Article

Synthesis of Modern Amides Thiazolidine Derived From L-Cysteine and Investigation of Their Biological Effects on Cancer Cells

Ramla Fayiq Ibrahim[a] , D. S. Abid[b]

Department of Chemistry, College of Education for Pure Sciences, University of Basra, Iraq

Email: aliali2088h@gmail.com [a], dawodsalim@yahoo.com [b]

Abstract

The study included the preparation and characterization of new compounds called thiazolidine-4-carboximaide in three main steps. In the first step, compound (R) was reacted from the reaction of cysteine with different aromatic aldehydes. Then the compound (R)was reacted with acetic anhydride to prepare the compound (RA). The compound (RA) was reacted with the aromatic amine (phenyl hydrazine) and DCC,HOBT to obtain different amides-4-thiazolidine compounds. All compounds were diagnostic using organic description techniques, FT-IR,magnetic resonance spectroscopy, ^{1}HNMR, and ^{13}CNMR, mass spectroscopy, every the prepared Compounds gave perfect returns. and biological effect prepared compounds:[ML1 ,ML2, ,ML4,ML7 ML8,ML9] was studied against cancer cells for two types of cancer lines: human breast cancer line (MCF7) cells, women's cervical cancer line (Hela), The biological effectiveness of the prepared compoundsML1,ML2, ML4 ML7,ML8 and ML9, was studied against cancer cells for two types of cancer lines, a Michigan cancer foundation (MCF7) and a female cervix carcinoma (Hela). The results of the inspection showed that the compounds [ML1,ML2, ML4,ML8 ,ML9] had high efficacy against a mankind breast cancer cell line and a cell line. Cervical cancer and compound[ML7] showed little activity on the two types of cancer cell lines. and calculated Inhibition percentage (%) of prepared compounds effects a Michigan cancer foundation (MCF7) and a female cervix carcinoma (Hela).

Key words: Anticancer MCF-7,HeLa,L-Cysteine, Thiazoldines-4- Carboximide.

1. Introduction

Thiazolidine is one of the most important heterocyclic compounds derived from thiazole (1). Researchers have been interested in Thiazolidine due to its biological and industrial importance (2)(3) as it has an important role in organic synthesis, bioorganic synthesis and medicinal chemistry, where most antibiotics are natural products such as penicillin and sulfosporin and also used as antibacterials and antioxidants. As an anti-cancer (4), the carboxylic acid-4-thiazolidine represents a

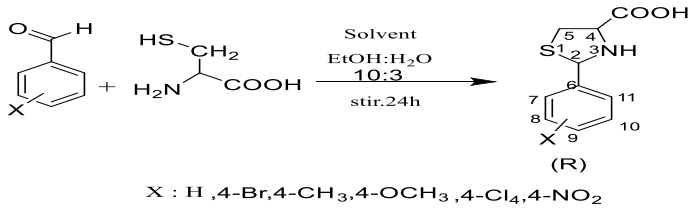
cyclic sulfur amino acid similarity in install it compound proline, so it is known thio-proline. It is produced from L-cysteine and formaldehyde (5), It also works as an effective detector of the nitrite pathway in the humans (6) and for compounds. Thiazolidine is important because it has biological activity against cancer, which is considerable occasion of demise in many countries of the world. The number of deaths in 2020 was about 20 thousand deaths due to cancer, where normal cells turn into malignant cells. This occurs between the interaction of the individual's genetic material with external factors are as ultra violet radiation and rays(7), Visible and chemical factors such as asbestos and arsenic. Breast cancer is one of the most dangerous types of cancer(8)(9), as uncontrolled cells that are different from the growth of cells are called breast cancer (10)(11). Among the factors that lead to breast cancer are smoking, pharmaceutical preparations, chemical radiation, alcohol consumption, and genetic factors. Cervix carcinoma prepare tertiary created wide spread tumor among women and the main occasion of demise in women with cervical cancer. It arises when changes occur in DNA and RNA, or other factors such as smoking, birth control pills, exposure to radiation, or weak immunity of the individual lead to the occurrence of Cervix carcinoma (12). Compounds were dealt with in this study are thiazolidine and its derivatives that have biological activity against breast cancer and cervical cancer, while 3-acetyl4-thiazolidine carboximide compounds have biological activity against two types of cancer cell lines. Important role in cellular homeostasis is a precursor for protein synthesis. In this study, we will show and represented of combining substituted benzaldehyde biological activity .

2. Materials and Methods

Melting points were measured and determined by with ^{1}HNMR spectra were also recorded on a Brucker-400MHz spectrometer in DMSO-*d6* in the presence of the TMS compound as an internal reference. Chemical shifts have been reported regarding the residual solvent coupling steady are conveyed in Hertz. The abbreviations for the signs used are as follows: s (single) , d (double) , t (triple), m (multiple) and dd (double doubling). Mass spectra were also recorded on an Agilent mass spectrometer 5975 quadruple analyzer with Electronic Impact Technology (EI) .

2.1.1General method for preparing compounds [R1,R2,R4, R7,R8,R9]

0.01 mole(1.49g) of L-Cysteine was mixed with an equivalent amount of aromatic aldehyde in a 250ml Erlenmeyer flask in a mixture of ethanol (16ml) and water (5ml) with continuous magnetic shaking at room temperature for 10 hours where a white precipitate was notice. The precipitate was filtered, dried and recrystallized with a mixture of ethanol and water at a percent of (10:3), and the chemical reaction was press by application Thin Layer Chromatography (TLC) using a eluent of (Ethanol: Chloroform) at a ratio of (1:9) [13].

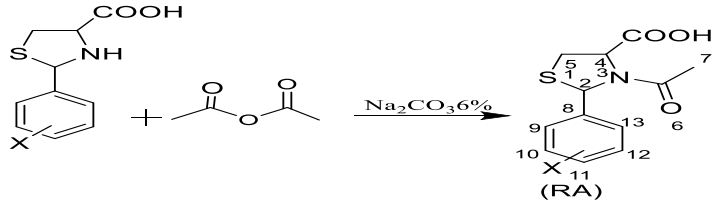


X : H ,4-Br,4-CH_3,4-OCH_3 ,4-Cl_4,4-NO_2

The method1: for making thiazolidine and its compounds

2. 2 General method for preparing compounds [RA1,RA2, RA4,RA7,RA8,RA9]

0.01 mole (2.36g) of [R1,R2,R4,R7,R8,R9] was dissolved in a (55 ml) solution of sodium carbonate (Na_2CO_3)%6) in a 150 ml Erlenmeyer bottle, then the reaction was cooled in an integument bath to 0°C percent (1.02mmol)) was added to the reaction mix. (10 m mole) of acetic anhydride in drops over five minutes. The reaction mixture was left on the magnetic shaker for an hour, after stopping the reaction mixture was acidified using a dilute solution of hydrochloric acid (HCl) 5%) and a satiate solution of sodium chloride (NaCl) was added to it for the purpose of equivalence, and the acidified reaction mix was extract by ethyl ethanoate (2x50ml) . The extracted organic layer was rinsed in water while dried exercise no aqueous salt cake (Na_2SO_4) and vaporization the solvent . After that, it formed a white solid.The crystallization mixture of methyl alcohol with distilled water in a ratio of (3:1) . The reaction was followed by Thin Layer of chromatography (TLC) technique using eluent (acetic ester: ethyl alcohol) by amount (6:4) [14].



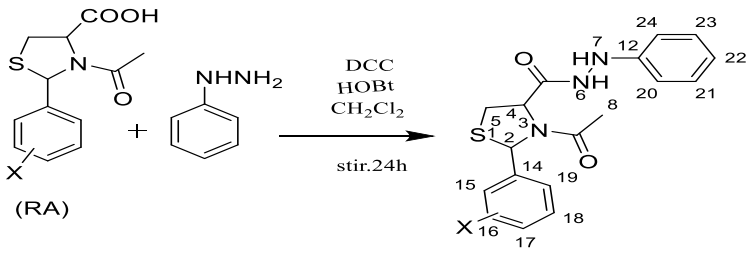
X : H ,4-Br,4-CH_3,4-OCH_3 ,4-Cl_4,4-NO_2

2.2Acetyl thiazolidine and its derivatives preparation equation

2.3 Preparation of 3 -acetyl-N',2-diphenylthiazolidine-4-carbohydrazide

0.004 mole (1.11 g) of [RA1, RA2, RA4, RA7, RA8, RA9] in a circular flask (100 ml) with (0.004 mole) (0.82g) of N,N -′dicyclohexylcarbodiimide (DCC) and 0.54 g (0.004 mole of 1-Hydroxybenzotriazole (HOBt) in (20 ml) of dichloromethane as a solvent and left the solution with continuous magnetic shaking at zero degrees Celsius for (10) minutes, until the solution became clear, then (0.004) mole of amine(Phenyl Hydrazine) was added to the reaction mix and left under continuous magnetic shaking for 24 hours at room temperature. after that, the formation a white solution be observed and then the reaction mixture was to get rid of the sediment by filtration, which one of them di-cyclohexylurea (DCU). The filtrate was taken and

diluted with dichloromethane (20 ml), then the mixture was washed successively with a solution.
(5%) sodium bicarbonate ($NaHCO_3$) , then with citric acid (10%), then a saturated solution of sodium chloride (NaCl) and finally with distilled water . The solution was dried, and I added anhydrous magnesium sulfate (MgSO4), then filtered and evaporated to make a precipitate recrystallized by a mixture of ethyl alcohol and H2O (1:1) . The chemical reactor was carried out by Thin Layer of Chromatography (TLC) the used a solvents (Formic acid, benzene and tetrahydrofuran) at a ratio of(2:6:) [15].

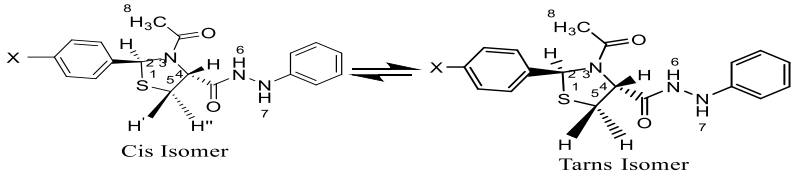


X : H ,4-Br,4-CH_3,4-OCH_3 ,4-Cl_4,4-NO_2

2.3Acetyl thiazolidinediamide and its derivatives preparation equation

2.4 Test the effectiveness of some prepared compounds on inhibiting breast cancer cells (MCF7) and cells Cervical cancer(Hela).

The Activity biological of the prepared samples was measured on MCF7 breast cancer line cells and cells Cervical Cancer line (Hela). It was conducted using bromide(3(4,5-dimethyl-2-thiazole-2-yl), 5- diphenyltetrazolium Bromide) (MTT), where the cells were grown in dishes, each dish contained 96 holes, for a full day under ideal conditions of temperature, pressure, and environmental conditions, including At 37 degrees Celsius, in an atmosphere of 5% carbon dioxide, and in a humid atmosphere. After that, the fetal serum media (10% FBS) was removed for cell growth, and the cells were washed twice with phosphate buffer solution (PBS). New culture media was used. It contained diluted concentrations of the compounds to be tested, which were (µg/ml 100), and the cells from the samples were incubated for 48 hours. The gaps in the plate were analyzed for each concentration by adding 10 microliters of freshly prepared 5 MTT in Buffer's phosphate solution and 100 ml of DMSO, then stirring the plates to ensure the crystals dissolved. Each concentration of the prepared compounds was re-measured three times, and the absorbance was measured at a wavelength of 570 nm using BioTak device, and calculated Inhibition percentage (%) of prepared compounds against breast cancer and cervical cancer cell line. [16].



(2S,4R)-3-acetyl-N',2- diphenylthiazolidine-4-carbohydrazide (trans Isomer)(70%) ML1

Yield: 70% m.p:103-104□C, ^{1}HNMR (400 MHz, DMSO-d6) δ 2.33 (s,3H) (H8), 3.10(s,1H)) (H5b), 3.33 (s,1H) (H5a), 4.94 (s,1H) (H4), 6.44(s,1H) (H2), 6.69-7.99(dd,10H) (HAr), 10.00 (s,1H) (H6), 10.20 (s,1H) (H7).FT-IR (KBr disk): 3387w (NH), (3286w)(C-H Ar), 2926w(C-H),1712 w (C=O Amide). Mass spectra (70 ev): M·+ =341.43 m\z

(2R,4R)-3-acetyl -N',2-diphenylthiazolidine-4-carbohydrazide (Cis Isomer)(30%) ML1

Yield: 70% m.p:103-104□C, ^{1}HNMR (400 MHz, DMSO-d6)δ 2. 04 (s,3H) (H8),, 1.68 (s,1H) (H5b), 2.55 (s,1H), (H5a), 4.69 (s,1H) (H4), 6.33(s,2H) (H2), 6.69-7.99, (dd,9H) (HAr), 10.01 (s,1H) (H6), 10.21 (s,1H) (H7), FT-IR (KBr disk): 3387w (NH), (3286w)(C-HAr), 2926w(C-H),1712 w (C=OAmide), Mass spectra (70 ev): M·+ =341.43 m\z

(2S,4R)3-acetyl-2-(4-bromophenyl)N,-phenyl thiazolidin-4- carbohydrazide (trans Isomer) (60%) ML2

Yield: 75% m.p:106-107□C, ^{1}HNMR (400 MHz, DMSO-d6) δ2.19 (t,3H) (H8), 3.14(dd,1H)(H5b),3.35 (dd,1H) (H5a), 4.95(m,1H) (H4),6.37 (t,2H) (H2), 6.85-8.60 , (d,t,9H) (HAr), 10.08 (s,1H) (H6), 10.32 (s,1H) (H7), FT-IR (KBr disk):3294w (NH),3061w (C-H Ar),2927w (C-H),1712 w (C=OAmide), Mass spectra (70 ev): M·+ =420.30 m\z

(2R,4R)-3-acetyl-2-(4-bromophenyl)N,-phenyl thiazolidin-4-carbohydrazide(Cis Isomer)(40%) ML2

Yield: 75% m.p:106-107□C, ^{1}HNMR (400 MHz, DMSO-d6) δ2.09 (t,3H) (H8), 3.12(dd,1H)(H5b),3.18 (dd,1H) (H5a), 4.66(m,1H) (H4),6.32 (s,2H) (H2), 6.85-8.60 , (d,t,9H) (HAr), 10.08 (s,1H) (H6), 10.32 (s,1H) (H7), FT-IR (KBr disk): 3294w (NH),3061w (C-H Ar),2927w (C-H),1712 w (C=OAmide), Mass spectra (70 ev): M·+ =420.30 m\z

(2S,4R)-3-acetyl-2-(4-methoxyphenyl)-N'-phenylthiazolidine-4-carbohydrazide)((trans Isomer)(77%) ML4

Yield: 59% m.p:100-101□C ,^{1} HNMR (400 MHz, DMSO-d6) δ2.33(d,3H) (H8), 3.19(d,1H), (H5b), 3.45(d,1H) (H5a), 4.49 (t,3H),(H9),4.49(s,1H) (H4), 4.87(m,1H) (H2), 6.79-7.55(d,t,9H) (HAr), 10.11(t,1H), (H6), 10.33(d,1H) (H7), FT-IR (KBr

disk):3325w (NH),3057w (C-H Ar),2927w (C-H),1629 w (C=OAmide), 1577, Mass spectra (70 ev): M·+ =371.46m\z

(2 S,4R)-3-acetyl-2-(4-methoxyphenyl)-N'-phenylthiazolidine-4-carbohydrazide (Cis Isomer)(23%) ML4

Yield: 59% m.p:100-101□C ,[1] HNMR (400 MHz, DMSO-d6) δ2.14(d,3H) (H8), 3.23(d,1H), (H5b), 3.45(d,1H) (H5a), 4.49 (t,3H),(H9),4.68(s,1H) (H4), 5.33(m,1H) (H2), 6.79-7.55(d,t,9H) (HAr), 10.11(t,1H), (H6), 10.33(d,1H) (H7), FT-IR (KBr disk):3325w (NH),3057w (C-H Ar),2927w (C-H),1629 w (C=OAmide), 1577, Mass spectra (70 ev): M·+ =371.46m\z

(2S,4S)-3-acetyl -2-(4-methyl phenyl) -N'-phenyl thiazolidine-4-carbohydrazide trans Isomer(92%)ML7

Yield: 64% m.p:179-180□C,[1] HNMR (400 MHz, DMSO-d6) δ , 2.33(m,3H) (H8), 2.93(s,3H) (H9), 3.55 (t,1H), ((H5b), 3.58(m,1H) (H5a), 5.50(d,3H) (H4), 6.37(d,t,1H)(H2) 6.77-8.16 (d,t,s,9H), (HAr), 10.20(s,1H) (H6), 10.76(s,1H) (H7 FT-IR (KBr disk):3325w (NH),3057w (C-H Ar),2927w (C-H),1629 w (C=OAmide), 1577, Mass spectra (70 ev): M·+ =355.46 m\z

(2R,4R)-3-acetyl -2-(methyl phenyl) -N'-phenyl thiazolidine-4-carbohydrazide (Cis Isomer)(8%) ML7

Yield: 64% m.p:179-180□C,[1] HNMR (400 MHz, DMSO-d6) δ , 2.01(m,3H) (H8), 2.67(s,3H) (H9), 3.52 (t,1H), ((H5b), 3.56(m,1H) (H5a), 5.56(d,3H) (H4), 5.40(d,t,1H)(H2) 6.77-8.16 (d,t,s,9H), (HAr), 10.20(s,1H) (H6), 10.76(s,1H) (H7 FT-IR (KBr disk):3325w (NH),3057w (C-H Ar),2927w (C-H),1629 w (C=OAmide), 1577, Mass spectra (70 ev): M·+ =355.46 m\z

(2S,4S)-3-acetyl-2-(4-chlorophenyl)-N'-phenylthiazolidine-4- carbohydrazide (transIsomer) (66%) ML8

Yield: 54% m.p:174-175□C, [1]HNMR (400 MHz, DMSO-d6) δ, 2.09 (d,3H) (H8), 3.08 (t,1H), (H5b), 3.51(s,1H) (H5a), 5.60(d,2H) (H4), 6.55(d,2H)(H2) 6.71-8.76(tt,9H) (HAr), 10.04(s,1H) (H6), 10.23(s,1H) (s,1H) (H7), FT-IR (KBr disk):34460w (NH),3284w (C-H Ar),2929w (C-H),1701w (C=OAmide), 1697w, Mass spectra (70 ev): M.+ =375.43 m\z

(2R,4R)-3-acetyl-2-(4-chlorophenyl)-N'-phenylthiazolidine-4-carbohydrazide(Cis Isomer)(44%)ML8

Yield: 54% m.p:174-175□C, [1]HNMR (400 MHz, DMSO-d6) δ, 2.09 (d,3H) (H8), 3.06 (t,1H), (H5b), 3.45(s,1H) (H5a), 5.58(s,2H) (H4), 6.45(d,2H)(H2) 6.71-8.76(tt,9H) (HAr), 10.04(s,1H) (H6), 10.23(s,1H) (s,1H) (H7), FT-IR (KBr disk):34460w (NH),3284w (C-H Ar),2929w (C-H),1701w (C=OAmide), 1697w, Mass spectra (70 ev): M.+ =375.43 m\z

(2R,4R)-3-acetyl-2-(4-nitrophenyl)-N'-phenylthiazolidine-4-carbohydrazide(trans Isomer)(55%)ML9

Yield: 60% m.p: 140-141□C, ^{1}HNMR (400 MHz, DMSO-d6) δ, 2.20 (d,3H) (H8), 3.20 (t,1H), (H5b), 3.69(t,1H) (H5a), 4.73(d,2H) (H4), 6.55(s,2H)(H2) 6.65-8.20(tt,9H) (HAr), 10.11(s,1H) (H6), 10.14(s,1H) (H7), FT-IR (KBr disk):3325w (NH),3282w (C-H Ar),2931w (C-H),1708w (C=OAmide), 1697w, Mass spectra (70 ev): M.+ =386.46m\z

(2R,4R)-3-acetyl-2-(4-nitrophenyl)-N'-phenylthiazolidine-4-carbohydrazide (Cis Isomer)(45%)ML9

Yield: 60% m.p: 140-141□C, ^{1}HNMR (400 MHz, DMSO-d6) δ, 2.05 (d,3H) (H8), 3.04 (t,1H), (H5b), 3.50(t,1H) (H5a), 4.69(d,2H) (H4), 6.42(s,2H)(H2) 6.65-8.20(tt,9H) (HAr), 10.11(s,1H) (H6), 10.14 (s,1H) (H7), FT-IR (KBr disk):3325w (NH),3282w (C-H Ar),2931w (C-H),1708w (C=OAmide), 1697w, Mass spectra (70 ev): M$^{\cdot}$+ =386.46m\z

3. Results and Discussion

3.1 Synthesis of materials

The study involved the synthesis of Thiazolidine derivatives by reacting L-Cysteine with one of its derivatives using ethanol and water (3:1) as a solvent to produce 2-phenylthiazolidine-4-carboxylic acid and its derivatives, (13) and the latter reacts with acetic anhydride to form 3-acetyl -2 -phenylthiazolidine-4-carboxylic acid and its derivatives [14]. with phenyl hydrazine using 1-hydroxybenzotriazole (HOBT) and N,N,N-dicyclohexyl carbodiimide (DCC) as coupling reagents to generate the necessary amides (15). DCC is a low-cost coupling reagent compared to other reagents, however it has a severe drawback that the DCU is weakly soluble.

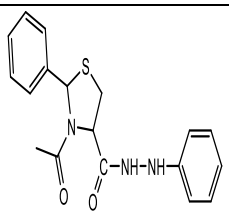
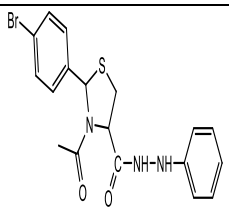
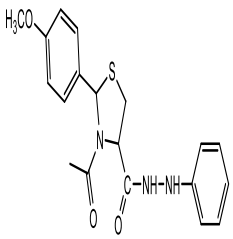
3 .2 Biological effectiveness against breast cancer and Cervical Cancer

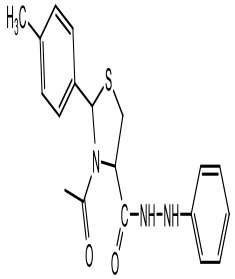
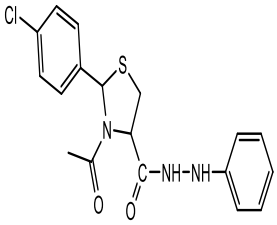
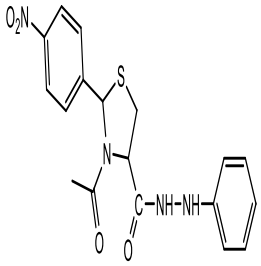
The effectiveness of the prepared compounds (ML1,ML2, ML4,ML7,ML8,ML9) against breast cancer and Cervical Cancer was studied using the method (Dimethyl thiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide [MTT] based on the value. inhibitor(%) of the compounds, as the results showed that the compounds (ML1,ML2,ML4, ML7,ML8,ML9) when treated with cancerous breast cells and Cervical Cancer and had good activity in the death of cancerous breast cells (Michigan Cancer Foundation-7) (MCF) and cancerous Cervical cells. The good activity is due to the compounds ML7. Compared to the rest of the compounds, it was attributed to the methyl group, while the other compounds, according to the data, did not have anti-breast cancer activity. This noticeable discrepancy in the effectiveness of the compounds on breast cancer lines is due to the difference in the ring substituents. In the prepared series, wi Table (3.2) shows the main inhibitor(%) values for the compounds under study.

4.Conclusion

Thiazolidine derivatives were obtained from the reaction of L-Cysteine with benzaldehyde and substituted Benzaldehyde easily and with very good yields, with a percentage ranging between 90- 95% under a few conditions. This intensification gave the product a mix of two isomers, Cis- (2S, 4S) and Trans- (2R, 4S), which could not be segregated. An equilibrium due to desiccation occurred at C(2) between the two isomers. The cis/trans ratios strongly depend on the type of solvent used. The dominant isomer in the DMSO-d6 solvent was the trans isomer while in $CDCl_3$, the main isomer was the cis isomer after complete equilibration, and we concluded from the results that every compound had excellent biologically effective anti-cells lines cancerous Michigan Cancer Foundation (MCF7) and a female cervix carcinoma (Hela).

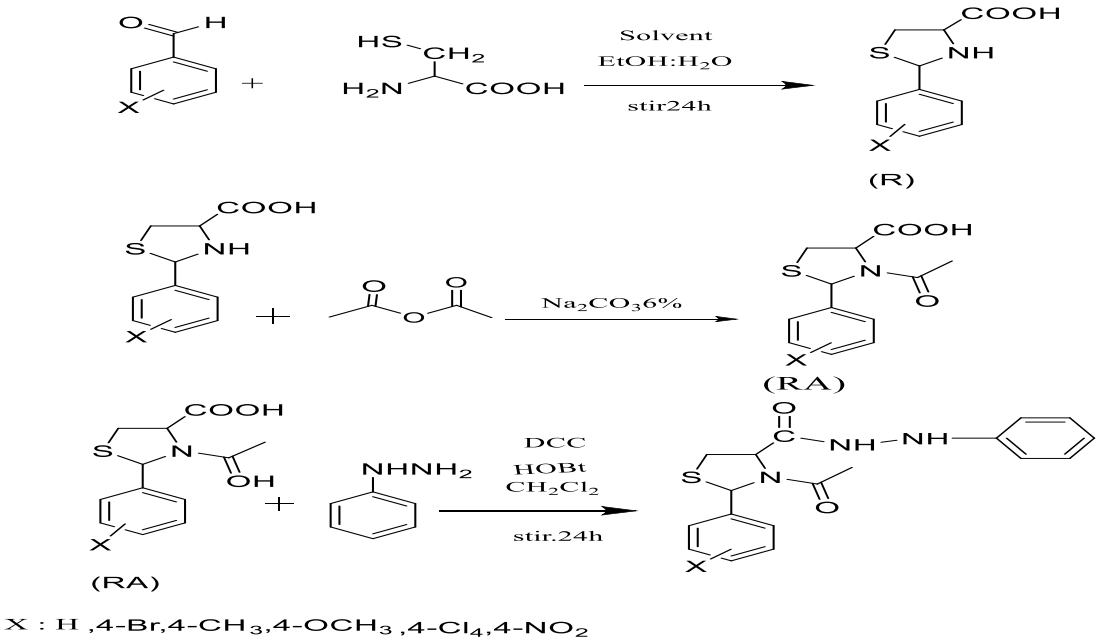
Table (2.3) Prepared compounds 3- acetylthiazolidine-carboximide, their melting points, yield, method of purification, crystal solvent, R*f* value, molecular formula, and molecular weight.

Abbreviation	Chemical Structure	Melting point ˚C	yield %	Solvent	R*f* Elunt	molecular formula, and molecular weight g\mol
ML1		103-104	70	Ethanol:water (2:1)	0.54 Ethanol : chloroform (1:9)	$C_{18}H_{19}N_3O_2S$ 341.43
ML2		106-107	75	Ethanol:water (2:1)	0.68 Ethanol : chloroform (1:9)	$C_{18}H_{19}BrN_3O_2S$ 420.33
ML4		100-101	59	Ethanol:water (2:1)	Ethanol : chloroform (1:9) 0.63	$C_{19}H_{21}N_3O_3S$ 371.46

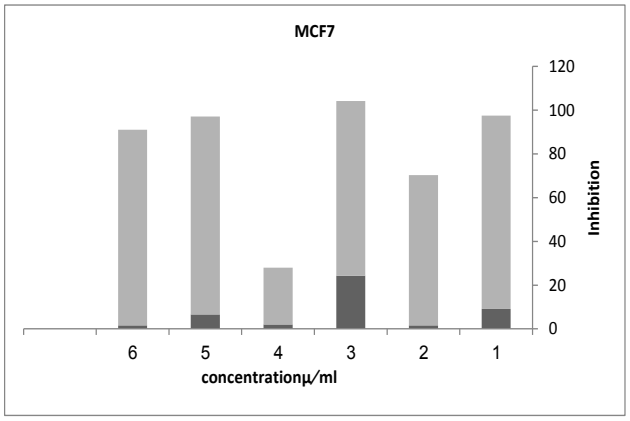
ML7		179-180	64	Ethanol:water (2:1)	0.48 Formic acid: Benzene: THF (2:6:2)	$C_{19}H_{21}N_3O_2S$ 355.46
ML8		174-175	54	Ethanol:water (2:1)	0.59 Formic acid: Benzene: THF (2:6:2)	$C_{18}H_{18}ClN_3O_2S$ 375.87
ML9		140-141	60	Ethanol:water (2:1)	0.38 Formic acid: Benzene THF (2:6:2)	$C_{18}H_{18}N_4O_4S$ 386.46

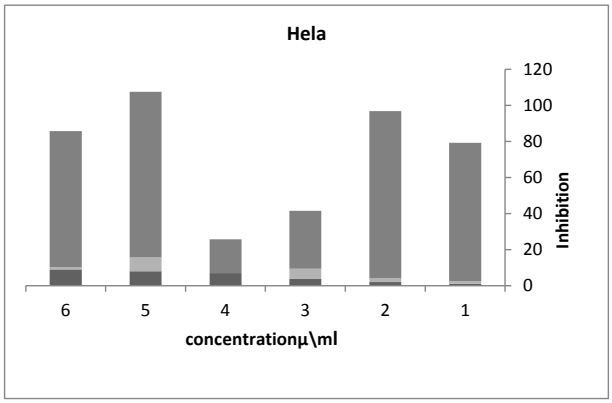
wi Table (3.2) shows the main inhibitor(%) values for the compounds under study

Symbol	mean inh(%)breast Cancer cells line	mean inh(%)cervical Cancer cells line	
ML1	88%	77%	Active
ML2	69%	93%	Active
ML4	80%	32%	Active
ML7	26%	19%	Inactive
ML8	89%	92%	Active
ML9	89%	75%	Active



Scheme3.1: procedures for making amides





Chem3.2: shows the inhibition rate (%) of the effectiveness of the prepared compounds on fighting human breast cervical cells (MCF7) and cervical cancer cells (Hela).

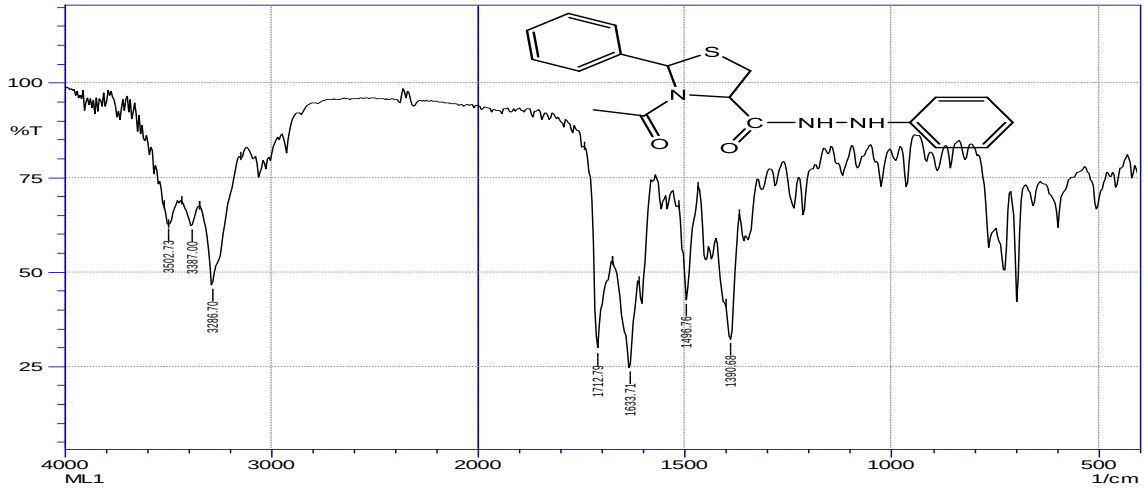


Fig1: FT-IR spectrum for Compound ML1

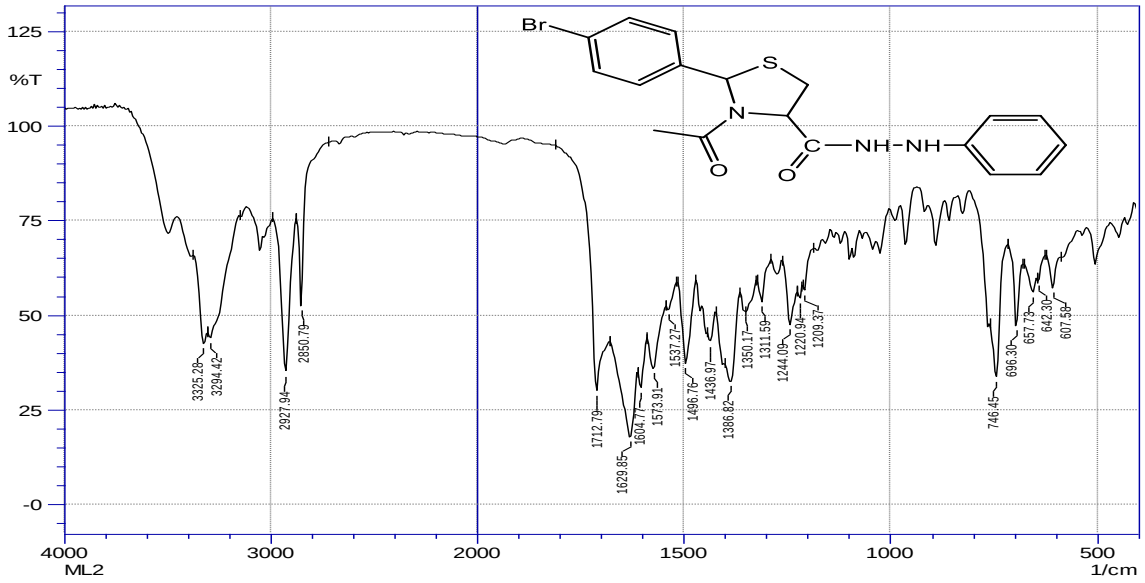


Fig2: FT-IR spectrum for Compound ML2

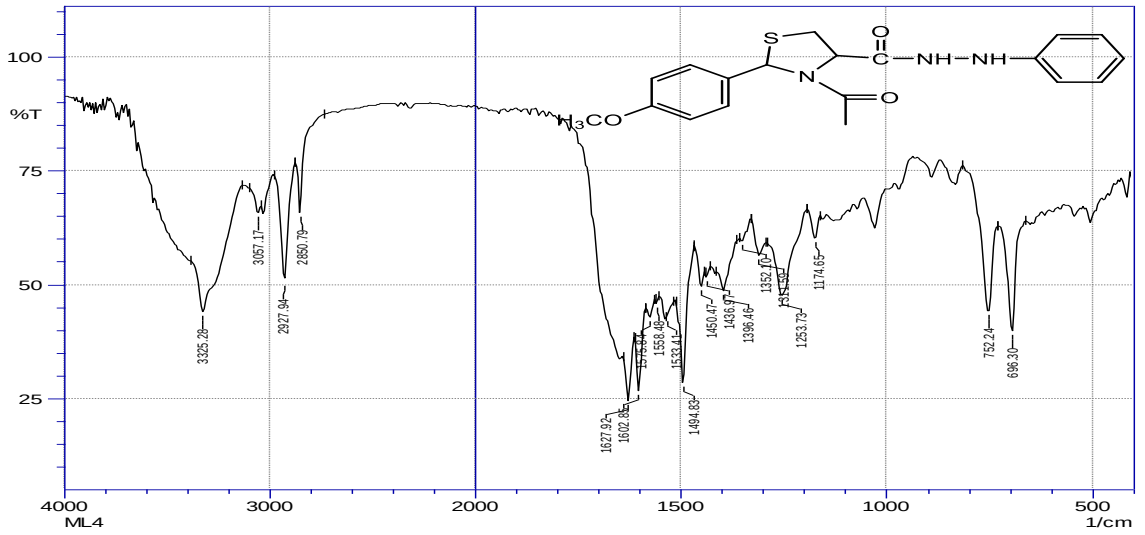


Fig3: FT-IR spectrum for Compound ML4

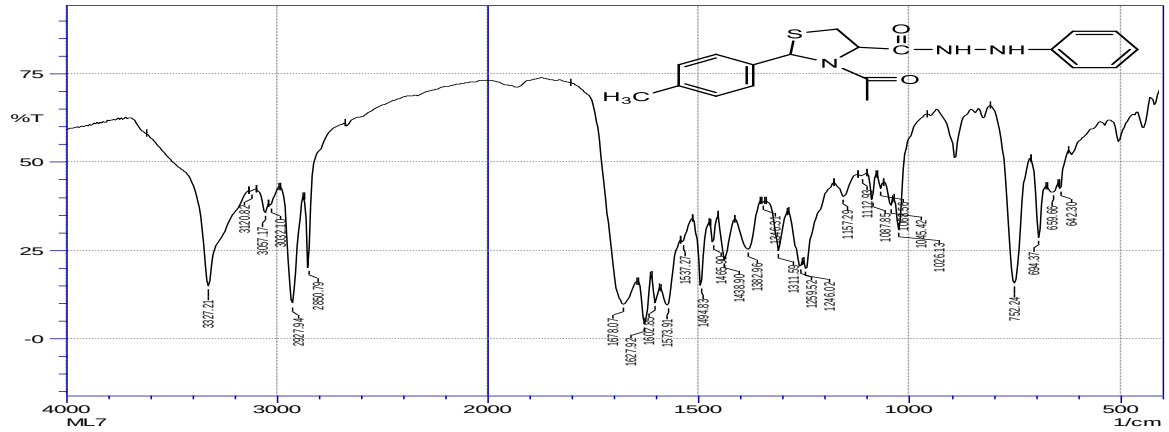


Fig4: FT-IR spectrum for Compound ML7

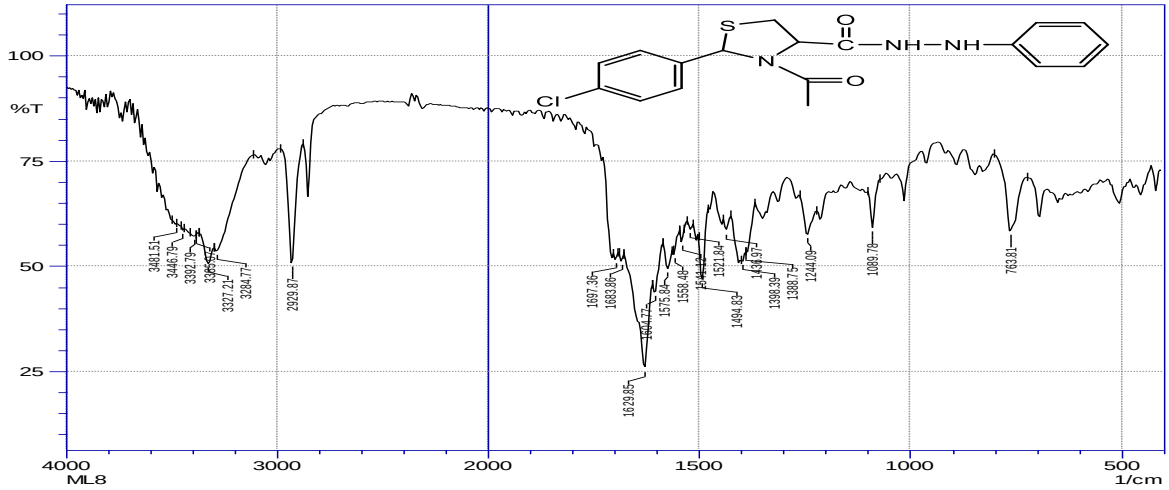


Fig5: FT-IR spectrum for Compound ML8

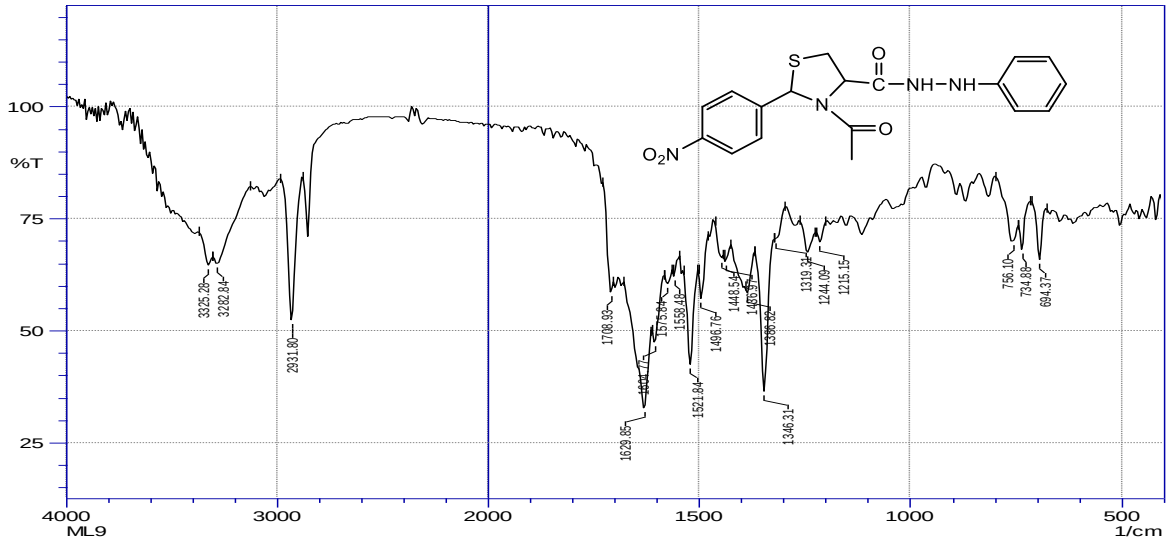


Fig6: FT-IR spectrum for Compound ML9

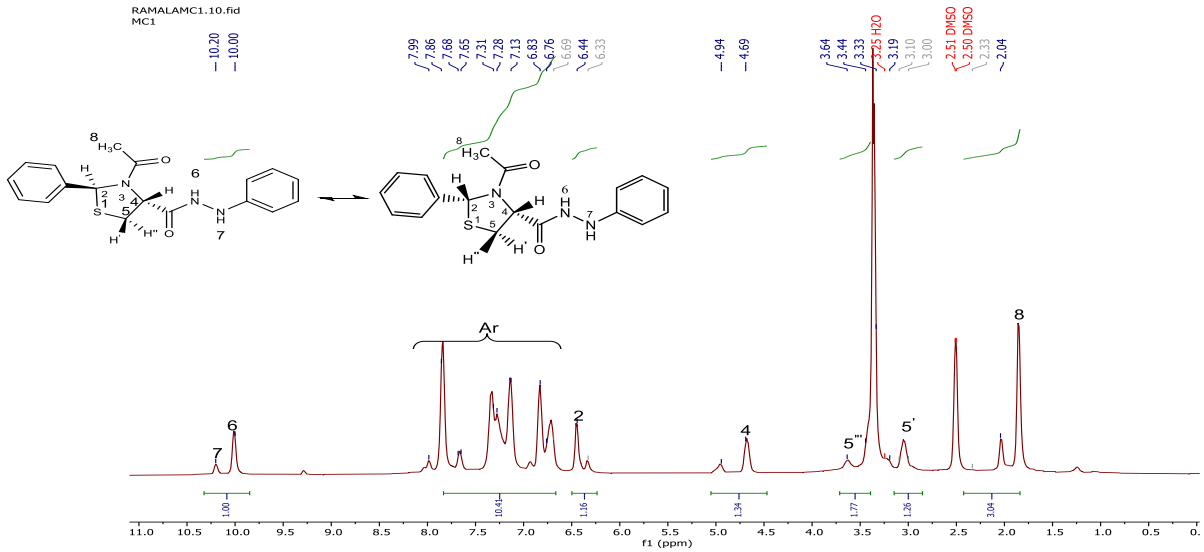


Fig1: [1]HNMR spectrum for Compound ML1

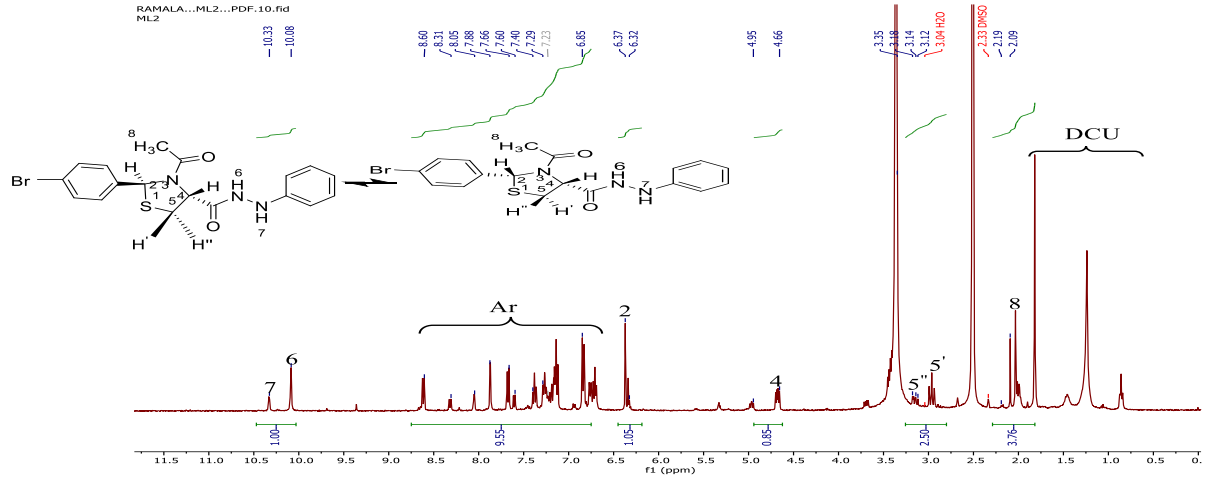


Fig2: [1]HNMR spectrum for Compound ML2

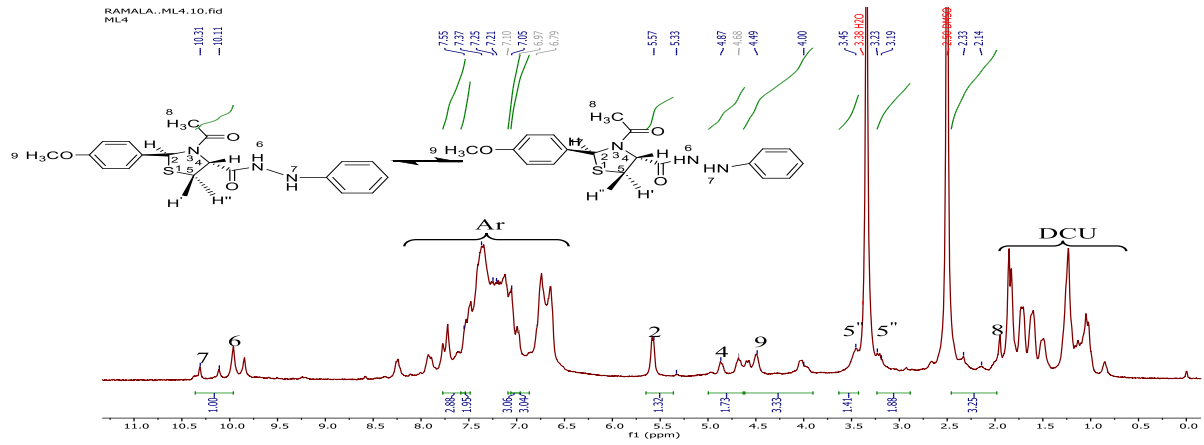


Fig3: [1]HNMR spectrum for Compound ML4

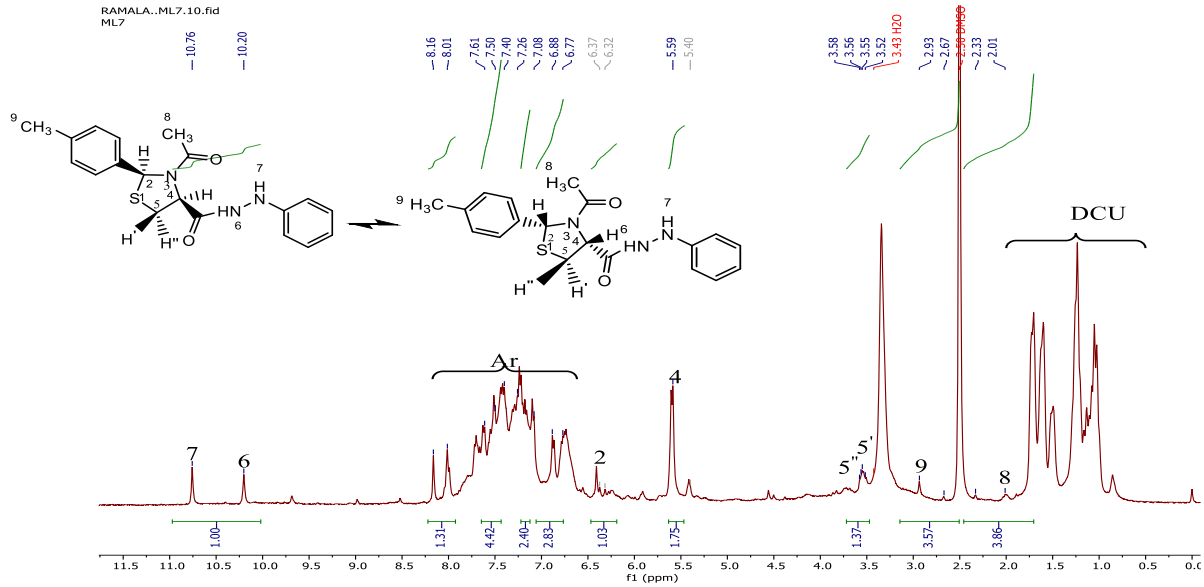


Fig4: [1]HNMR spectrum for Compound ML7

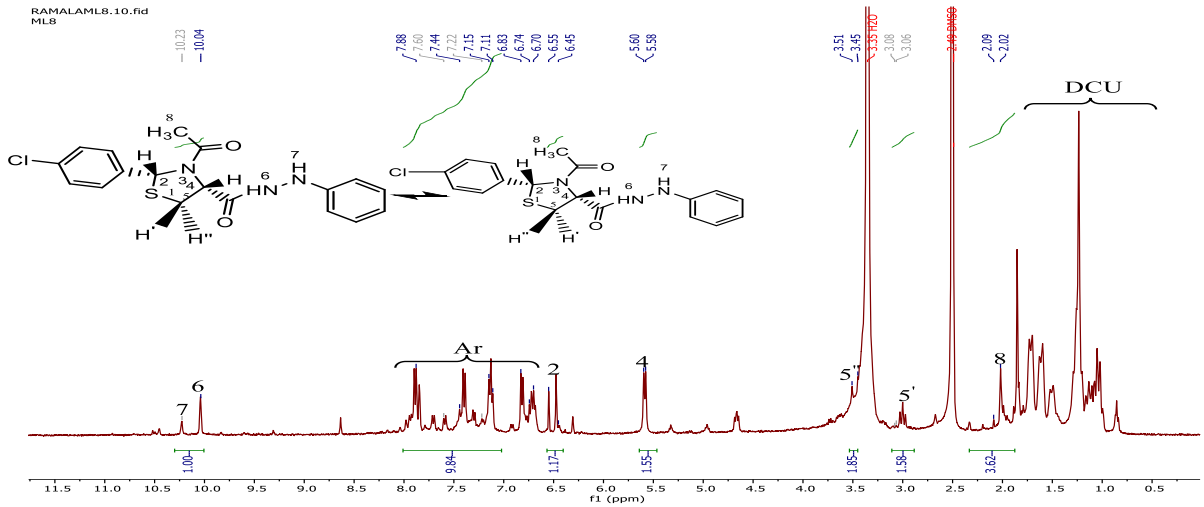


Fig5: [1]HNMR spectrum for Compound ML8

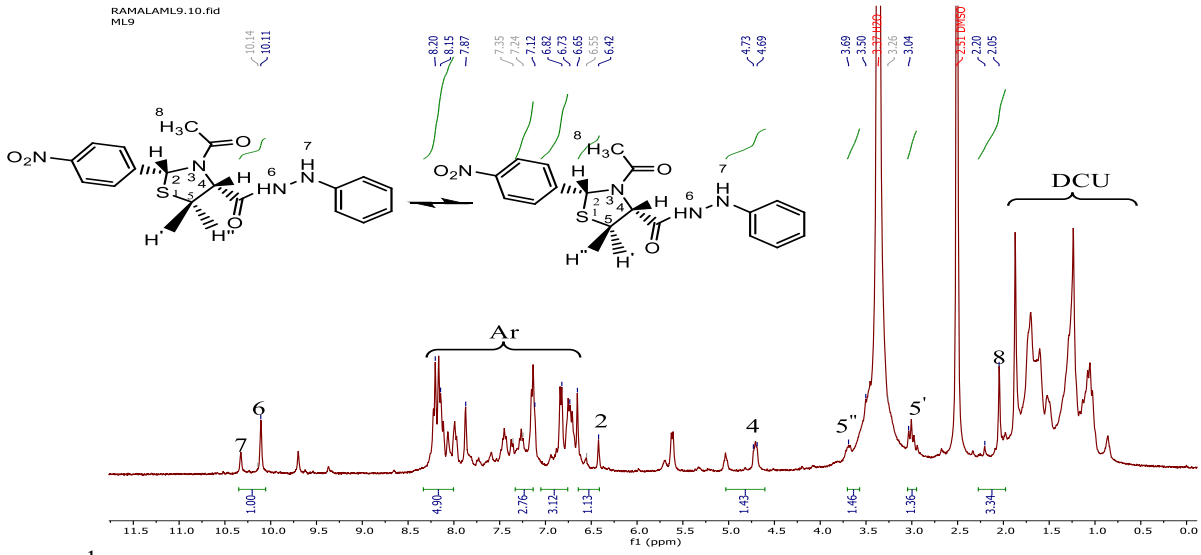


Fig6: ^{1}HNMR spectrum for Compound ML9

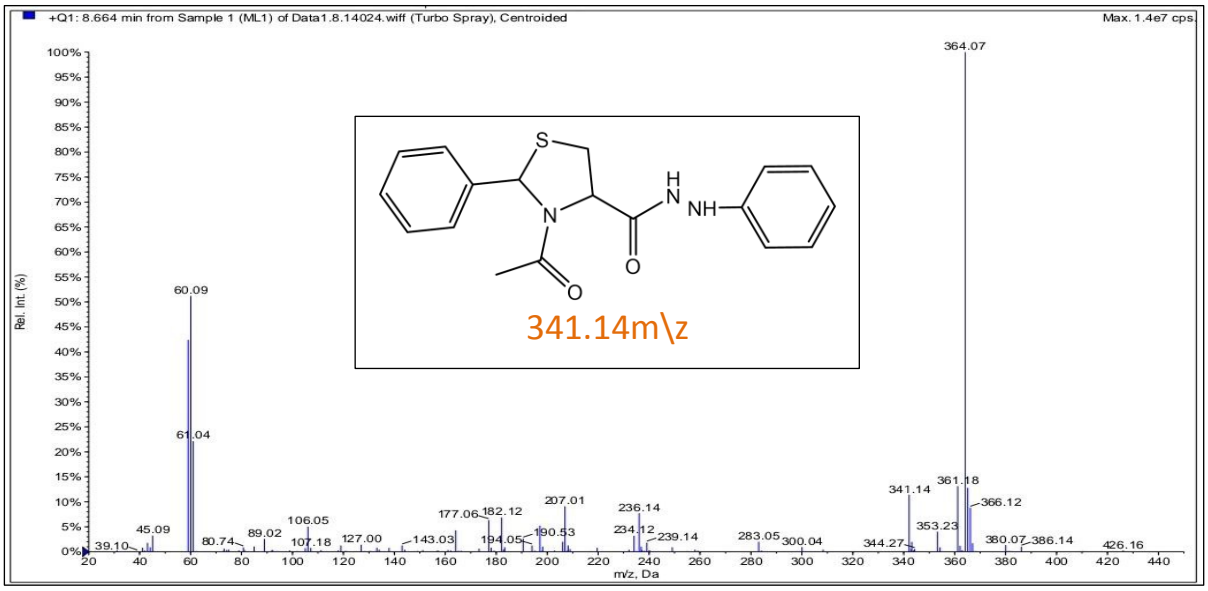


Fig1: Mass spectrum for Compound ML1

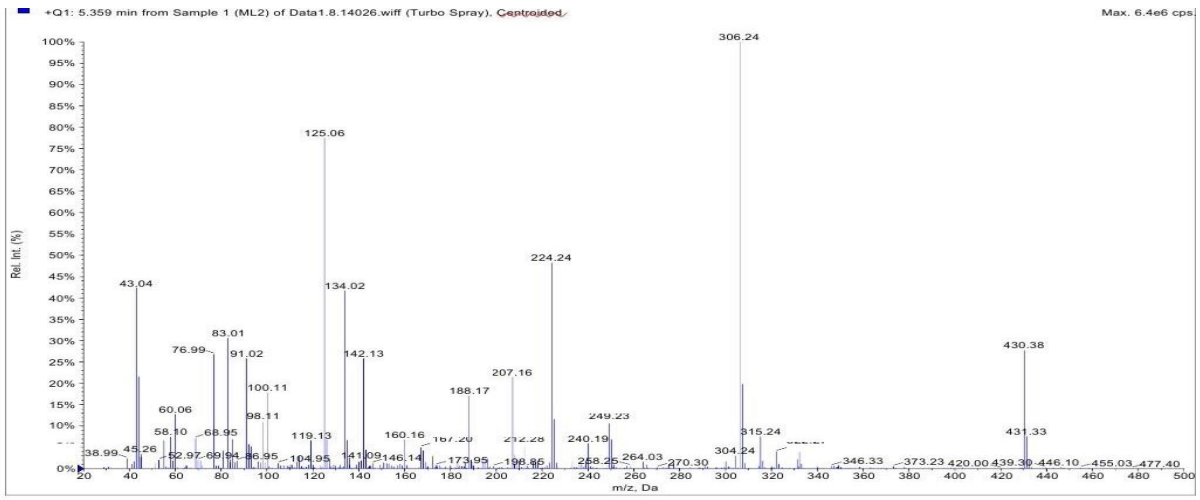


Fig2: Mass spectrum for Compound ML2

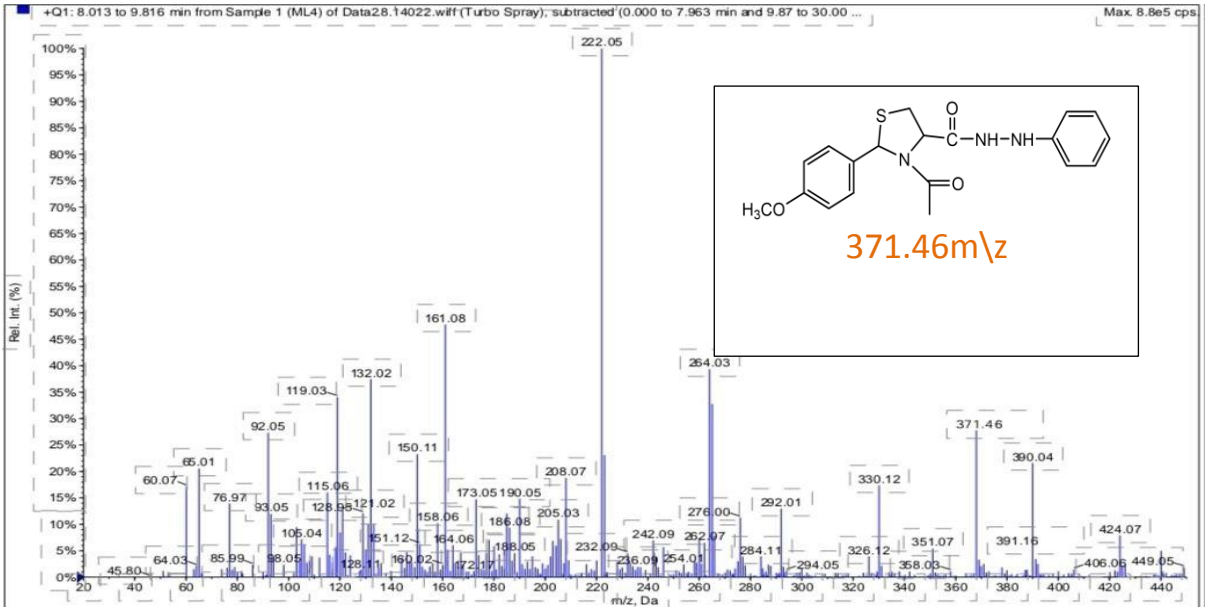


Fig3: Mass spectrum for Compound ML4

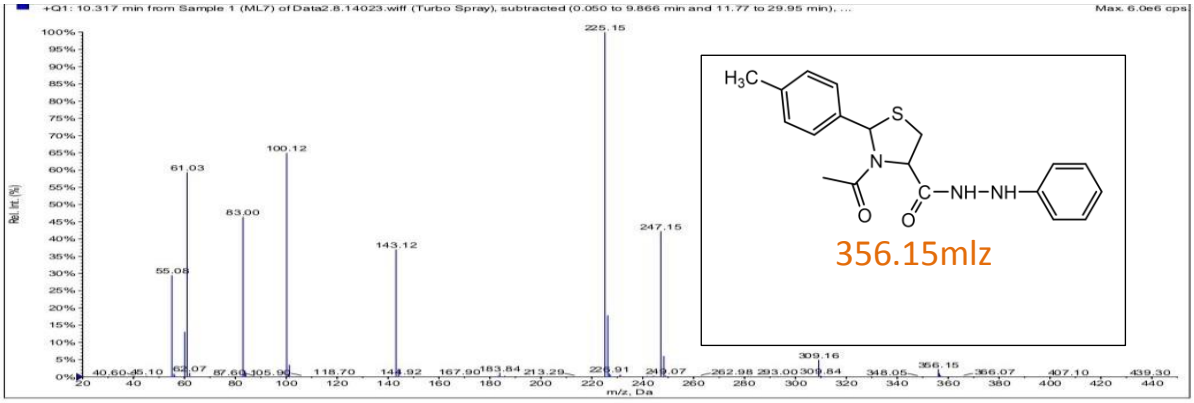


Fig4: Mass spectrum for Compound ML7

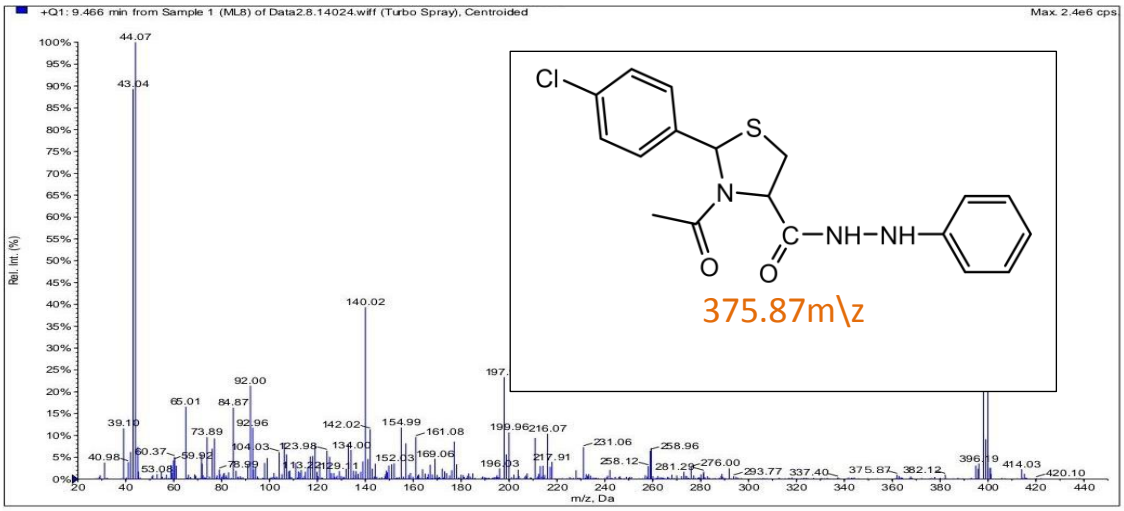


Fig5: Mass spectrum for Compound ML8

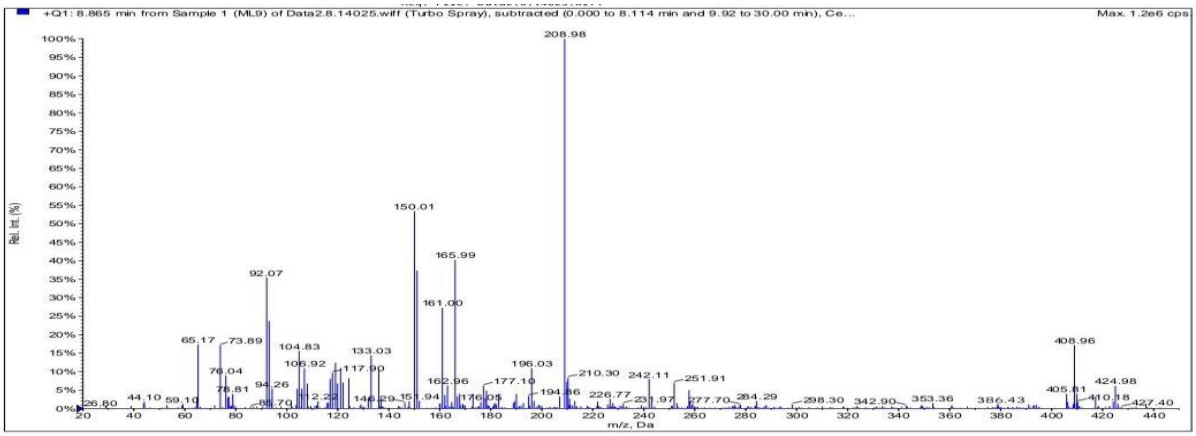


Fig6: Mass spectrum for Compound ML9

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