this study were prepared according to the synthetic pathways shown in Scheme 5.1.

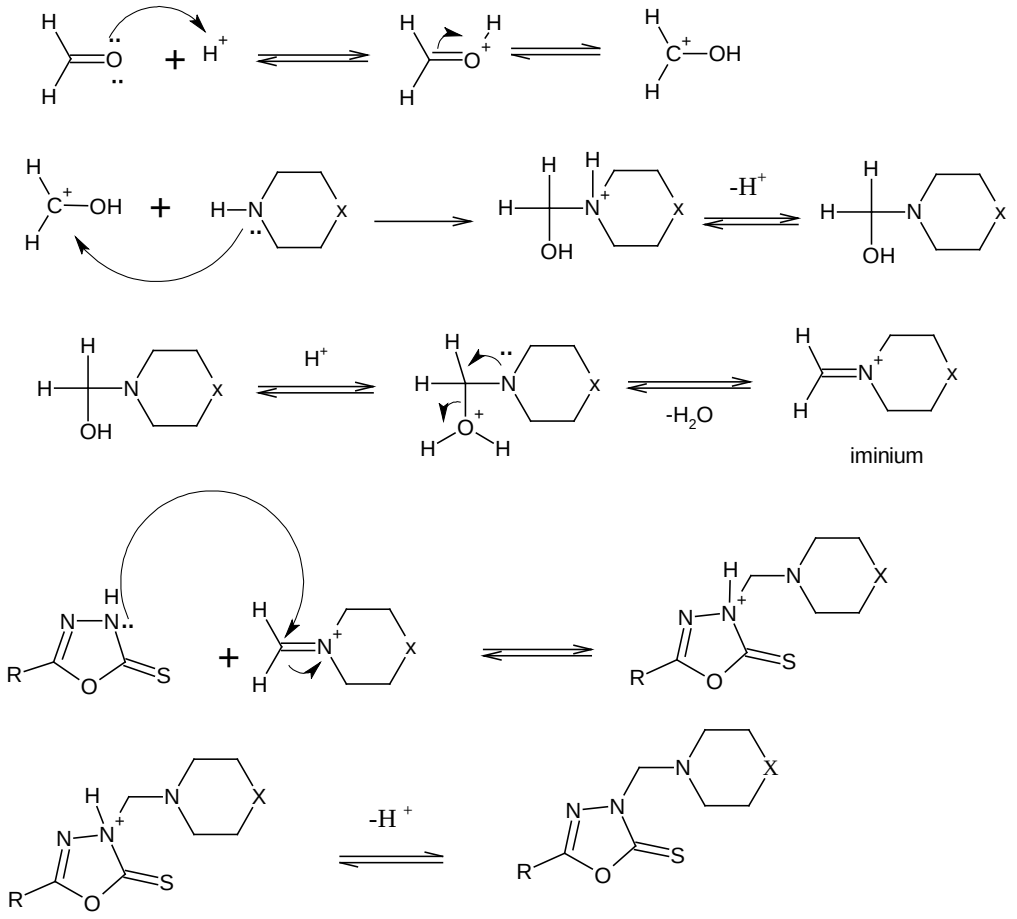


Scheme. 5.1. General synthesis pathway of the Compounds **5a-5h.**

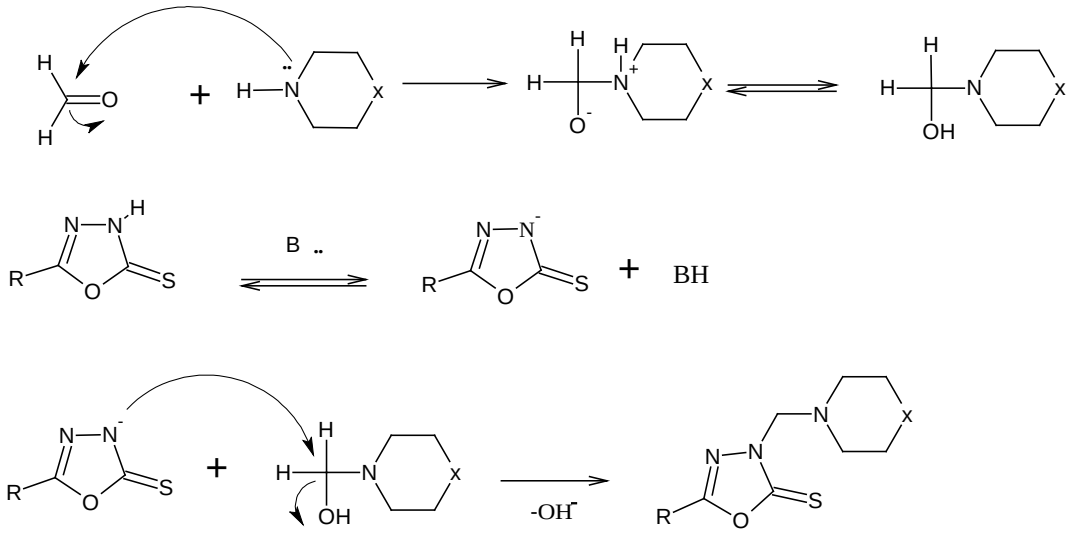
The final compounds are the *Mannich* bases of 5-(2-hydroxyphenyl)-1,3,4-oxadiazole-2-(3*H*)thione. 5-(2-Hydroxyphenyl)-1,3,4-oxadiazole-2-(3*H*)thione was synthesized according to the literature of 1,3,4-oxadiazole-2(3*H*)-thione ring cyclization through the reaction of salicylic acid hydrazide with carbon disulfide in potassium hydroxide [33].

The last step of the reaction pathway is achieved by *Mannich* reaction of 5-(2-hydroxypheny-1,3,4-oxadiazole-2-thione. In *Mannich* reaction, formaldehyde is condensed with primary/secondary amines, and a compound containing active hydrogen. In the first step of this method addition of the amine to aldehyde has occurred, followed by a nucleophilic substitution. Acidic or basic medium can be used for the reaction. The proposal of the mechanism of *Mannich* reaction was given below.

The acid-catalyzed reaction



The base-catalyzed reaction



After the synthesis of compounds, structure elucidation was carried out by spectral methods. In UV spectra, there are strong absorption bands at 206 and 318 nm which are similar to the literature data. These values represent $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions in conformity with maximum absorption of 2,5-diaryl-1,3,4-oxadiazole derivatives.

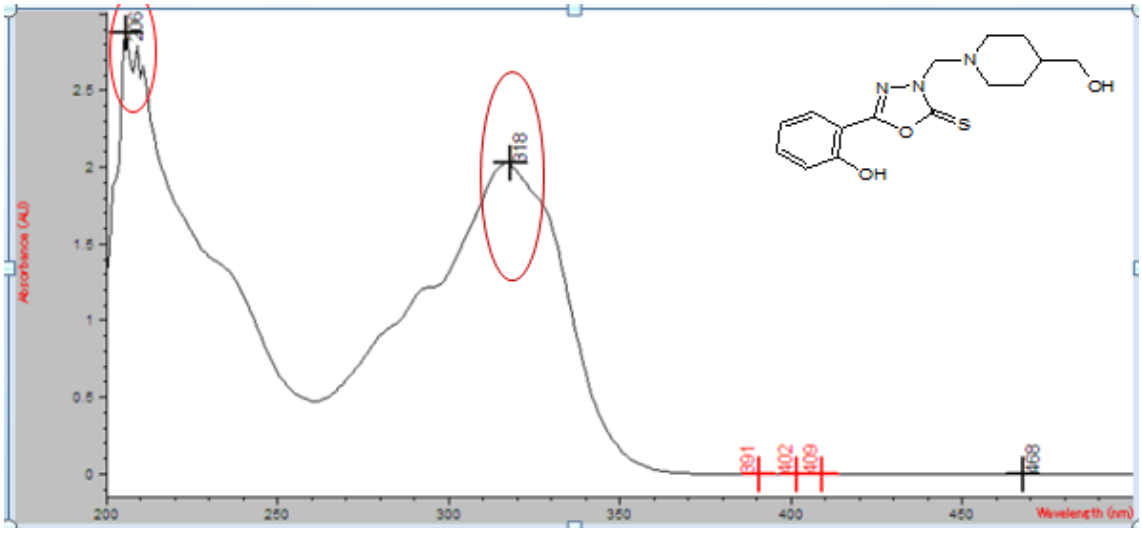


Figure 5.1. The UV spectrum of the Compound **5b**.

IR spectra of the synthesized compounds are similar to the IR values of which were stated in literature [33, 100-101]. For the compounds, no absorption band was determined at 3100-3400 cm^{-1}, indicating the absence of an NH group that is an evidence for the substitution reaction to 5-(2-hydroxyphenyl)-1,3,4-oxadiazole-2-thione with substituted piperidine or piperazine. IR spectrum of the all compounds exhibited C-H stretching at 2810-2936 cm^{-1}, C=C stretching at 1572-1597 cm^{-1}, and C=S stretching at 1436-1490 cm^{-1}.

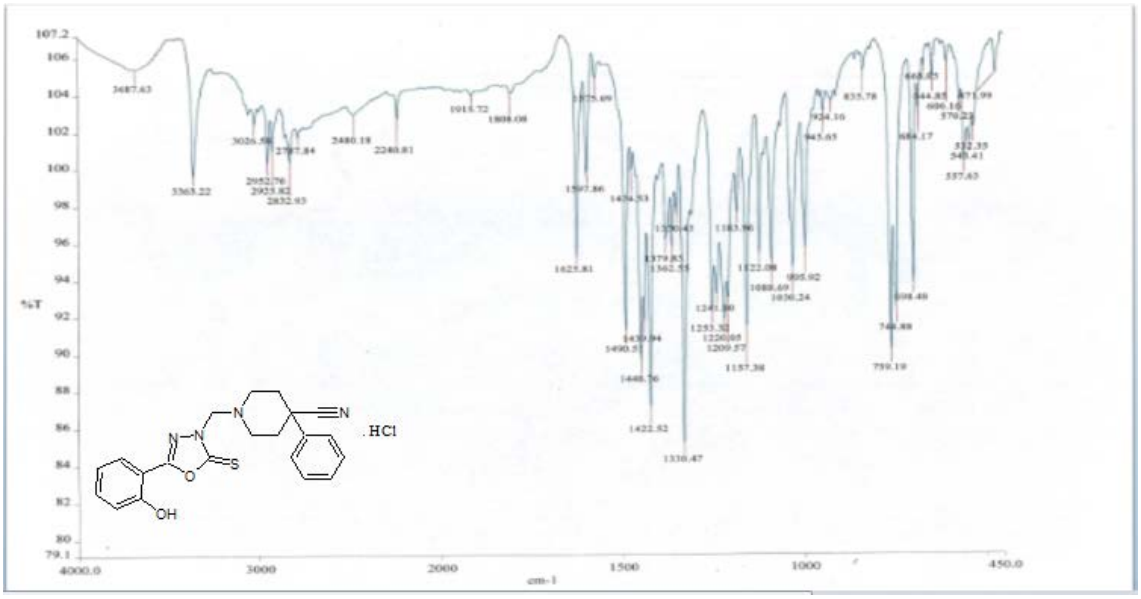


Figure 5.2. The IR spectrum of the Compound **5f**

In the ^{1}H-NMR of all synthesized compounds, the methylene protons representing the *Mannich* base formation were observed at about 5.00-5.45 ppm as a singlet.

The protons of 2-hydroxyphenyl group were seen at 7.61 (1H, dd, H^3, *J*= 8, *J'*= 1.6 Hz), 7.56-7.43 (1H, m, H^4), 7.06 (1H, d, H^6, J= 8 Hz), and 6.99-6.96 (1H, m, H^5) ppm, respectively. methylene protons occur at 5.00 ppm as a singlet peak.

H^2 proton of piperidine moiety is seen as multiplet at 2.94-2.91 ppm. At 2.64 ppm the peak of H^3 is observed as triplet, proton of H^6 is seen at 2.43 as doublet and finally H^5 proton appears at 1.99-1.93 as a multiplet.

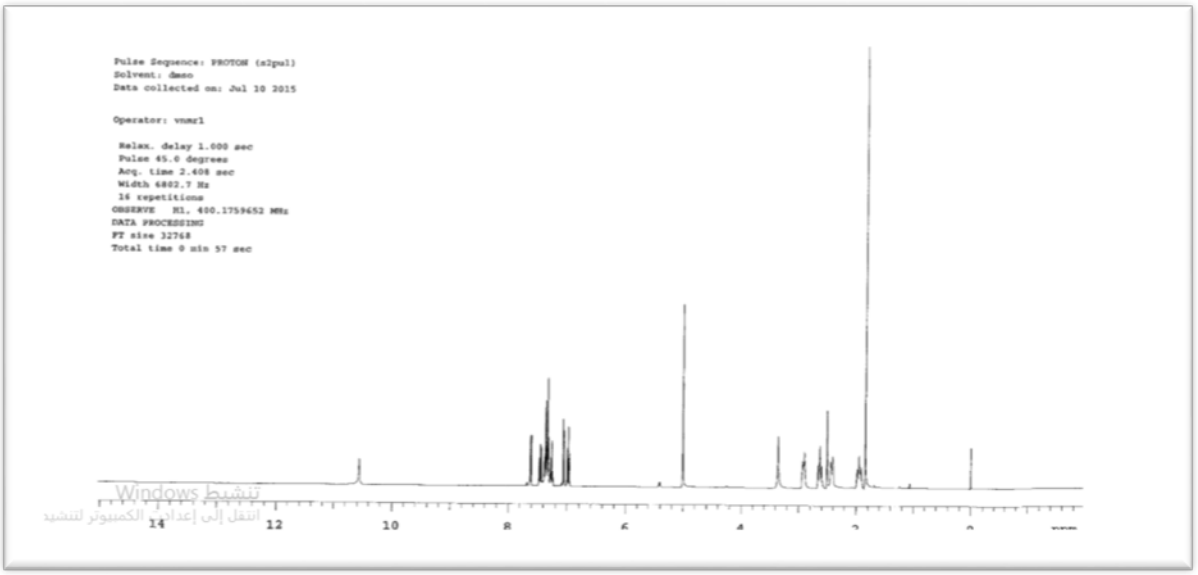


Figure 5.3. The [1]H-NMR spectrum of the Compound **5e** in DMSO.

The[13] C-NMR spectrum of the compound **5e** is taken by using dimethylsulfoxide (DMSO). In Figure 5.4, characteristic peaks of the compound were observed at 208.91 ppm for carbonyl group, 176.92 ppm for C_2 thione, 157.83 ppm for oxadiazole C_5, 69.98 pmm for methylene and 53.50-32.11 ppm peaks were seen for piperidine/piperizine moiety protons. Other carbon atoms of the compound have similar chemical shift indicated values indicating in the reference books and literature.

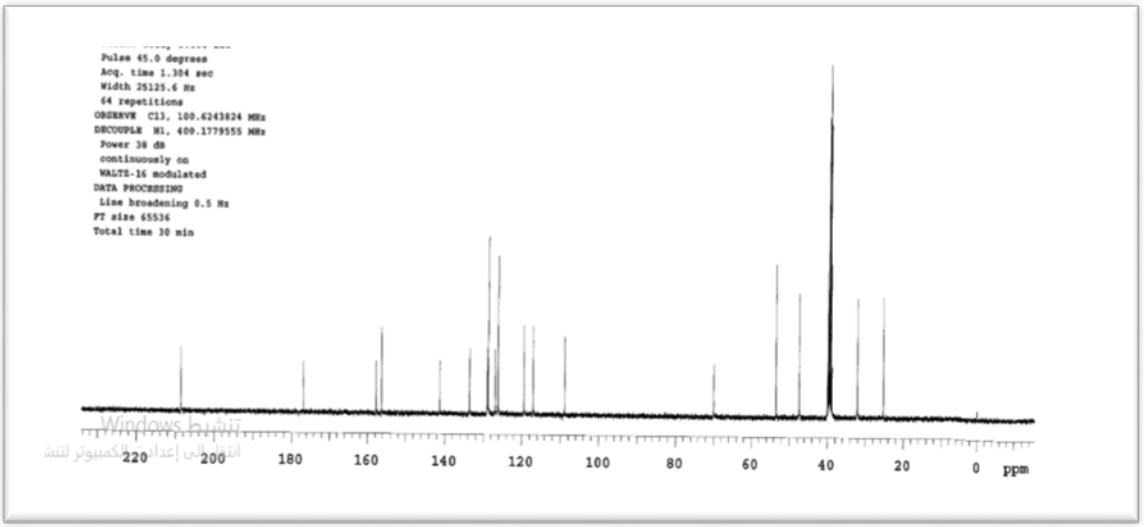
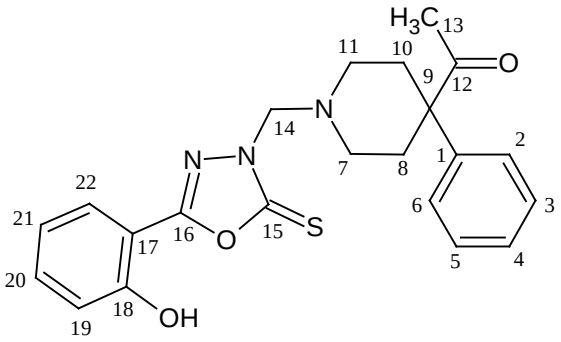


Figure 5.4. The [13]C-NMR spectrum of the Compound **5e** in DMSO.

Table 5.1. The ^{13}C-NMR spectrum interpretation of the Compound **5e**.



. HCl

C	Chemical Shift (δ)	C	Chemical Shift (δ)
1	141.26	12	208.91
2	126.97	13	25.47
3	128.75	14	69.98
4	126.22	15	176.92
5	128.75	16	157.83
6	126.97	17	133.36
7	53.50	18	156.36
8	32.11	19	129.02
9	47.40	20	117.03
10	32.11	21	108.86
11	53.50	22	119.40

In this study all the synthesized compounds (**5a-5h)** are evaluated for their cytotoxic activity against MCF-7 by using the Xcelligence system which allows us to obtain kinetic profiles throughout an entire experiment without need of labeling, and continuously capturing the cell morphology, proliferation and viability in real time. Due to the continuous nature of the monitoring used by Xcelligence system it is possible to distinguish between different disturbances in the cellular activity such as cell toxicity and reduced proliferation.

The cytotoxic effect of synthesized compounds determined at 24 and 48 hours were given below in the table.

Table.5.2. IC_{50} values of screened compounds on MCF-7 cell line by Xcelligence system.

Compound no	IC_{50}	
	24hrs	**48hrs**
5a	7.4 mM	250 mM
5b	73.8 µM	89.3 µM
5c	0.14 mM	0.17 mM
5d	0.13 mM	0.03 mM
5e	0.53 mM	1400 mM
5f	22 µM	32 µM
5g	30 µM	32.8 µM
5h	190 mM	1500 mM

According to cytotoxicity profile **5a**, **5c**, **5d** and **5e** compounds have cytotoxic effect. However, in high doses **5b** and **5f** may be show cytotoxic activity, in addition **5g**, and **5h** may be antiproliferative in higher doses. In contrast endpoint cell index values shows that **5f** neither antiproliferative nor cytotoxic, **5a**, **5c**, and **5e** compounds have antiproliferative effect. However, in high doses (>25µM) **5e** may be show cytotoxic activity, in addition **5g** may be antiproliferative in higher doses. **5d** has the maximal cytotoxic effect among the tested compounds, **5b** is cytotoxic also but not as strong as **5d**. In addition **5h** may be cytotoxıc in high doses.

In conclusion, we have prepared some new 3,5-disubstituted-1,3,4-oxadiazole-2(3*H*)-thione derivatives under environmentally mild conditions and their cytotoxicity profile were evaluated. According to promising compounds, it is hard to evaluate structure activity relationships.

The synthesized compounds can be used as a part of template for future development through modification and derivatization to design more potent cytotoxic agents that carry 1,3,4-oxadiazole core structure in order to produce a structure activity relationship. Future synthesis of similar derivatives will take place to create a larger set

of compounds. Also the active compounds represented in this study deserve to be studied further for their mechanistic studies such as protein kinase activity.

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