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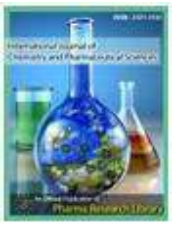

Research Article **Open Access**

Synthesis and Spectral Studies of Novel 3-(5-mercapto-1, 3, 4-oxadiazol-2-yl)-2H-chromen-2-one Derivatives as Anticancer Agents

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A B S T R A C T

In recent years, researchers hunt for a novel anticancer agent mainly from plants. Among various phytochemicals, coumarins have attracted considerable interest in the past few years due to their potential health benefits. Similarly Oxadiazole is a versatile heterocyclic nucleus which has attracted a wide attention of the medicinal chemists in search for new therapeutic molecules. A series of new of 3-(5-mercapto-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one **4** derivatives were synthesized by reacting it with some alkylating agents. The ethyl 2-oxo-2*H*-chromene-3-carboxylate **2** was synthesized in good yield by reacting 2-hydroxybenzaldehyde and diethyl malonate in presence of piperidine and AcOH. The 2-oxo-2*H*-chromene-3-carbohydrazide **3** was synthesized by refluxing **2** in presence of hydrazine hydrate 99% in ethanol. The 3-(5-mercapto-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one **4** was synthesized by refluxing **3** in presence of CS_2/KOH till evolution of H_2S ceased. This 3-(5-mercapto-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one **4** was further refluxed in presence of base and alkalyting agents to get different derivatives (WJ 1-10). By the anticancer screening we have observed that the derivatives have shown significant activity against Human leukemia Cancer (HL60) cell line, good to moderate activity against Human Breast Cancer (MCF7) cell line and moderate activity against Human Cervix Cancer (HeLa) cell line.

Keywords: cancer, coumarin, oxadiazole, leukemia, breast, cervix.

A R T I C L E I N F O

CONTENTS

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1. Introduction

Cancer:

Cancer is the second leading cause of death in Europe and North America. World Health Organization has estimated over 11 million global deaths due to cancer in 2030. Discovery and development of anticancer agents are the key focus of several pharmaceutical companies as well as non-profit government and non-government organizations, like the National Cancer Institute (NCI) in the United States, the European Organization for Research and Treatment of Cancer (EORTC) and the British Cancer Research Campaign (BCRC) [1]. Cancer is a genetic disease resulting from faulty DNA. Mutations can occur in genes, causing normal cell to become cancerous. More specifically, a defective gene can lead to increased cellular proliferation in one cell and it can be passed down to a daughter cell. The accumulation of mutations in subsequent generations of daughter cells can cause cells to proliferate even more rapidly and eventually undergo structural changes to become malignant. Cancer cells are believed to be result from at least two genetic mutations to a normal cell. These mutations cause the cells to divide uncontrollably [2]. Only 5–10% of all cancer cases can be attributed to genetic defects, whereas the remaining 90–95% has their roots in the environment and lifestyle. The lifestyle factors include cigarette smoking, diet (fried foods, red meat), alcohol, sun exposure, environmental pollutants, infections, stress, obesity, and physical inactivity [3].

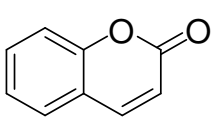
Coumarin:

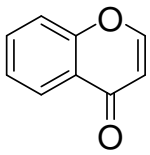
A variety of herbs and herbal extracts contain different photochemical and biological activity that can provide therapeutic effects. Several herbs can help to provide some protection against cancer & stimulate the immune system. Coumarin comprises a group of natural compounds and the synthesis of coumarin is an important reaction in organic chemistry because of their wide application in medicinal chemistry [4]. Coumarin is the parent substance of the benzo- -pyrone group which is a naturally occurring compounds being present in a wide variety of plant including cassia, lavender, yellow sweet clover and woodruff. It was first isolated from tonka beans in 1820. The name comes from a French word, coumarou, for the tonka bean. It is the simplest compound of a large class of naturally occurring phenolic substances made of fused benzene and -pyrone ring and is highly present in some essential oils, particularly cinnamon bark oil and lavender oil. Coumarin is also found in fruits (e.g. bilberry, cloudberry), green tea and other foods such as fixative enhances the odour of essential oils in perfumes, toilet soaps, tooth paste and hair preparations and in tobacco products to enhance and fix the natural taste, flavour and aroma [5]. Coumarins are naturally occurring as well as synthetic derivatives and having widespread applications such as HIV protease inhibitors, anticoagulant, spasmolytic and bacteriostatic agents. However, the most widely reported activities for coumarin derivatives are their anti-inflammatory and anti-cancer activities [6]. Coumarin derivatives (CDs) are often discussed because of their diverse biological properties. CDs have attracted considerable attention from organic and medicinal chemists, as they are widely used as fragrances, pharmaceuticals and agrochemicals. Their antioxidant, bacteriostatic and anti-cancer activities make these compounds attractive for investigators for further backbone derivatization and screening as novel therapeutic agents and other foremost topics of this field of research. Some reports have emphasized the efficacies of pure coumarins against Gram-positive and Gram-negative bacteria as well as fungi. In addition CDs have been shown to inhibit cell proliferation in a cancerous cell line.

Experimental investigations as well as clinical and epidemiological findings have provided evidence supporting the role of reactive oxygen metabolites or free radicals in the etiology of cancer. Certain aldehydes such as malonyldialdehyde, the end product of lipid peroxidation arising from free radical degeneration of polyunsaturated fatty acids, can cause cross-linking in lipids, proteins and nucleic acids leading to cellular damage. The human body is equipped with certain enzymatic and non-enzymatic antioxidants which can counteract the deleterious actions of free radicals and radical-induced cellular and molecular damage. Disruption of this sensitive balance between the free radicals and the antioxidants may lead to cellular damage and trigger carcinogenesis. The circumstantial literature concerning CDs cited above inspired us to undertake the present studies on selected CDs and evaluate them as possible antioxidant and anticancer agents. Different physicochemical descriptors have been calculated in silico to discuss the possible structure activity relationship [7].

Chemistry of Coumarin:

The fusion of pyrone ring with benzene nucleus gives rise to a class of heterocyclic compound known as benzopyrone, of which two distinct types are recognized: Benzo- -pyrone commonly called as coumarin and Benzo- -pyrone commonly called as chromon [Figure 1].



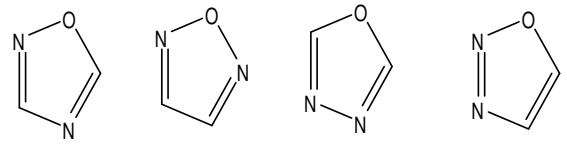


(**Figure 1) Benzo- -pyrone** **Benzo- -pyrone**

They are differing from each other only in the position of carbonyl group in heterocyclic ring. Several methods were developed for the synthesis of coumarin, such as the Pechmann, Perkin, Knoevegel, Witting and Reformastsky reaction. Among these, the Pechmann reaction has been the most widely used method, since it proceeds from very simple starting materials and gives good yield of variously substituted coumarin. Pechmann reaction consists of condensation of -ketonic ester with phenol to give coumarin [8].

Oxadiazole:

The most numerous and important heterocyclic systems are those having five and six membered rings having hetero atoms such as N, O, S, P, Si, B etc. Heterocyclic compounds have attracted the attention of medicinal chemists because of having broad spectrum of pharmacological activities and hence it continues to yield new medicinal agents. One such heterocyclic nucleus of medicinal importance is oxadiazole nucleus. Oxadiazole is a versatile heterocyclic nucleus which has attracted a wide attention of the medicinal chemists in search for new therapeutic molecules. Oxadiazoles are five membered heterocyclic compounds with two nitrogen atoms and one oxygen atom. Depending on the position of hetero atoms; they are named as 1,2,3-oxadiazole, 1,2,4-oxadiazole, 1,2,5-oxadiazole and 1,3,4-oxadiazole [Figure 2]. Out of its possible isomers; 1,3,4-oxadiazole is widely exploited for various applications as medicinal agents. This interesting group of compounds has diverse biological activities such as antibacterial, antifungal, antiviral, anticonvulsant, anticancer activities etc.



1,2,4-oxadiazole 1,2,5-oxadiazole 1,3,4-oxadiazole 1,2,3-oxadiazole

Figure 2: Chemical structures of isomers of oxadiazole nucleus

Biologically active molecules containing oxadiazole moiety includes antimicrobial, anticancer, anticonvulsant, anti-inflammatory and antiviral agents [9].

Physical Properties of Oxadiazole:

Oxadiazole is a five membered heterocycle having two carbons, two nitrogens, one oxygen and two double bonds. The first mono substituted 1, 3, 4-Oxadiazoles were reported in 1955 by two independent laboratories. Since 1955 other workers have extended this reaction; 1, 3, 4-Oxadiazole boils at 150^0 C. The percentage of C, H, N present in 1, 3, 4-Oxadiazole is given in table 1.

Table 1: Percentage of C, H, N present in 1,3,4-oxadiazole

Calculated %			Found %		
C	H	N	C	H	N
34.29	2.88	40.00	34.56	3.19	39.71

The IR spectra of 1,3,4-oxadiazole is characterized by the peaks at 1640-1560 cm-1 (C=N) and 1020 cm-1 (C=O). The position of both protons of 1,3,4-Oxadiazole in 1H-NMR is 1.27. The refractive index of 1,3,4-Oxadiazole is 1.43. The mass spectra showed that the base peak is the molecular ion peak [10].

2. Experimental

From the literature survey, 3-(5-mercapto-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one derivatives were synthesized by the following reaction **scheme 1**. The identification and characterization of the compounds were carried out by determining melting point, TLC, IR, ^{1}H NMR, ^{13}C NMR, DEPT, COSY, HSQS and Mass spectroscopy.

Materials and Methods

Chemicals used were of LR grade and purchased from Spectrochem, Merck India, S. D. fine-chem limited, Fisher Scientific, Loba Chemical, Changshu Yangyuan Chemical China. All solvents were used after distillation and drying. Silica gel pre-coated plates from e-Merck and Co. were used for TLC and spots located either by UV or by visualizing reagents. Various solvent systems used for developing the chromatograms were:

Methanol: Chloroform

Ethyl acetate: Hexane and Benzene: Hexane

Instruments used for characterization

Weighing of the samples was carried out by using Shimadzu balance Model: AX-200. All the melting points were determined in open-capillary tubes by heating in paraffin bath. The IR spectra were recorded by placing the solid sample on ATR crystal by using Bruker's FTIR and the transmittance spectra were obtained by using OPUS 6.5 software. ^{1}H NMR, ^{13}C NMR, DEPT 135, HSQC, COSY spectra of the synthesized compounds were recorded in $CDCl_3$/DMSO solution on a Bruker Avance II 400 MHz NMR spectrometer at SAIF, Punjab University, Chandigarh. Proton chemical shift are relative to Tetramethyl silane (TMS) as internal standard. Mass spectra were recorded on Waters, Q-TOF Micromass (LC-MS) at SAIF, Punjab University, Chandigarh.

Synthesis of ethyl 2-oxo-2H-chromene-3-carboxylate (2)

Procedure

The mixture of 2-hydroxybenzaldehyde and diethyl malonate in 100 ml of ethanol was placed in a 500-ml round-bottomed flask equipped with a reflux condenser. To this mixture, 2.5 ml of piperidine and 0.25 ml of glacial acetic acid was added and the solution was heated under reflux for 3 hrs. The reaction was monitored with the help of TLC by using solvent system methanol: chloroform (5%) and when difference was observed between the spots of reactants and product, it means TLC complies.

Then the hot solution was transferred to an Erlenmeyer flask, the reaction flask was rinsed with 10 ml ethanol and the rinsed ethanol and 200 ml of water was added to the solution. The product crystallized readily as the solution cooled, the mixture was stirred from time to time as crystallization proceeded and was finally stored overnight in a refrigerator. The crystallized product was collected by filtration and washed with a solution of ethanol and water. The product was dried at room temperature [11].

Observation:

Melting Point	76-78°C
Percentage Yield	80%
Solvent system	Methanol : Chloroform (5%)
TLC	Reaction Complies
Molecular Weight (g)	218.06

Table 2: Spectral Data of Compound 2

IR (cm^{-1})		^{1}H NMR ($CDCl_3$, 400 MHz, ppm)	
1559	**(Ar. C=C)**	**1.39-1.43**	**(t,3H,-CH_3)**
1728	**(Ester C=O)**	**4.38-4.43**	**(q,2H,-CH_2-)**
2978	**(C-H str.)**	**7.31-7.67**	**(m,4H,Ar-H,coumarin)**
3060	**(Ar. C-H str.)**	**8.53**	**(s,1H,-CH=, coumarin)**

Synthesis of 2-oxo-2H-chromene-3-carbohydrazide (3)
Procedure: Hydrazine hydrate 99% was added to a mixture of ethyl 2-oxo-2*H*-chromene-3-carboxylate **2** in 200 ml ethanol. The mixture was refluxed for 6 hrs under constant stirring and the reaction was monitored with the help of TLC, developed with methanol: chloroform (5%) and the spots were visualized by spraying with ninhydrin reagent. When TLC complies, the reaction mass was crystallized out on cooling. It was then filtered, washed with excess of water and recrystallized from ethanol [12].
Observation:

Melting Point	136-138^{0}C
Percentage Yield	81.9%
Solvent system	Methanol: Chloroform (5%)
TLC	Reaction Complies
Molecular Weight (g)	204.18

Table 3: Spectral Data of Compound 3

IR (cm^{-1})		^{1}H NMR ($CDCl_3$, 400 MHz, ppm)	
1564	**(Ar. C=C)**	**5.42**	**(s,2H,-NH_2)**
1610	**(Amine N-H bend)**	**6.83-7.25**	**(m,4H,Ar-H,coumarin)**
1753	**(Ester C=O)**	**7.85**	**(s,1H,-CH=,coumarin)**
3276	**(Amine N-H str.)**	**11.07**	**(s,1H,-CO-NH-)**
3372	**(Amide N-H str.)**		

Synthesis of 3-(5-mercapto-1, 3, 4-oxadiazol-2-yl)-2H-chromen-2-one (4)

Procedure:
To a solution of 2-oxo-2*H*-chromene-3-carbohydrazide **3** in ethanol, eq. amount of KOH in water (q.s.) was added followed by carbon disulfide. The reaction mixture was heated under reflux till evolution of H_2S ceased. The reaction was monitored with the help of TLC, developed with ethyl acetate: hexane (20%). When TLC complies, the reaction mass was cooled, diluted with cold water and acidified with 10% HCl. The separated solid was collected by filtration, washed with water and crystallized from ethanol [13].
Observation:

Melting Point	228-2300C
Percentage Yield	75%
Solvent system	Ethyl acetate: Hexane (20%)
TLC	Reaction Complies
Molecular Weight (g)	246.24

IR (cm^{-1})		^{1}H NMR (DMSO, 400 MHz, ppm)		MS Q-TOF (m/z)
1021	**(C=O, oxadiazole)**	**3.47**	**(br. s,1H,-SH)**	**246.3 [*M*] 247.3[*M*+1]**
1566	**(Ar. C=C)**	**6.90-7.63**	**(m,4H,Ar-H,coumarin)**	
1613	**(C=N, oxadiazole)**	**8.96**	**(s,1H,CH=,coumarin)**	
1753	**(lactone, coumarin)**			
2372	**(S-H)**			

Table 4: Spectral Data of Compound 4

Synthesis of ethyl 2-(5-(2-oxa-2H-chromen-3-yl)-1,3,4-oxadiazol-2-ylthio)acetate (WJ-1)
Procedure:
A mixture of **4** and anhydrous potassium carbonate in 50 ml dry acetone was stirred at room temperature for 30 min. To this mixture, ethyl bromoacetate was added and the mixture was refluxed with stirring for 16 hrs. The reaction was monitored with the help of TLC, developed with ethyl acetate: hexane (30%). When TLC complies, the reaction mass was cooled, evaporated, then the residue was treated with dilute acetic acid, the solid product that obtained was collected by filtration, washed with water and crystallized from dioxane [14].
Observation:

Melting Point	118-120^{0}C
Percentage Yield	67%
Solvent system	Ethyl acetate: Hexane (30%)
TLC	Reaction Complies
Molecular Weight (g)	332.33

Table 5: Spectral Data of Compound WJ-1

IR (cm^{-1})		^{1}H NMR ($CDCl_3$, 400 MHz, ppm)	
1022	**(C=O, oxadiazole)**	**1.26-1.33**	**(m,3H,-CH_3)**
1160	**(C-S)**	**4.23-4.29**	**(q,2H,-CH_2-)**
1485	**(Ar. C=C)**	**4.72**	**(s,2H,-S-CH_2-CO-)**
1671	**(C=N, oxadiazole)**	**6.81-8.16**	**(m,4H,Ar-H,coumarin)**
1748	**(C=O, ester)**	**9.14**	**(s,1H,-CH=,coumarin)**

Various reagents used for synthesis of compound WJ-2
Procedure
A solution of **WJ-1** in 20 ml ethanol and NaOH in sufficient amount of water was stirred at room temperature for 2 hrs. The reaction was monitored with the help of TLC, developed with ethyl acetate: hexane (30%). When TLC complies, the reaction mass was cooled, then acidified with diluted HCl, the solid product that obtained was collected by filtration, washed with water and dried.

Observation:

Melting Point	226-228^{0}C
Percentage Yield	74.17%
Solvent system	Ethyl acetate: Hexane (30%)
TLC	Reaction Complies
Molecular Weight (g)	304.28

Table 5: Spectral Data of Compound WJ-2

IR (cm^{-1})		^{1}H NMR (DMSO, 400 MHz, ppm)	
1163	**(C-S)**	**4.70**	**(s,2H,-S-CH$_2$-CO-)**
1485	**(Ar. C=C)**	**6.92-7.99**	**(m,4H,Ar-H,coumarin)**
1707	**(C=O, carboxylic acid)**	**8.99**	**(s,1H,-CH=,coumarin)**
3400-2400	**(O-H, carboxylic acid)**	**10.40**	**(s,1H,-OH)**

Synthesis of Compounds (WJ-3,4,5,6)
General Procedure

To a solution of **4** in 20 ml ethanol, 5 ml of 10 % aqueous sodium hydroxide was added. To this solution, alkyl halide was added and the reaction mass was refluxed for 6 hrs. The reaction was monitored with the help of TLC, developed with benzene : hexane (9:1). When TLC complies, the reaction mass was cooled, evaporated, then the residue was treated with dilute acetic acid, the solid product that obtained was collected by filtration, washed with water and crystallized from dioxane [81].

Observation:

Compound No.	Melting Point	Percentage Yield
WJ-3	154-156^{0}C	52.1%
WJ-4	120-122^{0}C	51.2%
WJ-5	128-130^{0}C	43.5%
WJ-6	108-110^{0}C	45.6%

Solvent system	Benzene : Hexane (9:1)
TLC	Reaction Complies

Table 7: Spectral Data of Compounds WJ-3,4,5,6

Compound No.	IR (cm^{-1})
WJ-3	1021 (C=O, oxadiazole), 1154 (C-S), 1458 (-CH$_3$ bend), 1483 (Ar. C=C)
WJ-4	1038 (C=O, oxadiazole), 1155 (C-S), 1449 (-CH$_3$ bend), 1483 (Ar. C=C)
WJ-5	1147 (C-S), 1447 (-CH$_3$ bend), 1593 (Ar. C=C), 2924 (C-H str., alkane), 2961 (Ar. C-H str.)
WJ-6	1025 (C=O, oxadiazole), 1156 (C-S), 1595 (Ar. C=C), 2863 (C-H str., alkane), 2946 (Ar. C-H str.)

Compound No.	^{1}H NMR (CDCl$_3$, 400 MHz, ppm)
WJ-3	3.89 (s,3H,-CH$_3$), 6.91-8.09 (m,4H,Ar-H,coumarin), 8.68 (s,1H,-CH=,coumarin)
WJ-4	1.44-1.48 (m,3H,-CH$_3$), 4.07-4.13 (m,2H,-CH$_2$-), 6.91-8.13 (m,4H,Ar-H,coumarin), 9.12 (s,1H,-CH=,coumarin)
WJ-5	1.05-1.09 (t,3H,-CH$_3$), 1.81-1.90 (m,2H,-CH$_2$-), 3.97-4.01 (t,2H,-S-CH$_2$-), 6.91-8.13 (m,4H,Ar-H,coumarin),9.11 (s,1H,-CH=,coumarin)
WJ-6	0.89-0.93 (t,3H,-CH$_3$), 1.41-1.50 (sextet,2H,-CH$_2$-), 1.72-1.76 (m,2H,-CH$_2$-), 3.94-3.97 (t,2H,-S-CH$_2$-), 6.83-8.06 (m,4H,Ar-H,coumarin), 9.02 (s,1H,-CH=,coumarin)

Synthesis of Compounds (WJ-7,8,9)
General Procedure

A mixture of 3-(5-mercapto-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one **4**, 4-substituted phenacyl halides and base in sufficient amount of solvent was refluxed with stirring. The reaction was monitored with the help of TLC, developed with benzene: hexane (9:1). When TLC complies, the reaction mass was cooled, then acidified, the solid product that obtained was collected by filtration, washed with water and dried.

Observation

Compound No.	Melting Point	Percentage Yield
WJ-7	162-164^{0}C	44.7%
WJ-8	140-142^{0}C	47.9%
WJ-9	130-132^{0}C	45.8%

Solvent system	Benzene : Hexane (9:1)
TLC	Reaction Complies

Table 8: Spectral Data of Compounds WJ-7,8,9

Compound No.	IR (cm^{-1})
WJ-7	1056 (C-Br), 1121 (C-O), 1152 (C-S), 1574 (Ar. C=C), 1642 (C=C,alkene)
WJ-8	1083 (C-Cl), 1126 (C-O), 1182 (C-S), 1582 (Ar. C=C), 1644 (C=C,alkene)
WJ-9	1186 (C-F), 1094 (C-O), 1148 (C-S), 1592 (Ar. C=C), 1643 (C=C,alkene)

Compound No.	^{1}H NMR ($CDCl_3$, 400 MHz, ppm)
WJ-7	7.26-7.54 (m,4H,Ar-H,coumarin), 7.55 (s,1H,-S-CH=), 7.63-7.96 (m,4H,Ar-H)
WJ-8	7.32-7.53 (m,4H,Ar-H,coumarin), 7.55 (s,1H,-S-CH=), 7.62-8.04 (m,4H,Ar-H)
WJ-9	7.12-7.46 (m,4H,Ar-H,coumarin), 7.48 (s,1H,-S-CH=), 7.56-8.09 (m,4H,Ar-H)

Synthesis of 3-(5-(benzylthio)-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (WJ-10)

Procedure

A mixture of **4** and potassium tertiary butoxide in 20 ml dry acetone was stirred at room temperature for 30 min. To this mixture, benzyl bromide was added and the mixture was refluxed with stirring for 6 hrs. The reaction was monitored with the help of TLC, developed with benzene: hexane (9:1). When TLC complies, the reaction mass was cooled, evaporated, then the residue was treated with dilute acetic acid, the solid product that obtained was collected by filtration, washed with water and crystallized from dioxane.

Observation

Melting Point	172-174^{0}C
Percentage Yield	41%
Solvent system	Benzene : Hexane (9:1)
TLC	Reaction Complies
Molecular Weight (g)	336.36

Table 9: Spectral Data of Compound WJ-10

IR (cm^{-1})		^{1}H NMR ($CDCl_3$, 400 MHz, ppm)	
1041	**(C=O, oxadiazole)**	**5.14**	**(s,2H,-S-CH_2-)**
1148	**(C-S)**	**6.98-8.14**	**(m,9H,Ar-H)**
1595	**(Ar. C=C)**	**9.13**	**(s,1H,-CH=, coumarin)**
2866	**(C-H, alkane)**		
3150-3050	**(Ar. C-H)**		

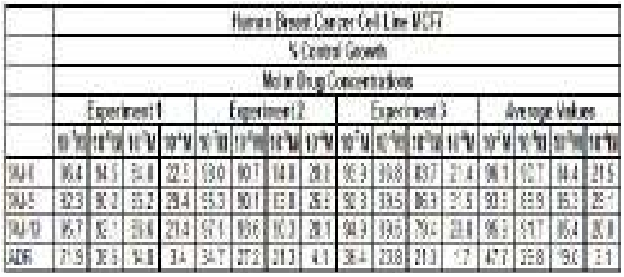
3. Results and Discussion

As per the proposed reaction scheme, the synthesis of 3-(5-mercapto-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one **10** derivatives (**WJ 1-10)** was carried out. The reaction was monitored by Thin layer chromatography using suitable solvent system such as Methanol : Chloroform, Ethyl acetate : Hexane and Benzene : Hexane. After the completion of reaction the product were purified by appropriate method. The melting point of the derivatives was determined. The melting point obtained was different from each other which confirmed the formation of new derivatives. All the derivatives were further characterized by various spectral techniques; IR, NMR, MS. IR data shows peaks at 1760-1730 cm^{-1} indicating presence of C=O, ester in compounds, peaks at 1600-1475 cm^{-1} indicating presence of C=C, aromatic ring in compounds, peaks at 1180-1100 cm^{-1} indicating presence of C-S in compounds.

The IR spectra of 1,3,4-oxadiazole is characterized by the peaks at 1640-1560 cm^{-1} (C=N) and 1020 cm^{-1} (C=O) . These derivatives were further characterized by ^{1}H NMR spectral data where the signals at 6.8 to 8.1 ppm confirmed the presence of aromatic protons. Finally some compounds were confirmed by the Mass Spectral data. Compound **4** showed [*M*] peak at 246.3 and [*M*+1] peak at 247.3. All the synthesized compounds were screened for anticancer activity against three cell lines: Human Breast Cancer (MCF7), Human Leukemia Cancer (HL60), Human Cervix Cancer (HeLa) by the Advanced Centre for Treatment Research and Education in Cancer (ACTREC) Mumbai, India.

All the environmental conditions were maintained throughout the experiment for all the groups. The growth curve was plotted against molar drug concentration of compounds and % control growth. *In vitro* testing was done using SRB assay protocols, each derivative was tested at 4 dose levels ($1x10^{-7}$M, $1x10^{-6}$M $1x10^{-5}$M $1x10^{-4}$M). Appropriate positive control was run in each experiment and each experiment was repeated thrice. Results are obtained in terms of GI_{50}, TGI and LC_{50} values. The experiment data were estimated using linear regression method of plots of the cell viability against the molar drug concentration of tested compounds. By the anticancer screening we have observed that the derivatives have shown significant activity against Human leukemia Cancer (HL60) cell line, good to moderate activity against Human Breast Cancer (MCF7) cell line and moderate activity against Human Cervix Cancer (HeLa) cell line. The results of the cytotoxicity assay show that the compounds have different responses of cytotoxicity against the selected cancer cell lines.

Anticancer Drug Screening Results



ISSN: 2321-3132 | CODEN (USA): IJCPNH

MCF7	LC50	TGI	GI50*
WJ-1	>100	>100	61.7
WJ-2	>100	>100	83.1
WJ-3	>100	>100	62.0
WJ-4	>100	>100	60.0
WJ-5	>100	>100	65.9
WJ-6	>100	>100	78.1
WJ-7	>100	>100	68.3

MCF7	LC50	TGI	GI50*
WJ-8	>100	>100	61.1
WJ-9	>100	>100	67.7
WJ-10	>100	>100	60.9
ADR	>100	>100	<0.1

	Human Leukemia Cell Line HL60															
	% Control Growth															
	Molar Drug Concentrations															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M
WJ-1	73.9	36.1	31.3	13.0	71.7	43.2	22.4	5.1	56.0	62.1	26.3	3.1	68.5	53.5	26.7	8.1
WJ-2	73.0	48.8	45.7	30.7	82.3	76.0	41.7	40.4	100.0	70.0	65.8	39.6	83.3	65.3	61.4	36.9
WJ-3	62.8	63.7	13.2	-8.1	55.5	53.5	41.2	-6.2	76.1	56.9	28.7	-6.1	65.2	54.7	26.7	-4.1
WJ-4	100.0	100.0	39.3	30.4	100.0	93.5	47.7	42.5	100.0	100.0	78.2	51.0	100.0	94.6	66.6	41.5
WJ-5	100.0	62.5	33.2	22.4	95.6	71.2	53.3	5.4	100.0	71.7	34.1	17.8	97.9	65.2	40.1	15.9
WJ-6	100.0	38.3	35.2	25.5	73.4	75.5	32.1	21.6	100.0	100.0	38.9	36.8	92.8	70.8	36.1	28.0
WJ-7	100.0	54.5	45.0	14.3	100.0	93.3	93.3	34.5	100.0	99.5	39.2	18.2	100.0	66.8	49.5	22.7

	Human Leukemia Cell Line HL60															
	% Control Growth															
	Molar Drug Concentrations															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M
WJ-8	83.1	74.2	72.5	55.3	85.5	73.3	50.9	13.4	79.5	69.7	62.2	23.2	84.0	74.4	61.9	32.6
WJ-9	100.0	100.0	77.8	75.8	100.0	100.0	54.3	47.5	78.4	70.6	60.3	24.2	92.1	91.2	61.6	49.1
WJ-10	76.4	41.7	41.2	40.1	75.5	63.3	43.3	37.5	58.7	40.0	31.5	21.6	70.3	48.5	38.8	33.1
ADR	48.8	37.7	4.7	-2.2	43.1	21.3	-4.3	-2.8	26.1	17.6	-3.5	-51.1	39.3	26.7	4.3	-10.0

HL60	LC50	TGI	GI50*
WJ-1	>100	>100	15.0
WJ-2	>100	>100	56.9
WJ-3	>100	90.6	3.8
WJ-4	>100	>100	77.7
WJ-5	>100	>100	37.0
WJ-6	>100	>100	43.9
WJ-7	>100	>100	46.9

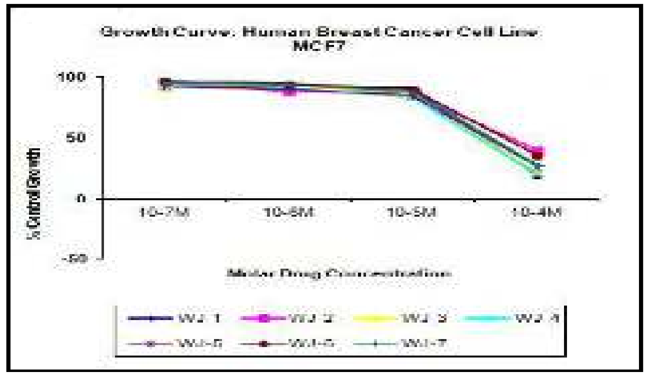
HL60	LC50	TGI	GI50*
WJ-8	>100	>100	59.1
WJ-9	>100	>100	93.7
WJ-10	>100	>100	19.6
ADR	>100	64.1	<0.1

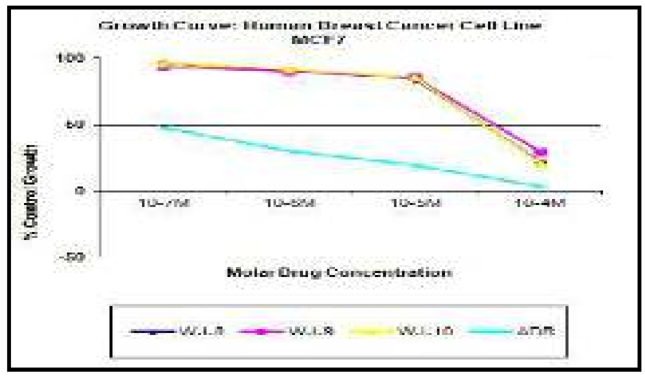
	Human Cervix Cancer Cell Line HeLa															
	% Control Growth															
	Molar Drug Concentrations															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M
WJ-1	100.0	102.0	100.0	68.4	100.0	100.0	102.0	60.0	100.0	100.0	100.0	64.4	100.0	100.0	102.0	66.6
WJ-2	100.0	102.0	100.0	84.3	100.0	100.0	102.0	77.9	100.0	100.0	100.0	75.1	100.0	100.0	102.0	79.3
WJ-3	100.0	102.0	100.0	72.1	100.0	100.0	102.0	79.6	100.0	100.0	100.0	76.1	100.0	100.0	102.0	75.6
WJ-4	100.0	102.0	100.0	68.2	100.0	100.0	102.0	59.0	100.0	100.0	100.0	54.3	100.0	100.0	102.0	58.1
WJ-5	100.0	102.0	100.0	78.7	100.0	100.0	102.0	82.5	100.0	100.0	100.0	73.2	100.0	100.0	102.0	78.1
WJ-6	100.0	102.0	100.0	62.5	100.0	100.0	102.0	63.2	100.0	100.0	100.0	90.1	100.0	100.0	102.0	95.3
WJ-7	100.0	102.0	100.0	72.1	100.0	100.0	97.2	62.0	100.0	100.0	100.0	76.6	100.0	100.0	95.1	71.2

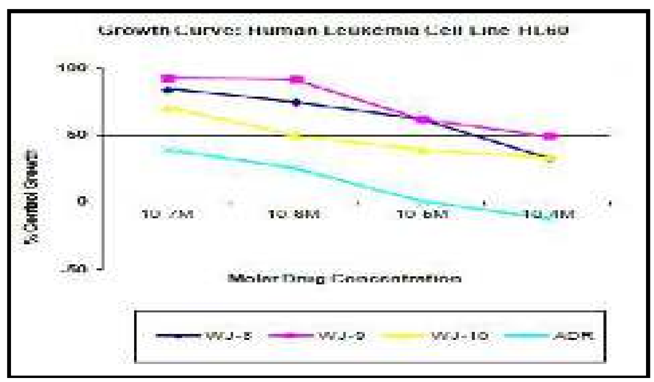
	Human Cervix Cancer Cell Line HeLa															
	% Control Growth															
	Molar Drug Concentrations															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M
WJ-8	100.0	100.0	87.2	13.4	100.0	100.0	100.0	14.1	100.0	100.0	90.3	16.3	100.0	100.0	95.2	14.5
WJ-9	100.0	88.5	96.4	42.1	100.0	100.0	100.0	34.9	100.0	100.0	90.4	47.6	100.0	96.5	98.3	43.2
WJ-10	100.0	100.0	100.0	-2.5	100.0	100.0	100.0	13.3	100.0	100.0	81.8	8.6	100.0	100.0	93.7	5.2
ADR	65.6	29.5	30.5	-29.4	54.5	30.4	16.1	-21.5	95.3	21.1	10.8	-31.7	61.5	36.0	18.4	-27.6

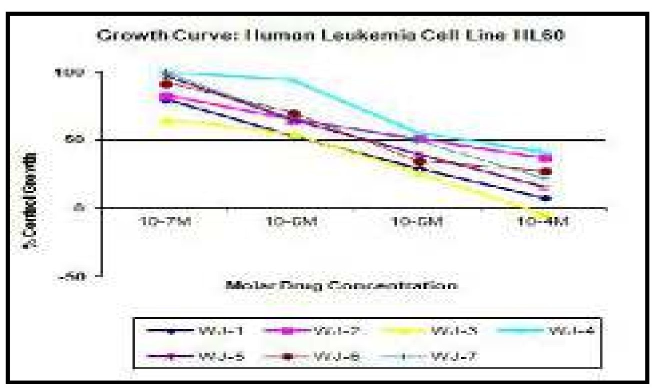
HeLa	LC50	TGI	GI50*
WJ-1	>100	>100	>100
WJ-2	>100	>100	>100
WJ-3	>100	>100	>100
WJ-4	>100	>100	>100
WJ-5	>100	>100	>100
WJ-6	>100	>100	>100
WJ-7	>100	>100	>100

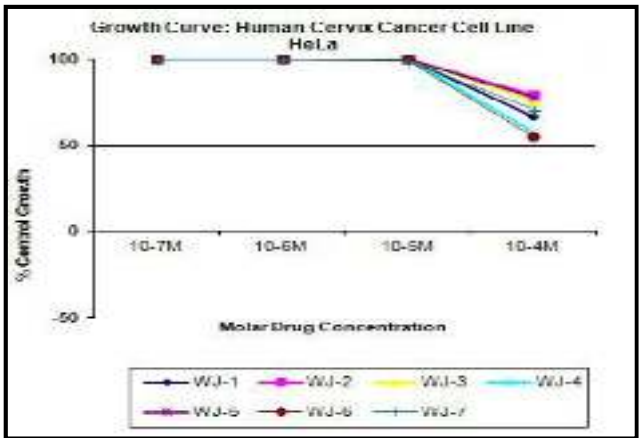
HeLa	LC50	TGI	GI50*
WJ-8	>100	>100	60.4
WJ-9	>100	>100	98.0
WJ-10	>100	>100	55.0
ADR	>100	58.8	<0.1

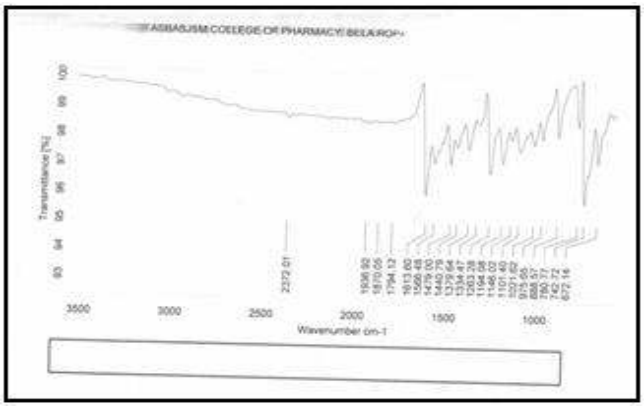




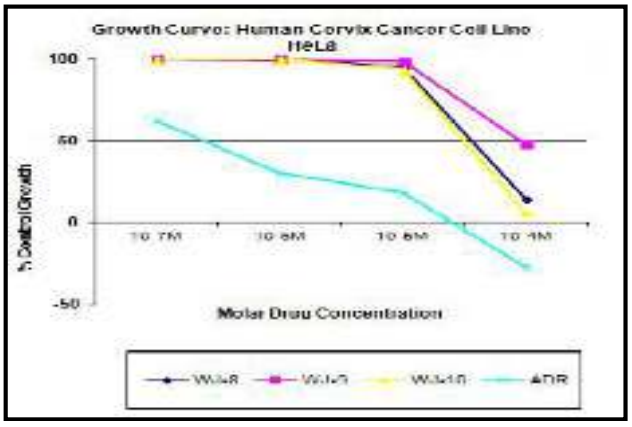


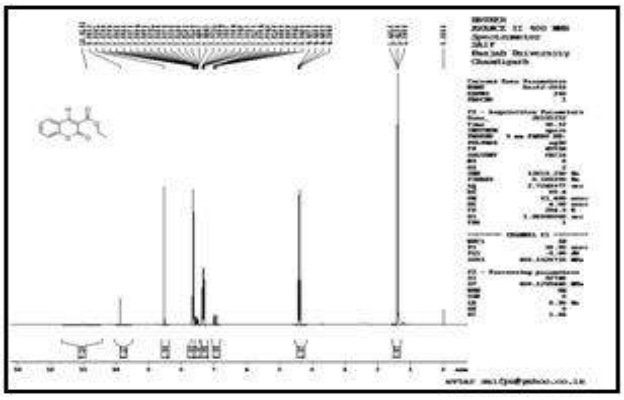




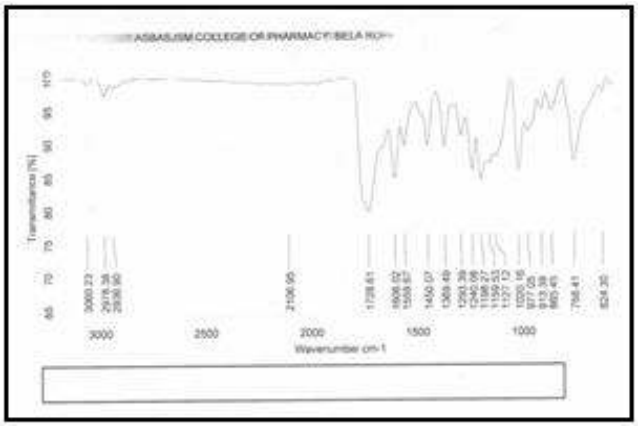


IR Spectra of Compound 4

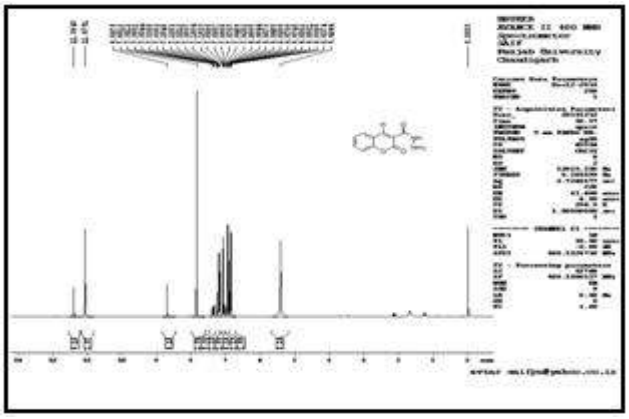




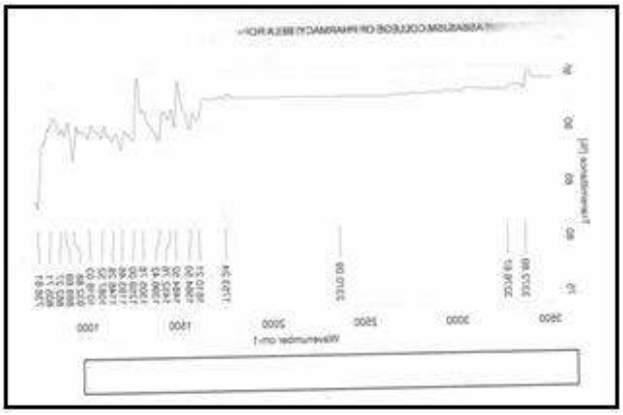
1H NMR Spectra of Compound 2



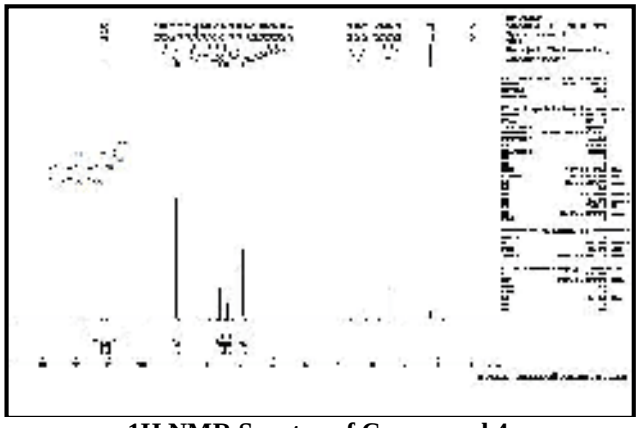
IR Spectra of Compound 2



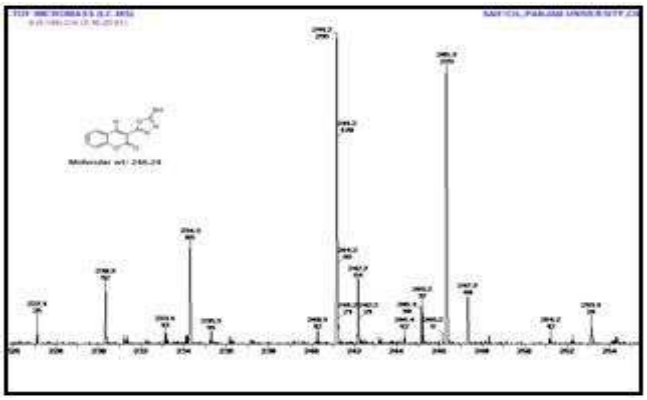
1H NMR Spectra of Compound 3



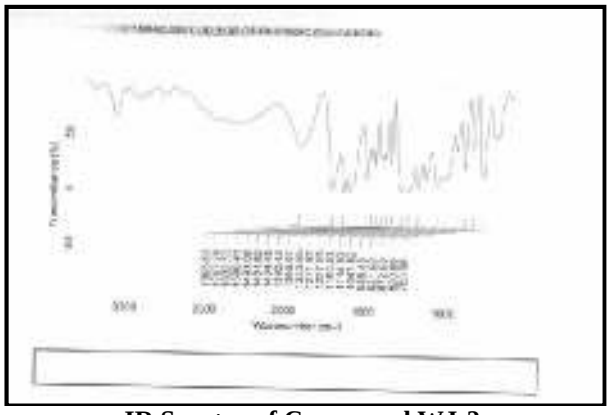
IR Spectra of Compound 3



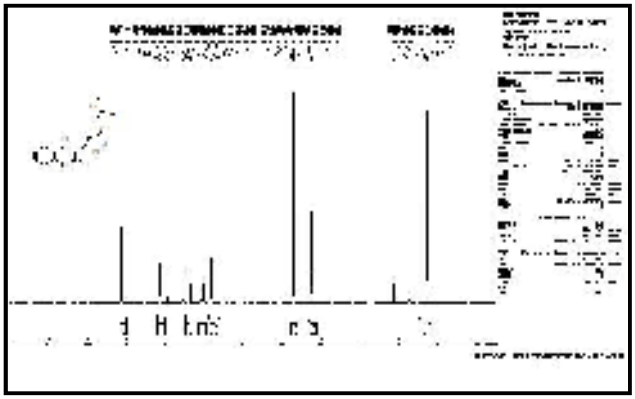
1H NMR Spectra of Compound 4



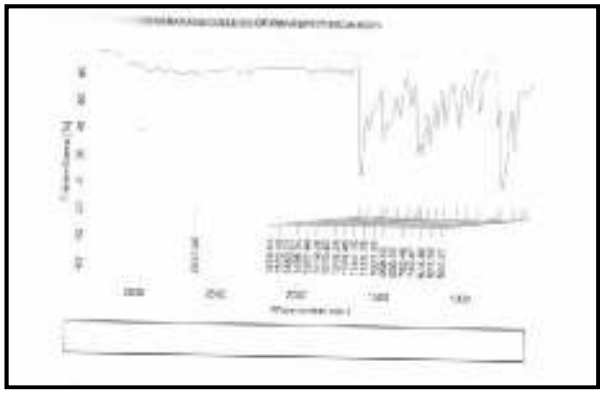
MASS Spectra of Compound 4



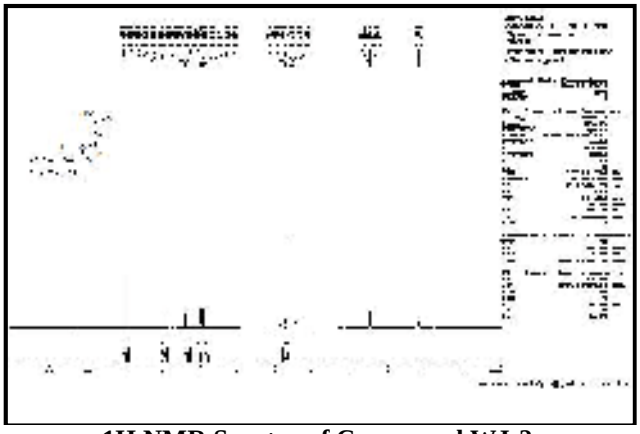
IR Spectra of Compound WJ-2



1H NMR Data of Compound WJ-1



IR Spectra of Compound WJ-3



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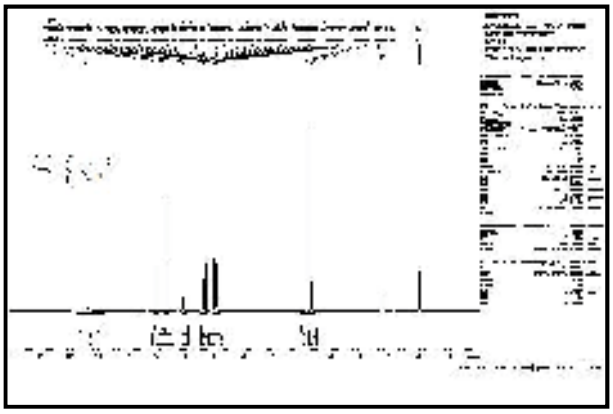
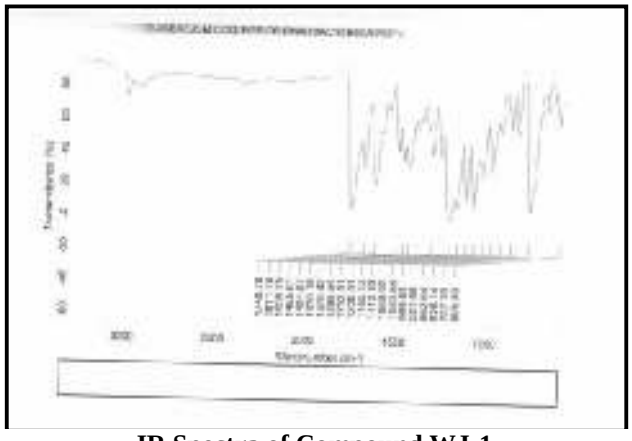
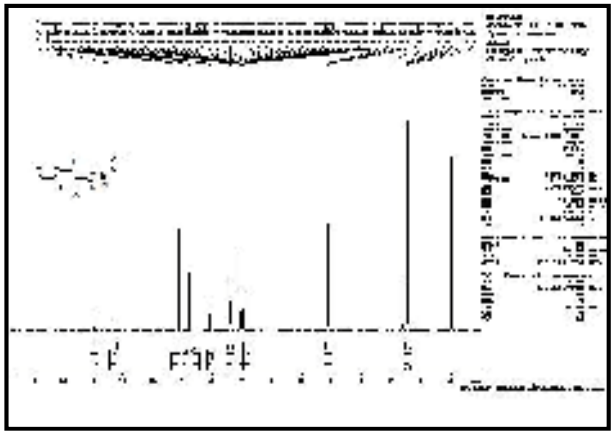


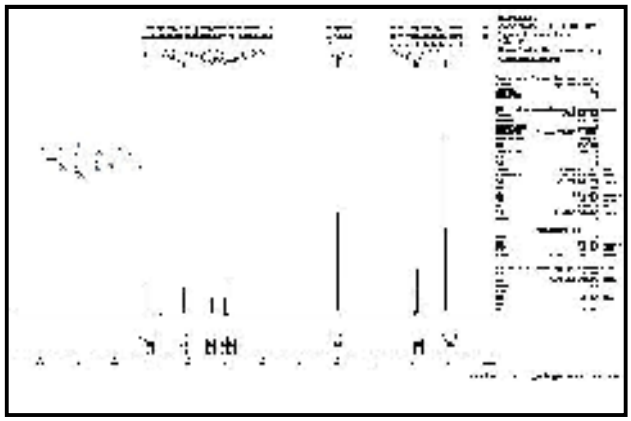
1H NMR Spectra of Compound WJ-3



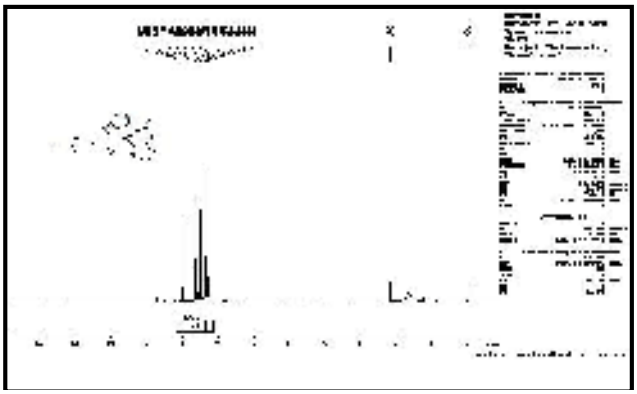
IR Spectra of Compound WJ-1



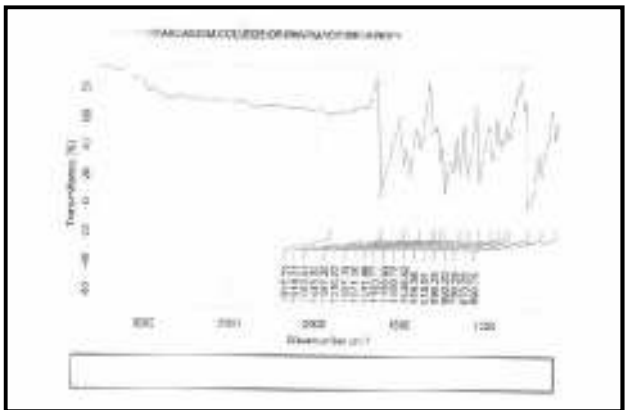
1H NMR Spectra of Compound WJ-4



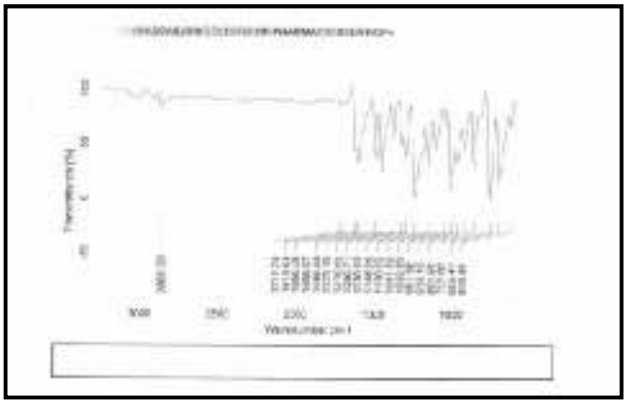
1H NMR Spectra of Compound WJ-5



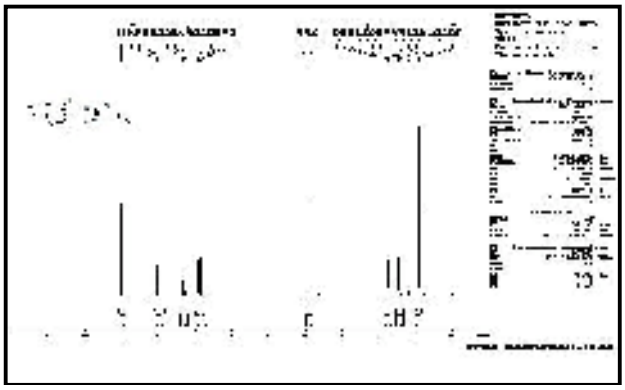
1H NMR Spectra of Compound WJ-8



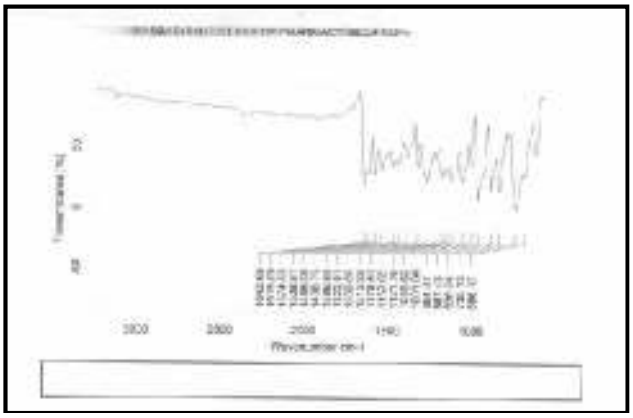
IR Spectra of Compound WJ-6



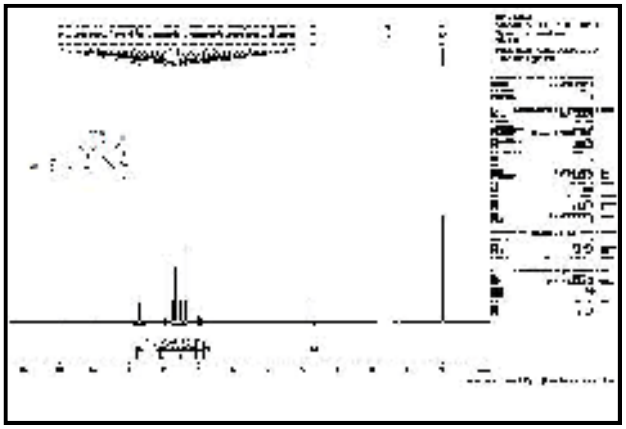
IR Spectra of Compound WJ-7



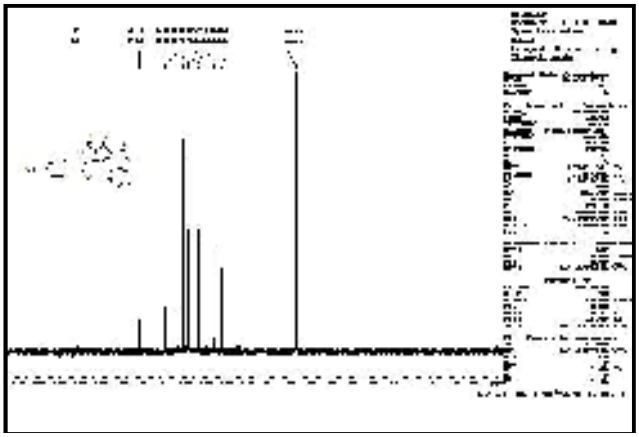
1H NMR Spectra of Compound WJ-6



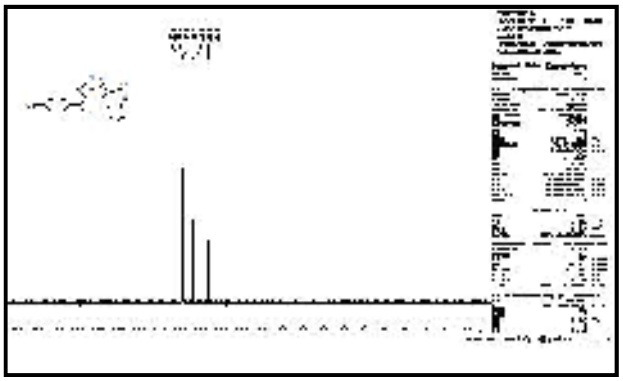
IR Spectra of Compound WJ-8



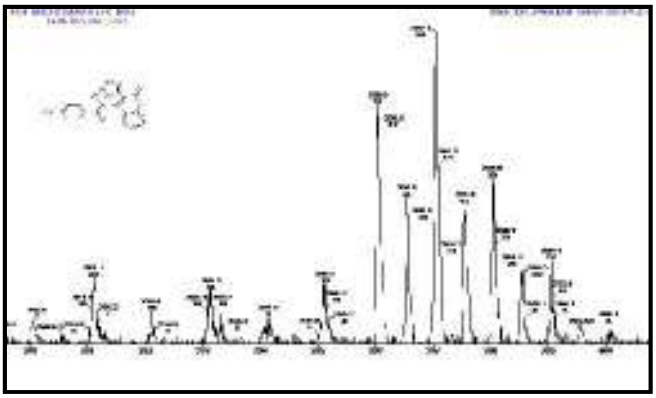
1H NMR Spectra of Compound WJ-7



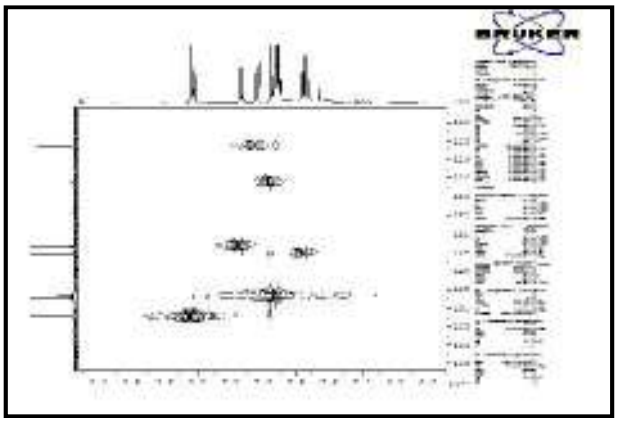
13C NMR SPECTRA OF COMPOUND WJ-8



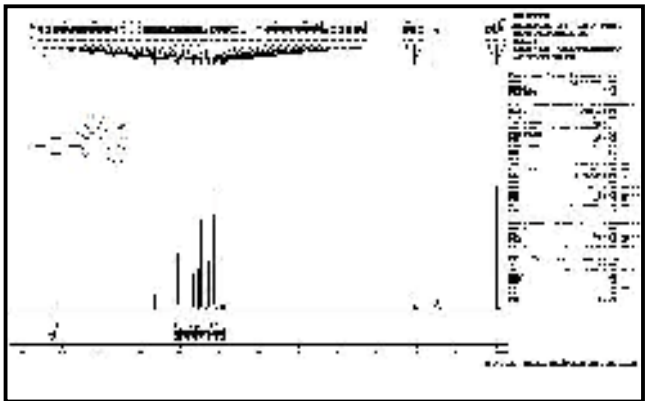
DEPT-135 NMR Spectra of Compound WJ-8



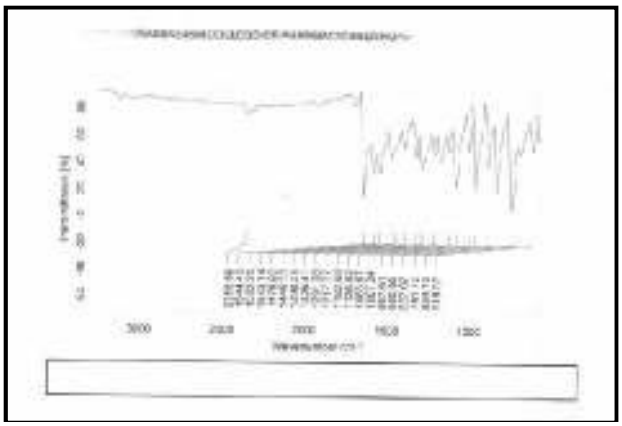
MASS SPECTRA OF COMPOUND WJ-8



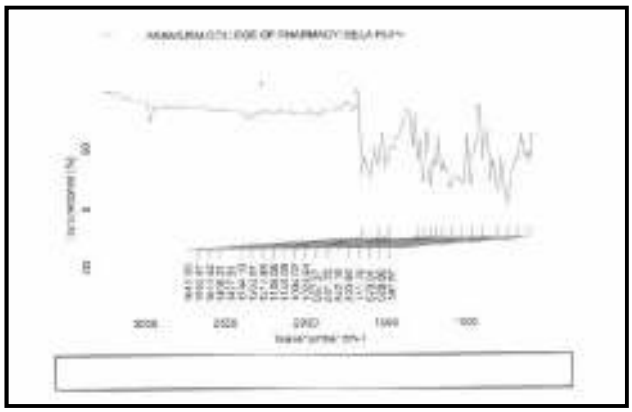
HSQC Spectra of Compound WJ-8



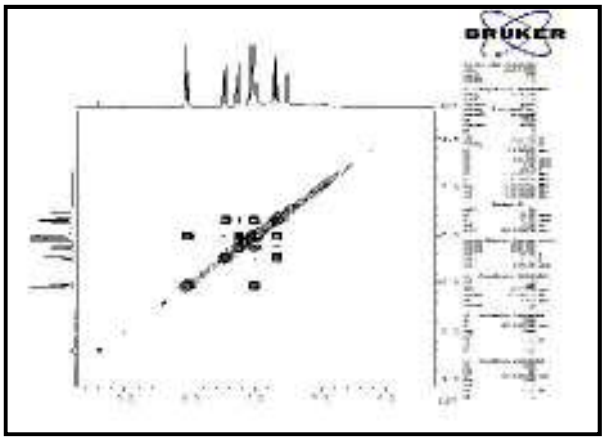
1H NMR Spectra of Compound WJ-9



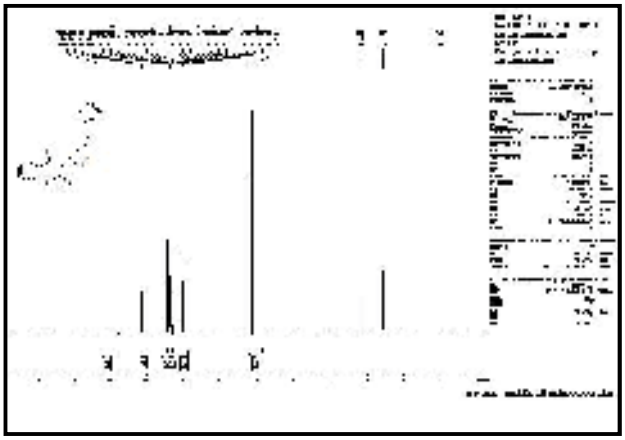
IR Spectra of Compound WJ-9



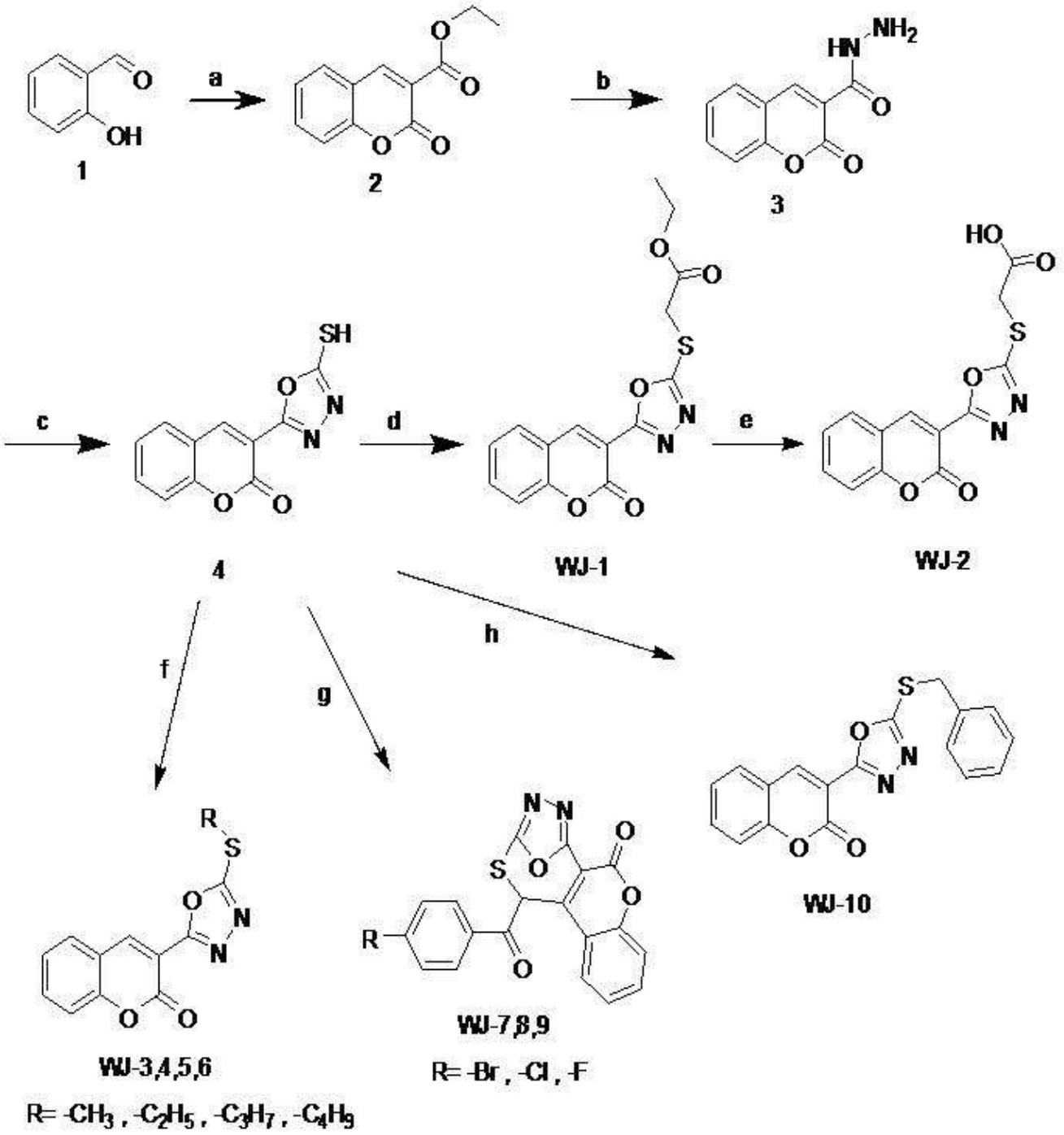
IR Spectra of Compound WJ-10



Cosy Spectra of Compound WJ-8



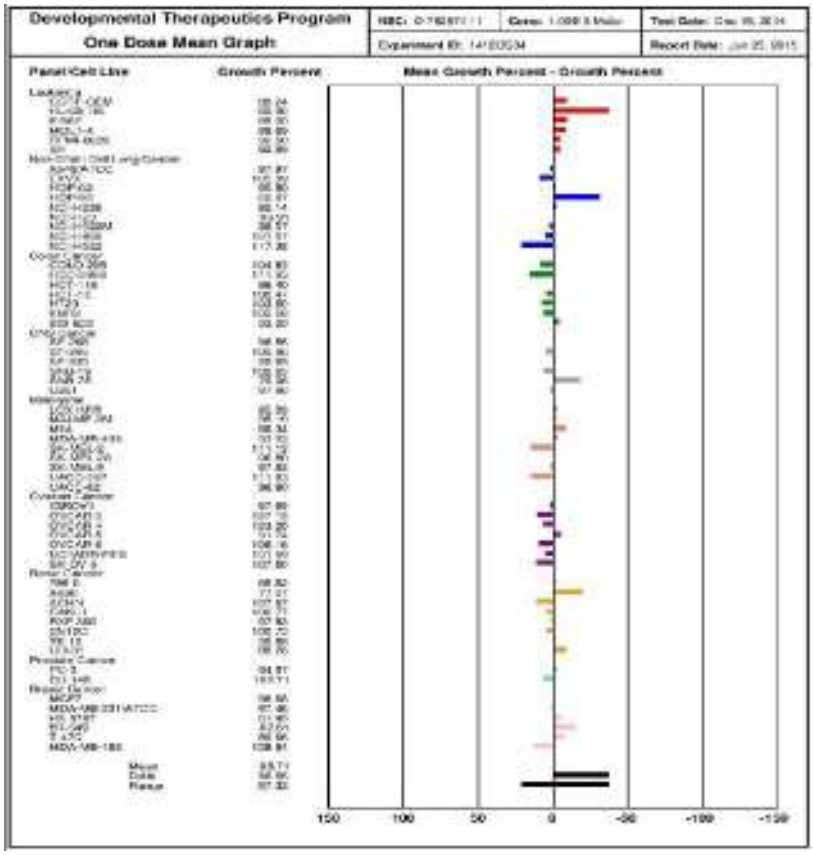
1H NMR Spectra of Compound WJ-10



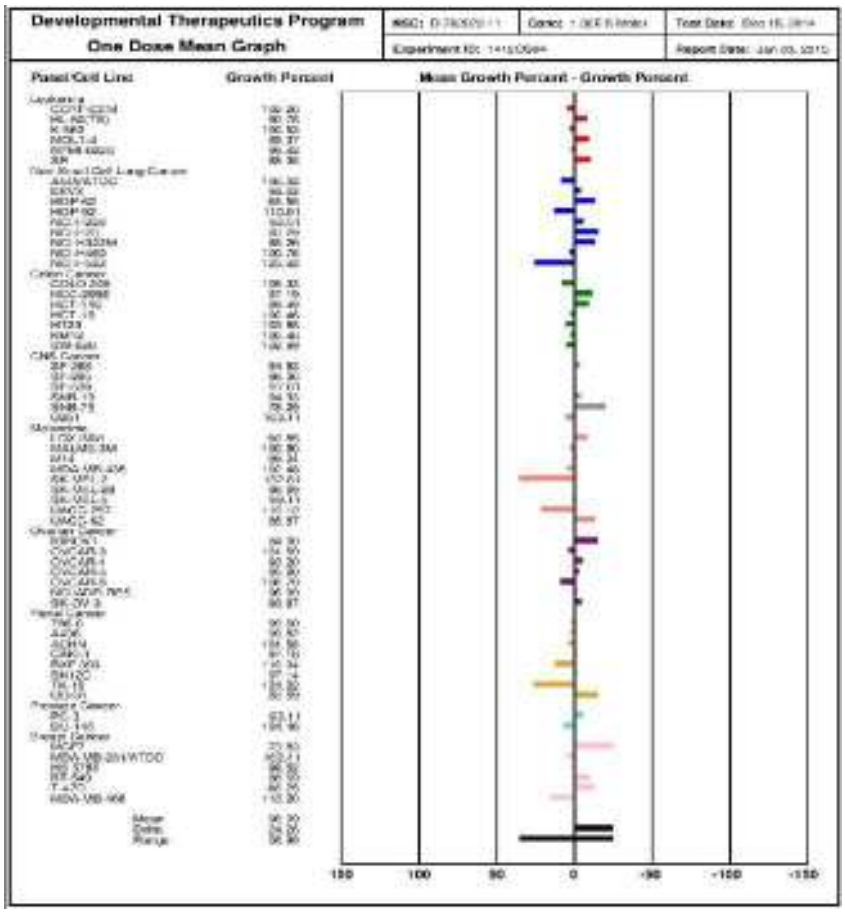
Scheme 1

Reaction conditions:

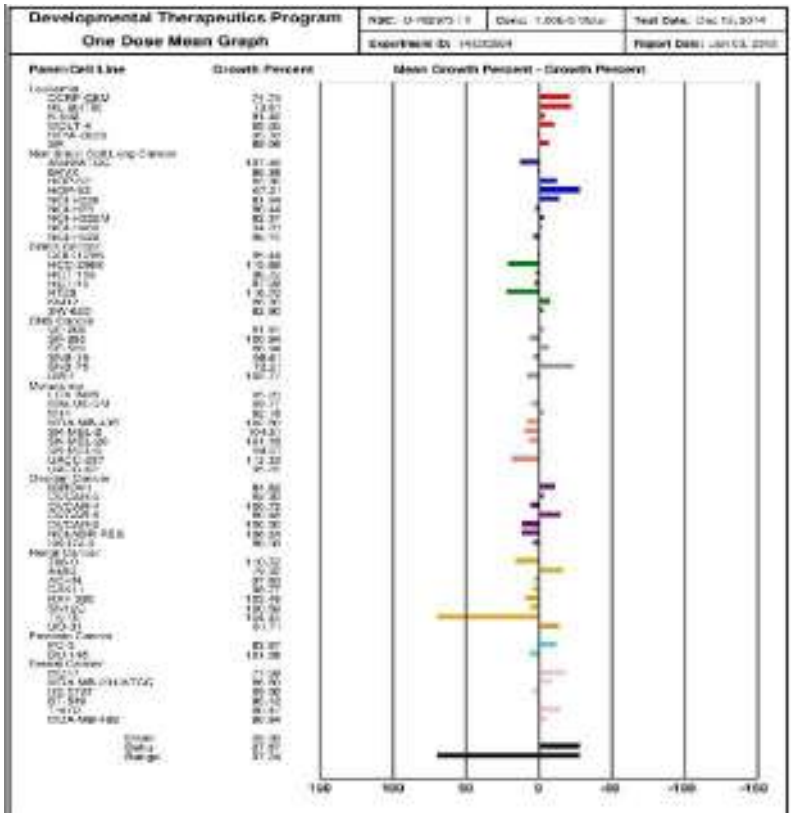
a. Diethyl Malonate, Piperidine, glacial acetic acid, refluxed for 3 hrs.
b. Hydrazine Hydrate 99%, ethanol, refluxed for 6 hrs.
c. CS_2, KOH, ethanol, refluxed till evolution of H_2S ceased.
d. Ethyl Bromoacetate, anhyd. K_2CO_3, dry acetone, refluxed with stirring for 16 hrs.
e. NaOH in water, ethanol, stirring for 2 hrs.
f. Alkyl Halides, ethanol, 10% NaOH solution, refluxed for 6 hrs.
g. 4-substituted phenacyl halides, base, solvent, refluxed with stirring
h. Benzyl Bromide, potassium t-butoxide, dry acetone, refluxed with stirring for 6 hrs.



Anticancer Drug Screening Results (NCI) (WJ-10)

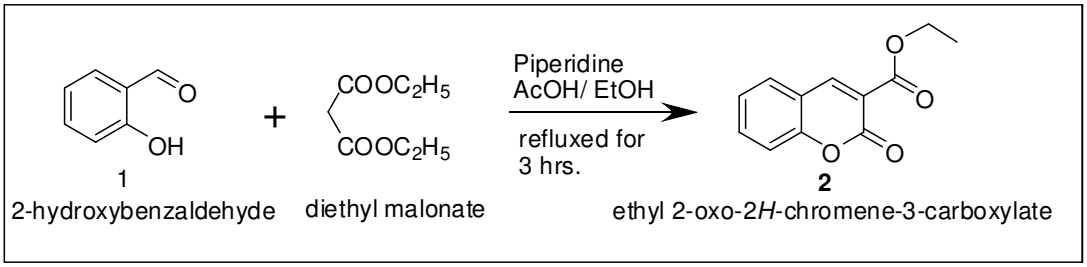


Anticancer Drug Screening Results (NCI) (WJ-8)



Anticancer Drug Screening Results (NCI) (WJ-4)

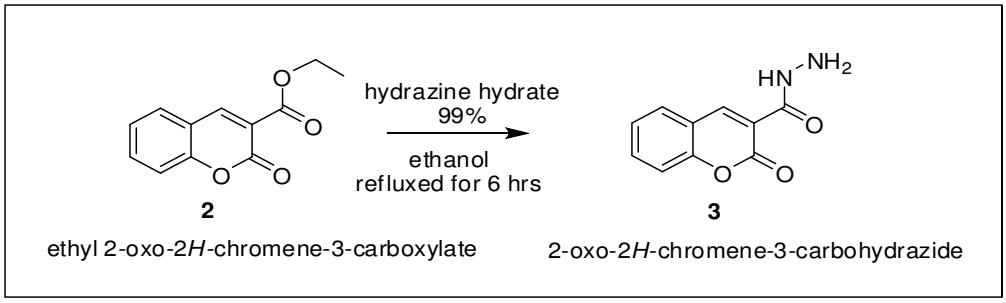
Synthesis of ethyl 2-oxo-2*H*-chromene-3-carboxylate (2)
Reaction



Various reagents used for synthesis of compound 2

Sr. No.	Chemicals used	Molecular weight (g)	Moles	Density (g/ml)	Equivalents
1.	2-hydroxybenzaldehyde **1**	122.12	0.250	1.15	1
2.	diethyl malonate	160.17	0.275	1.05	1.1
3.	glacial acetic acid	60.05	-	1.05	-
4.	piperidine	85.15	-	85.15	-

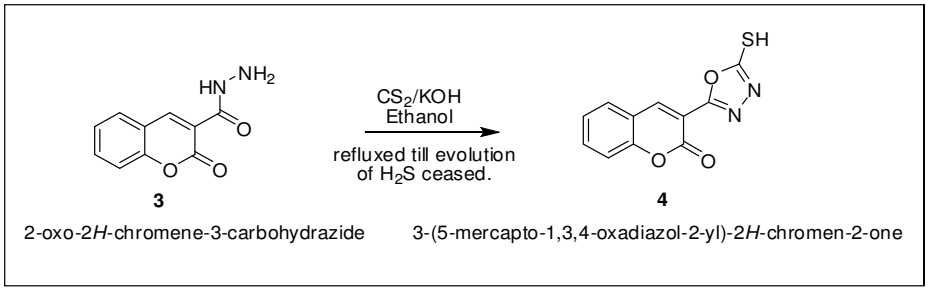
Synthesis of 2-oxo-2*H*-chromene-3-carbohydrazide (3)
Reaction



Various reagents used for synthesis of compound 3

Sr. No.	Chemicals used	Molecular weight (g)	Moles	Density (g/ml)	Equivalents
1.	ethyl 2-oxo-2*H*-chromene-3-carboxylate **2**	218.06	0.138	-	1
2.	hydrazine hydrate 99%	50.06	0.276	0.204	2

Synthesis of 3-(5-mercapto-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one (4)
Reaction



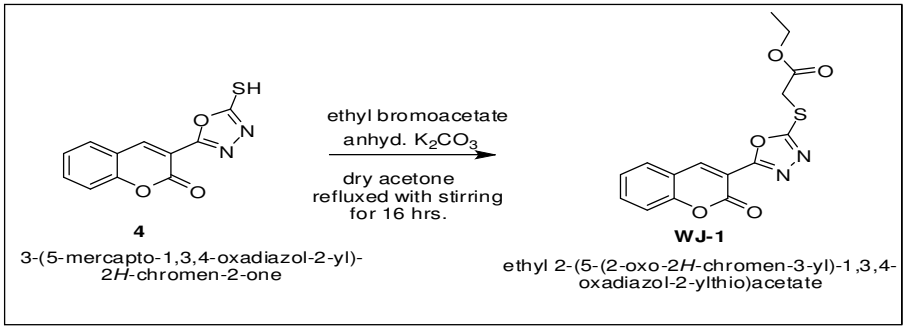
3 2-oxo-2*H*-chromene-3-carbohydrazide

4 3-(5-mercapto-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one

Various reagents used for synthesis of compound 4

Sr. No.	Chemicals used	Molecular weight (g)	Moles	Density (g/ml)	Equivalents
1.	2-oxo-2*H*-chromene-3-carbohydrazide **3**	204.18	.073	-	1
2.	CS_2	76	.220	1.260	3
3.	KOH	45	.073	-	1

Synthesis of ethyl 2-(5-(2-oxa-2*H*-chromen-3-yl)-1,3,4-oxadiazol-2-ylthio)acetate (WJ-1)
Reaction



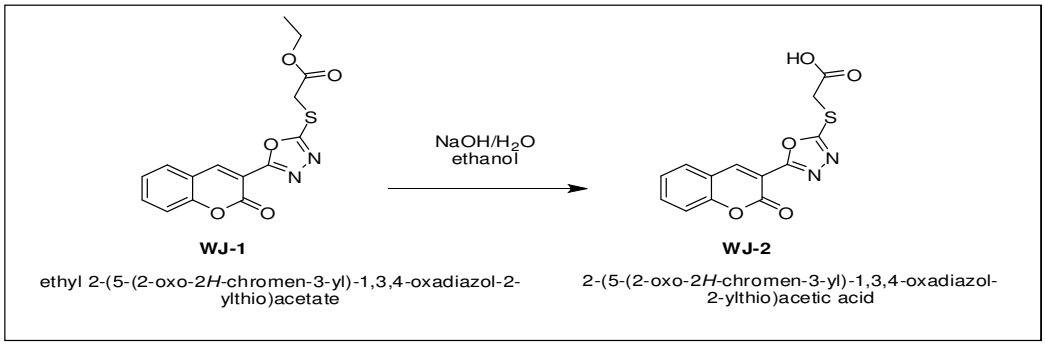
4 3-(5-mercapto-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one

WJ-1 ethyl 2-(5-(2-oxo-2*H*-chromen-3-yl)-1,3,4-oxadiazol-2-ylthio)acetate

Various reagents used for synthesis of compound WJ-1

Sr. No.	Chemicals used	Molecular weight (g)	Moles	Density (g/ml)	Equivalents
1.	3-(5-mercapto-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one **4**	246.24	.0081	-	1
2.	ethyl bromoacetate	167.01	.0162	1.51	2
3.	anhyd. K_2CO_3	138.21	.0243	-	3

Synthesis of 2-(5-(2-oxa-2*H*-chromen-3-yl)-1,3,4-oxadiazol-2-ylthio)acetic acid (WJ-2)
Reaction



WJ-1 ethyl 2-(5-(2-oxo-2*H*-chromen-3-yl)-1,3,4-oxadiazol-2-ylthio)acetate

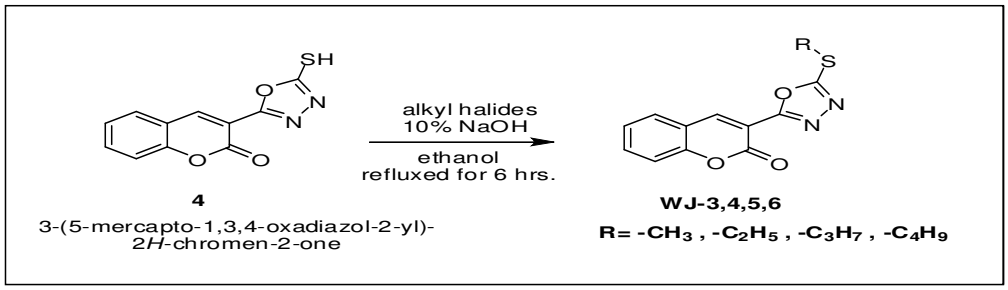
WJ-2 2-(5-(2-oxo-2*H*-chromen-3-yl)-1,3,4-oxadiazol-2-ylthio)acetic acid

Various reagents used for synthesis of compound WJ-2

Sr. No.	Chemicals used	Molecular weight (g)	Moles	Density (g/ml)	Equivalents
1.	**WJ-1**	332.33	.00063	-	1
2.	NaOH	40.00	.00126	-	2

Synthesis of Compounds (WJ-3,4,5,6)

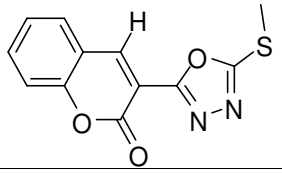
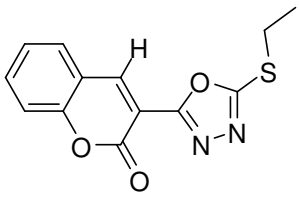
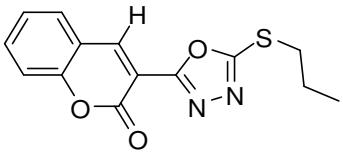
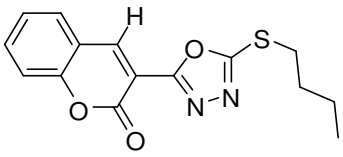
General Reaction



Various reagents used for synthesis of compounds WJ-3,4,5,6

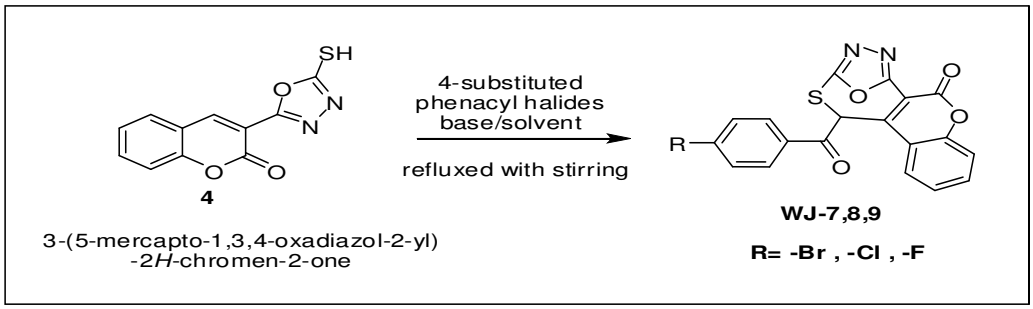
Sr. No.	Chemicals used	Molecular weight (g)	Moles	Density (g/ml)	Equivalents
1.	3-(5-mercapto-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one **4**	246.24	0.002	-	1
2. **Alkyl halide**	WJ-3 methyl iodide	141.94	0.004	2.28	2
	WJ-4 ethyl iodide	155.97	0.004	1.95	2
	WJ-5 n-propyl bromide	122.99	0.004	1.35	2
	WJ-6 n-butyl bromide	137.02	0.004	1.27	2

Products

Compound No.	Name	Molecular Weight (g)	Structure
WJ-3	**3-(5-(methylthio)-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one**	**260.27**	
WJ-4	**3-(5-(ethylthio)-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one**	**274.30**	
WJ-5	**3-(5-(propylthio)-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one**	**288.32**	
WJ-6	**3-(5-(butylthio)-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one**	**302.35**	

Synthesis of Compounds (WJ-7,8,9)

General Reaction

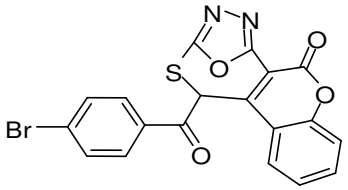
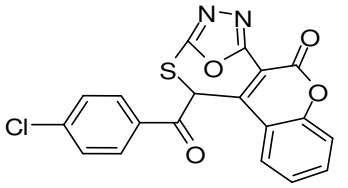
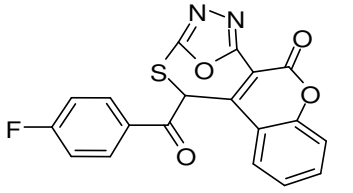


3-(5-mercapto-1,3,4-oxadiazol-2-yl) -2*H*-chromen-2-one

Various reagents used for synthesis of compounds WJ-7,8,9

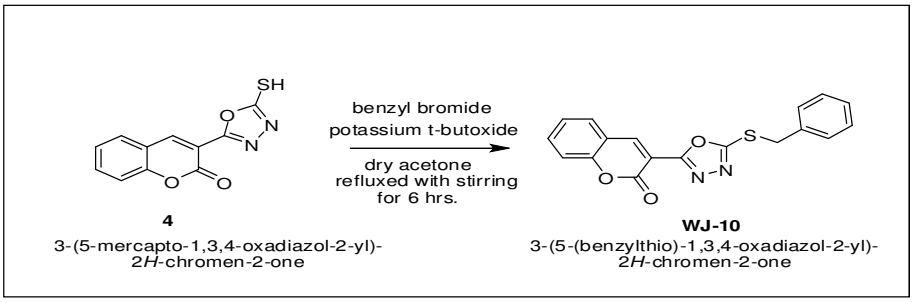
Sr. No.	Chemicals used	Molecular weight (g)	Moles	Density (g/ml)	Equivalents
1.	3-(5-mercapto-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one **4**	246.24	.002	-	1
2.	4-substituted phenacyl halides	-	.002	-	1

Products

Compound No.	Molecular Weight (g)	Structure
WJ-7	**441.25**	
WJ-8	**396.80**	
WJ-9	**380.35**	

Compound No.	Supporting Spectral Data for Structure Confirmation
WJ-8	**^{13}C NMR ($CDCl_3$,400MHz, ppm)** 112.58 (-S-CH=), 116.50, 123.38, 124.13, 126.38, 128.58, 128.92 (2C,Ar.-C,meta-position), 130.95 (2C,Ar.-C,ortho-position), 135.41 (Ar.-**C**-CO-), 139.47 (Ar.-C-Cl), 152.11, 156.04, 182.98 (-CO-) **DEPT-135 ($CDCl_3$,400MHz, ppm)** Positive peaks**(CH):** 112.58, 116.50, 123.38, 124.13, 128.58, 128.92, 130.95 Negative peaks**(CH_2)**: absence **MS Q-TOF (m/z)** 396[*M*], 397.1[*M*+1], 398[*M*+2], 399[*M*+3], 400[*M*+4] **COSY & HSQC NMR SPECTRA** also confirms the structure.

Synthesis of 3-(5-(benzylthio)-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one (WJ-10)
Reaction



4
3-(5-mercapto-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one

WJ-10
3-(5-(benzylthio)-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one

Various reagents used for synthesis of compound WJ-10

Sr. No.	Chemicals used	Molecular weight (g)	Moles	Density (g/ml)	Equivalents
1.	3-(5-mercapto-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one **4**	246.24	.002	-	1
2.	benzyl bromide	171.04	.004	1.44	2
3.	potassium t-butoxide	112.22	.006	-	3

4. Conclusion

All the synthesized compounds were screened for anticancer activity against three cell lines: Human Breast Cancer (MCF7), Human Leukemia Cancer (HL60), Human Cervix Cancer (HeLa) by the Advanced Centre for Treatment Research and Education in Cancer (ACTREC) Mumbai, India. The anticancer data reveals that the derivatives have shown significant activity against Human leukemia Cancer (HL60) cell line, good to moderate activity against Human Breast Cancer (MCF7) cell line and moderate activity against Human Cervix Cancer (HeLa) cell line. It was concluded that the synthesized derivatives act as anticancer agents and the compounds have different responses of cytotoxicity against the selected cancer cell lines.

5. References

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