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Synthesis and characterization of new 1,3-thiazinane derivatives and study their effect on breast cancer (MCF-7)

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الخلاصة

حضرت بعض مشتقات الثايازينان (هكساهايدروثايازين) من خلال تفاعل الجالكونات مع الثايو يوريا. شخصت هذه المركبات بطرق التشخيص الطيفية (الاشعة تحت الحمراء، طيف الكتلة و الرنين النووي المغناطيسي للبروتون). مركبات الثايازينان المحضرة (S_H , S_{p-NH2}, S_{m-NH2}, S_{p-NO2}) طبقت كمثبطات لسرطان الثدي على الخط الخلوي MCF-7 حيث اعطت هذه المركبات نسب تثبيط مختلفة اعتماداً على نوع المجموعة المعوضة. الثايازينان المعوض بمجموعة الامين بالموقع ميتا (S_{m-NH2}) اعطى اعلى نسبة تثبيط حيث كانت 75% ، ثم يليه الثايازينان غير المعوض حيث كانت نسبة تثبيطه 47% والثايازينان المعوض بمجموعة الامين بالموقع بارا اعطى نسبة تثبيط 26% وكان اقلهم نسبة تثبيط هو الثايازينان المعوض بمجموعة النايترو بالموقع بارا حيث كانت 5%.

Abstract

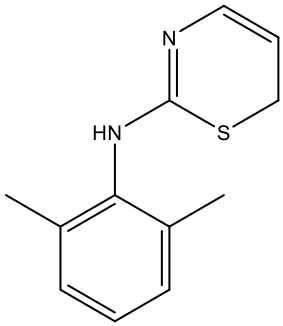
The research includes synthesized some thiazinane (hexahydrothiazine) derivatives were synthesized from the reaction of chalcons with thiourea. FT-IR, Mass, and H^1-NMR spectra identified the compounds. The new thiazinane derivatives (S_H, S_{m-NH2}, S_{p-NH2}, S_{p-NO2}) applied on breast cancer (MCF-7) gave a different results. The inhibition ratio of prepared compounds according to the type of the substituted group where the thiazinane derivative with an amino group on meta position (S_{m-NH2}) was (75%). The thiazinane derivative without a substituted group ($S_{.H.}$) was (47%), then with an amino group on para position (S_{p-NH2}) was (26%), and with a nitro group on para position (S_{p-NO2}) was (5%).

Keywords:

Chalcone , thiazinane derivatives, breast cancer, MCF-7.

Introduction

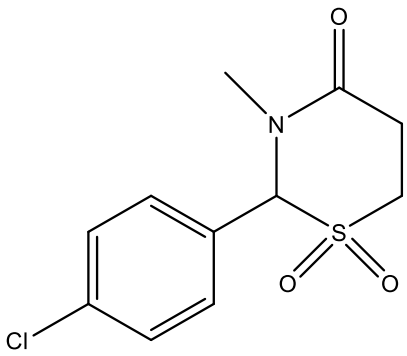
1,3 Thiazines compounds are an essential type of heterocyclic compounds that showed a wide range used in anaesthesia, relaxing the muscle, calm in animals, and pharmacological properties [1]. Thiazine also used as an anticancer drug it is due to the N-C-S group. Show it essential in some medical compounds such as xylazine (Figure 1) [2]:



xylazine.

Figure (1): The chemical structure of xylazine.

Thiazinane derivative (hexahydrothiazine) it's one of the thiazine derivatives that enter the important area as one medication is Chlormezanone (Figure 2), which is used in the treatment of anxiety and relaxing muscle [3].



Chlormezanone

Figure (2): The chemical structure of chlormezanone.

Breast cancer (MCF-7):

Cancer is a complex disease that is hard to treat, beginning when cells grow out of rule. There are many types of cancer, it can grow anywhere in the body, and it's named for the portion of the body where it is ongoing. Breast cancer that starts in the breast is still called breast cancer, even if it extends to other body parts [4]. (Figure 3); it's the most common malignancy in women globally [5].

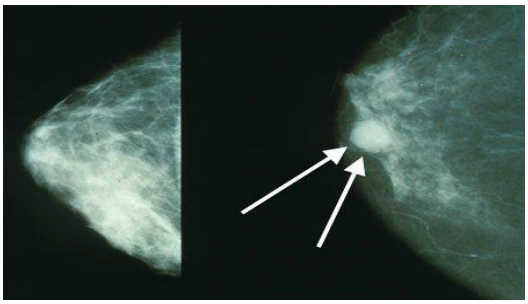
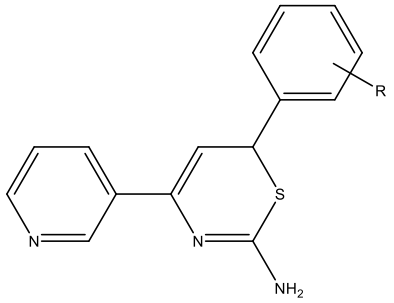


Figure (3): Normal breast (left) and a breast with a cancer cell (right).

MCF-7 is a breast cancer cell line isolated in 1970 from a 69-year-old Caucasian woman [6]. MCF-7 is the acronym of Michigan Cancer Foundation-7, referring to the institute in Detroit where the cell line was established in 1973 by Herbert Soule and coworkers [7].

The human breast cancer cell line MCF-7, which contains estrogen receptors, provides another experimental system to study hormone-regulated genes. Specific proteins are induced in response to estrogen [8].

Researchers everywhere in the world are working to find healthy ways to avoid, notice, and treat breast cancer and advance the value of the life of patients. An important example of thiazine amine used to inhibit breast cancer is the compound (A) (Figure 4) [9].



R= m-NO2, p-OH, m-Br, m-OCH3, p-N(CH3)2

Compound (A)

Figure (4): The chemical structure of compounds were used as an anticancer.

Materials and Methods:

The incorrect melting point was measured with the Electrothermal melting point apparatus. I.R. spectra were recorded using KBr disk on shimadzuFT-IR-8300 spectrophotometer in Basrah University, Science college, Chemistry department. H^1-NMR spectra were taken in Tehran University (IRAN) on Avance DRX 500 MHz (from Bruker), using dimethylsulphoxide (DMSO) as solvent. The breast cancer experience was done on Biotech Technology in Basrah (IRAQ).

preparation of chalcones:

We were used two different ways to prepare chalcone as well as the type of the substituted group on the acetophenone. If the substituted group was a withdrawing group such as the NO_2 group, it's prepared in the acidic medium. And if the substituted group was a donating group such as NH_2, it's prepared in a basic medium [10].

preparation of thiazinane derivative:

Un aqueous KOH 40% (1ml) was added to a mixture of chalcones' derivatives (2mmole), and thiourea (1mmole) in ethanol absolute (15 ml). The reaction mixture was heated with reflux for 3-5h, then powered into ice-cold water after acidified with 3drops of (1:1) HCl: Water. The precipitate formed was filtered and recrystallized in a mixture of absolute ethanol: water.

Maintenance of cell cultures of the breast cancer MCF-7 and the normal cell L20B:

MCF-7cancer cell line was obtained from the IRAQ Biotech with 10% Fetal bovine, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were passaged using Trypsin-EDTA reseeded at 50% confluence twice a week and incubated at 37°C and 5% CO_2 [11]. Cell Bank Unit in Basrah and maintained in RPMI-1640 supplemented.

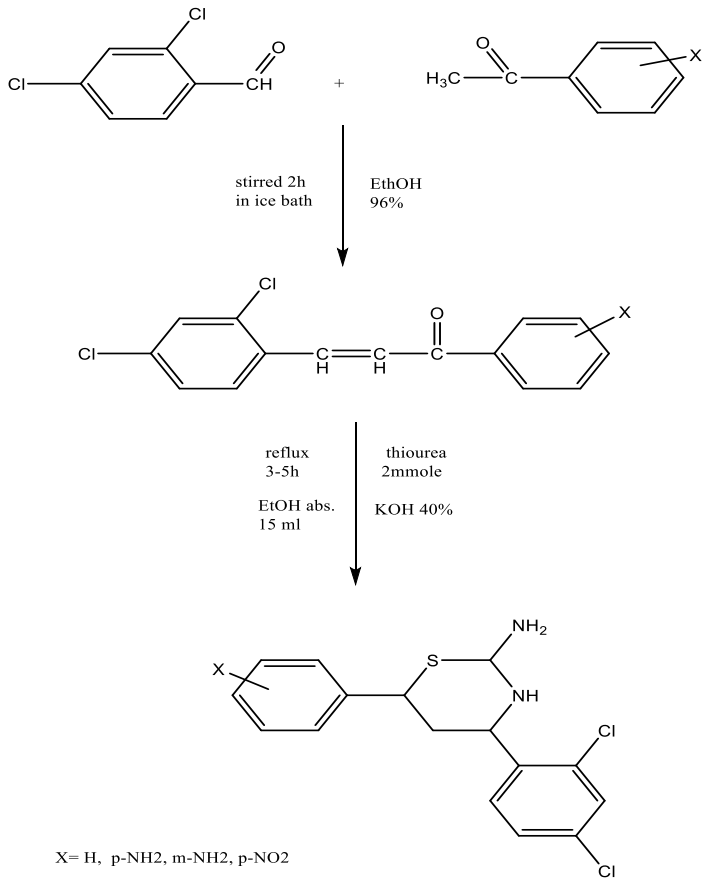
The MTT cell viability assay was conducted on 96-well plates to determine the cytotoxic effect. Cell line MCF-7 were seeded at 1×10^4 cells/well. After 24h or a confluent monolayer was achieved, cells were treated with the tested compound with a final concentration (1000)µg/ml. Cell viability was measured after 72 hrs. of treatment by removing the medium, adding 28 µL of 2 mg/mL solution of MTT (and incubating the cells for two h at 37 °C. After removing the MTT solution, the crystals remaining in the wells were solubilized by adding 100 µL of DMSO (Dimethyl Sulphoxide) followed by 37°C incubation for 15 min with shaking. The absorbency was determined on a microplate reader at 620 nm (test wavelength); the assay was performed in triplicate [12]. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation:

Proliferation rate as (PR) = B/A*100

where A is the mean optical density of untreated wells and B is the optical density of treated wells, and I.R. = 100- P.R.

Results and discussion:

Thiazinane derivatives derivatives were prepared using chalcones derivatives with thiourea to give the products (scheme1).



Scheme (1) : preparation of chalcons and thiazinane derivatives.

The most suitable method for synthesis chalcone compound is the Claisen-Schmidt condensation [13] of equimolar quantities of aryl methyl ketone with aryl aldehyde in the presence of alcoholic alkali. If the substituted group was a withdrawing group such as NO_2, it's prepared in the acidic medium. The substituted group was a donating group such as NH_2; it's prepared in a basic medium. That may depend on the difference in the electronic density from one group to another. Acetophenone has electron-withdrawing groups due to decreasing the electron cloudy on the methyl group's protons, increasing the acidity. To avoid that, we were used the acidic way to prepare the chalcone, which contains an electron-withdrawing group , and the yield was excellent. Also, we can use the basic medium, but it gives a meagre yield.

Thiazinane derivative was prepared by the cyclization between the chalcone and thiourea [14] in the presence of an alkali catalyst (KOH). The yield was excellent in the thiazinane derivative S_H. The final product was a powder and melted in different ranges.

The products were characterized by infra-red(Table2), H^1-NMR(Table3), and Mass spectra (Table 4). The physical properties are shown in (Table1):

Table (1) physical properties of synthesized compounds.

No. of comp.	IUPAC name	Yield	Colour	M.P.
S_H	4-(2,4-dichlorophenyl)-6-phenyl-1,3-thiazinan-2-amine	80%	Brown	74-76
$S_{m\text{-}NH2}$	6-(3-aminophenyl)-4-(2,4-dichlorophenyl)-1,3-thiazinan-2-amine	80%	Brown	98-100
$S_{p\text{-}NH2}$	6-(4-aminophenyl)-4-(2,4-dichlorophenyl)-1,3-thiazinan-2-amine	76%	Brown	150-152
$S_{p\text{-}NO2}$	4-(2,4-dichlorophenyl)-6-(4-nitrophenyl)-1,3-thiazinan-2-amine	71%	Brown	170

1- FT-IR Spectra:

The spectra of the prepared compounds show that all compounds have typical peaks, such as the stretching (N-H) group at (3414-3336) cm^{-1}, which belongs to the primary NH_2 group in the heterocyclic ring. These facts enhance the correct expected chemical structure of these compounds. Also, the appear peaks at (3225-3186) cm^{-1} due to stretching vibration of secondary (N-H) group of the heterocyclic ring [15]. The strong band showed in the region (3097-3055) $cm^{-1,}$ which were attributed to the (C-H) aromatic group, and two very strong bands at (1566- 1585 cm^{-1} and 1473- 1465 cm^{-1}) to the (C=C) stretching vibration aromatic ring. All spectra showed absorption bands in the (1103-1076) cm^{-1} belonging to the (C-Cl) stretching. See the figures (5-8). The absorption bands data of these compounds are shown in Table (2):

Table (2): FT-IR Spectra data of synthesized compounds.

Compounds	IR data(cm^{-1})
S_H	N-$H_{pry.}$(3414),N-$H_{sec.}$(3217), C-$H_{aliphatic.}$(2931), C=$C_{Aromatic}$ (1585,1469), Ar-H(3090),C-Cl (1103).
$S_{m\text{-}NH2}$	N-$H_{pry.}$ (3336), N-H_{sec} (3205), C-$H_{aliphatic}$ (2966,2928), C=$C_{Aromatic}$ (1585,1465), Ar-H(3082), C-Cl (1103).
$S_{p\text{-}NH2}$	N-H_{pry} (3414), N-H_{sec} (3186), C-$H_{aliphatic}$ (2974,2928), C=$C_{Aromatic}$ (1566,1473), Ar-H(3070), C-Cl (1076).
$S_{p\text{-}NO2}$	N-H_{pry} (3352), N-H_{sec} (3225), C-$H_{aliphatic}$ (2928,2870), C=$C_{Aromatic}$(1597,1508), N-O (1558,1361), Ar-H(3082), C-Cl(1103).

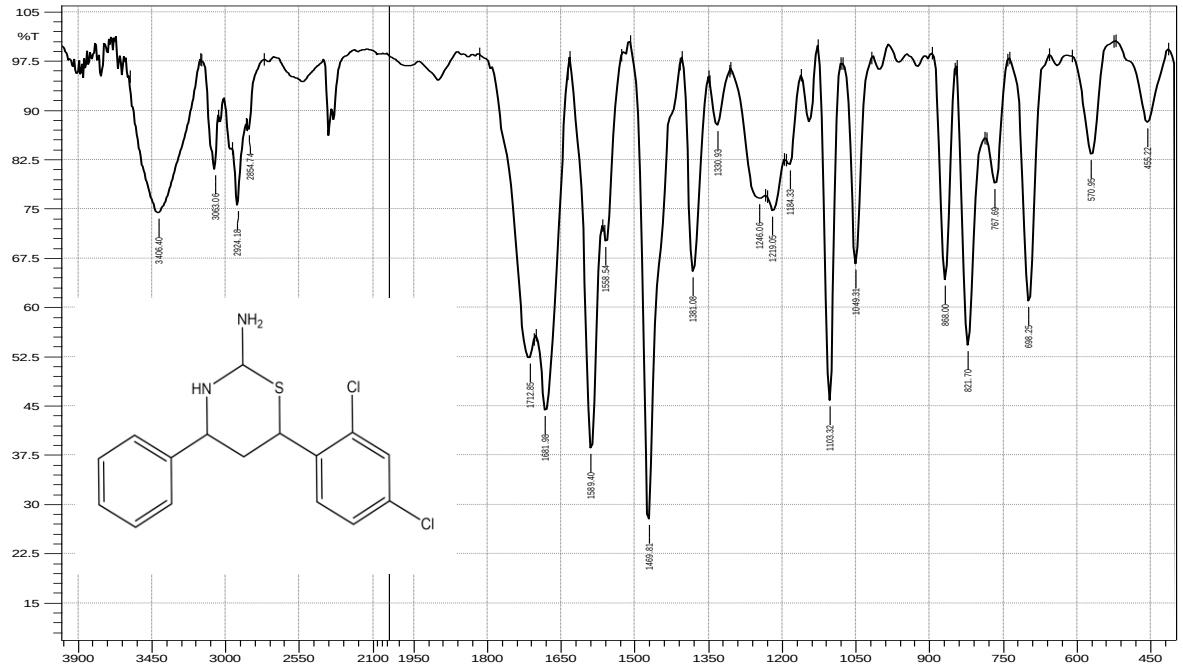


Figure 5: FT-IR spectrum of S_H compound.

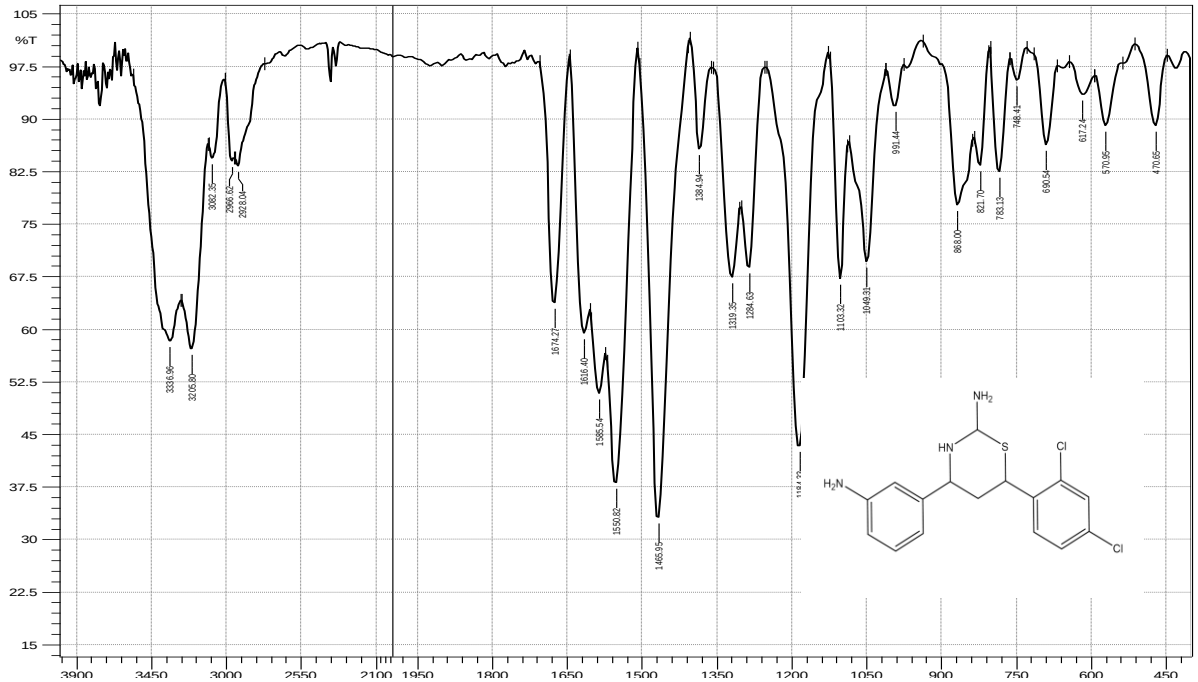


Figure 6: FT-IR spectrum of $S_{m\text{-}NH2}$ compound.

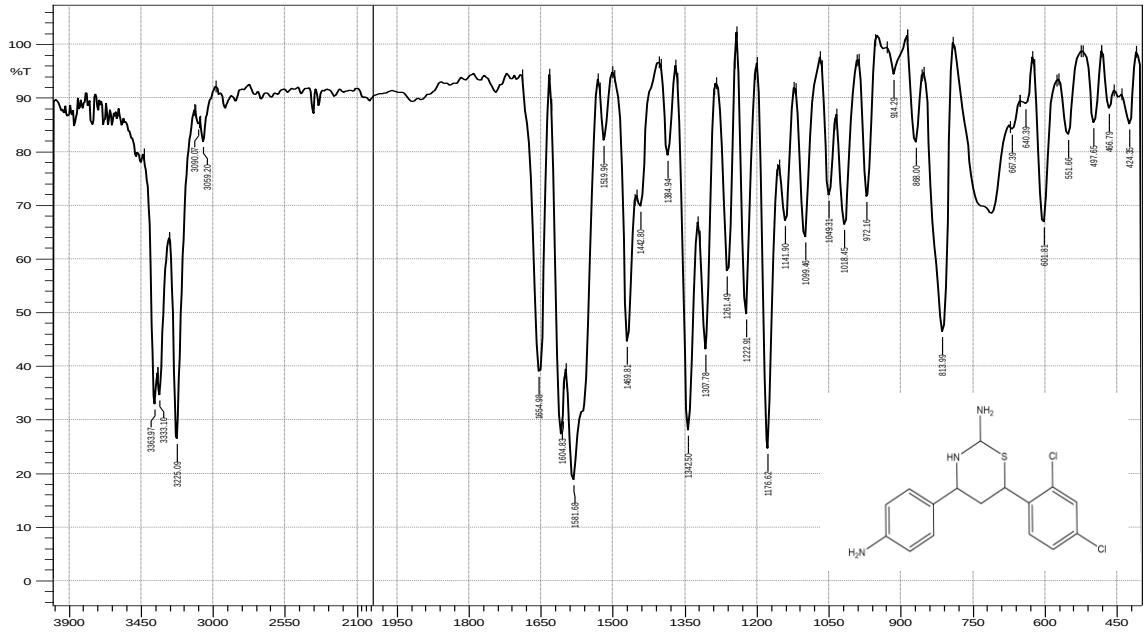


Figure 7: FT-IR spectrum of $S_{p\text{-}NH2}$ compound.

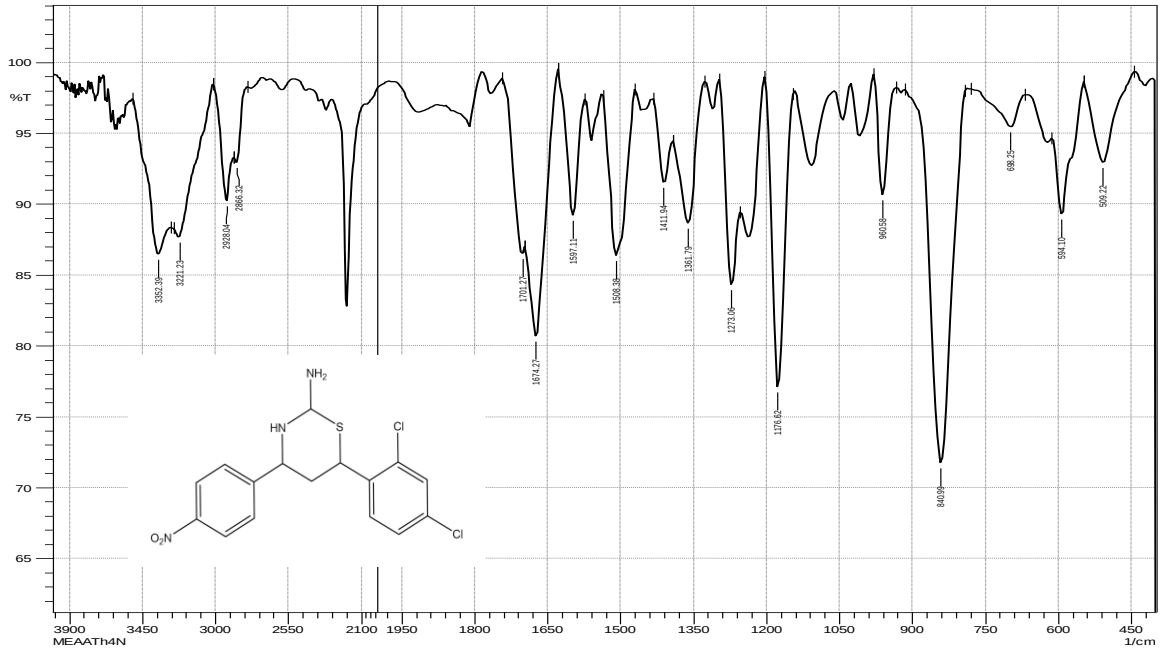
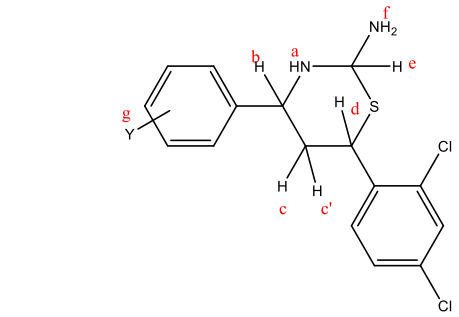


Figure 8: FT-IR spectrum of $S_{p\text{-}NO2}$ compound.

2- Proton Nuclear Magnetic Resonance (^{1}H-NMR).

The ^{1}H-NMR spectrum of the thiazinane derivative compounds where it shows a signal at which is ascribed to each the protons of aliphatic amine groups[16] (NH_2 (f)) was (1.26, 1.26, 1.16, and 1.26) ppm to S_H, $S_{m\text{-}NH2}$, $S_{p\text{-}NH2}$ and $S_{p\text{-}NO2}$ respectively. The protons of aromatic amine groups (NH_2 (g)) at (4.76, 4.49 ppm) to $S_{m\text{-}NH2}$ and $S_{p\text{-}NH2}$ respectively. The NH protons of the thiazinane derivative ring at (0.87, 0.96, 1.06, and 0.89) to S_H, $S_{m\text{-}NH2}$, $S_{p\text{-}NH2}$ and $S_{p\text{-}NO2}$

respectively as such we can have observed signal at which is ascribed to each the proton of thiazinane derivative ring (as appears in the figure(9), also we can have observed doublate signal for $1H_b$, and multiplate signals for each of c&c' protones, and triplate signal for $1H_d$, and doublate signal for $1H_e$ as shown in table 3. In addition to the appearance of signals for the aromatic ring, also it was observed that bundles of dimethyl sulfoxide DMSO-d^6 solvent in their specific locations upon displacement (2.50) ppm, and for the chloroform $CDCl_3$ at 7.26 ppm. See the figures (10-13) The chemical shifts of H^1-NMR spectra of the prepared compounds are shown in Table 3.



Y= H, p-NH2, m-NH2, p-NO2

Figure (9): The chemical structure of the prepared compounds.

Table(3): Chemical Shifts H^1NMR Spectra of the prepared compounds.

Compounds	NMR data(ppm)
S_H	0.87(d. , 1H (a)), 5.67(dd.,1H(b)), 3.48(m,1H(c)), 3.05(m,1H(c')), 5.29(dd,1H(d)), 6.80(d.,1H(e)), 1.26(s.,2H(f)), (7.03-7.22, m.,4H , 7.30-7.60, m., 4H) (Ar-H (8H)), (400 MHz, Chloroform-*d* (7.26)).
$S_{m\text{-}NH2}$	0.96 (d. , 1H (a)), 5.67 (dd.,1H(b)), 3.78 (m,1H(c)), 3.47(m,1H(c')), 5.25 (dd,1H(d)), 6.55 (d.,1H(e)), 1.26 (s.,2H(f)), 4.76 (s.,2H(g)), (7.11 – 7.21 (m, 3H), 7.33-7.43 (m, 4H)(Ar-H)), (400 MHz, Chloroform-*d* (7.26).
$S_{p\text{-}NH2}$	1.06(d. , 1H(a)), 2.09(d.,1H(b)), 3.66(m,1H(c)), 3.46(m,1H(c')), 1.66 (t,1H(d)), 4.89 (d.,1H(e)), 1.16 (s.,2H(f)), 4.49 (s.,2H(g)), (7.46 – 7.63) (m, 7H, Ar-H)), (301 MHz, DMSO-d_6) (2.50)ppm.

$S_{p\text{-}NO2}$	0.89 (d., 1H(a)), 6.10 (d.,1H(b)), 3.60(m,1H(c)), 4.31(m,1H(c')), 5.37 (d,1H(d)), 7.27 (d.,1H(e)), 1.26 (s.,2H(f)), (7.59 – 8.03) (m, 7H, Ar-H)), (301 MHz, DMSO-d_6) (2.50)ppm.

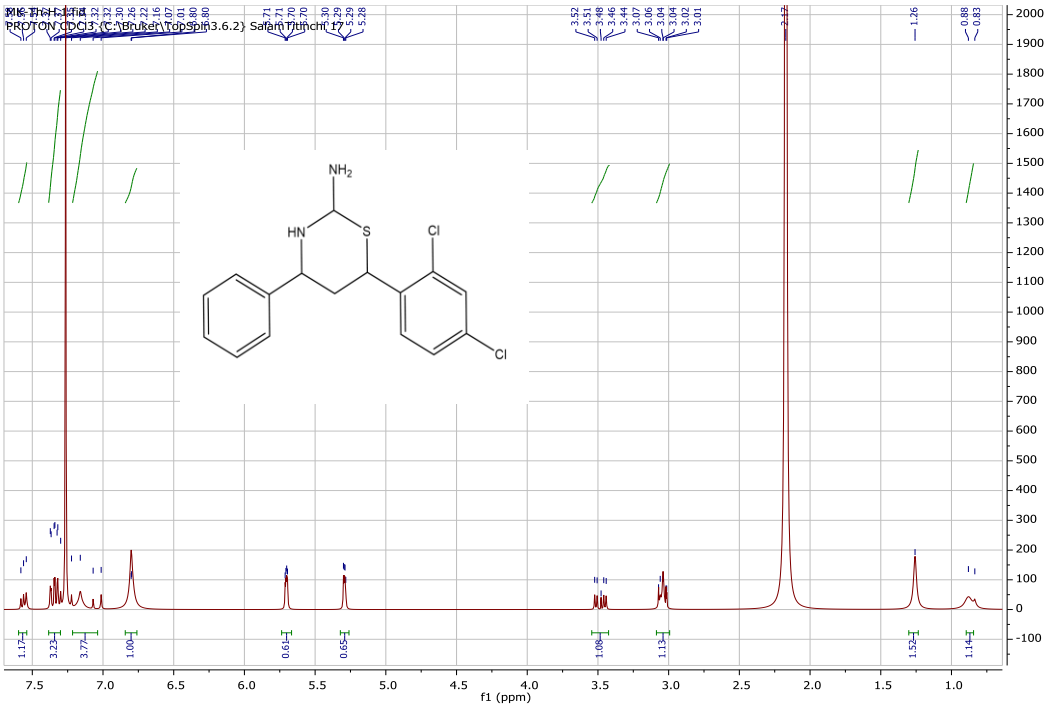


Figure 10: ^{1}H-NMR spectrum of S_H compound.

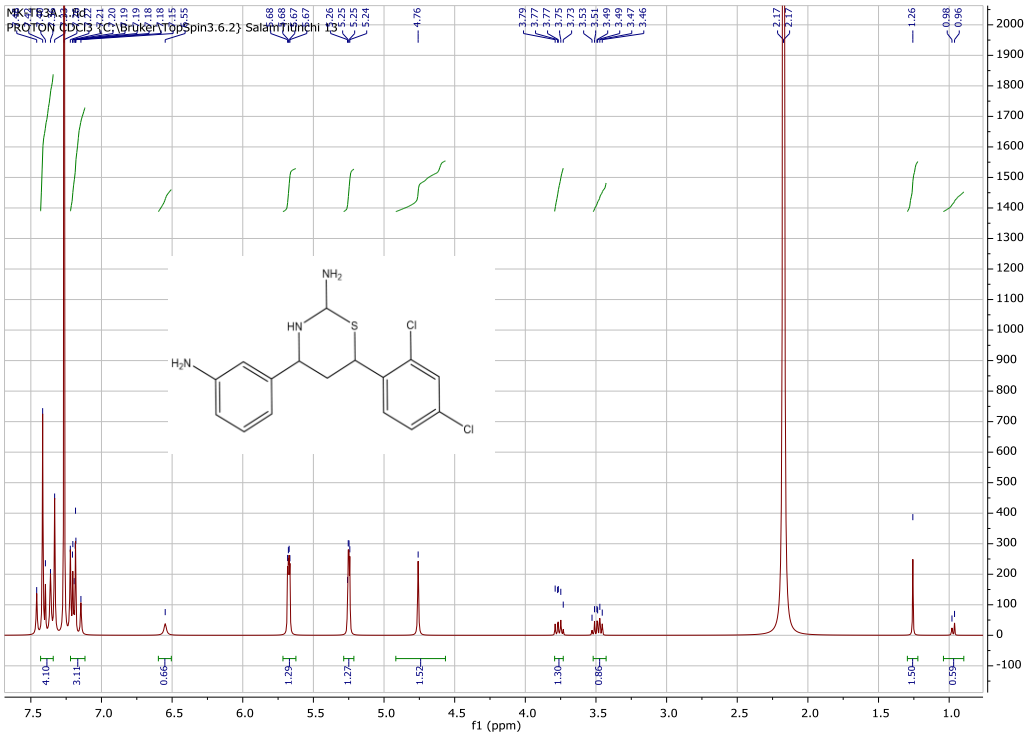


Figure 11: ^{1}H-NMR spectrum of $S_{m\text{-}NH2}$ compound.

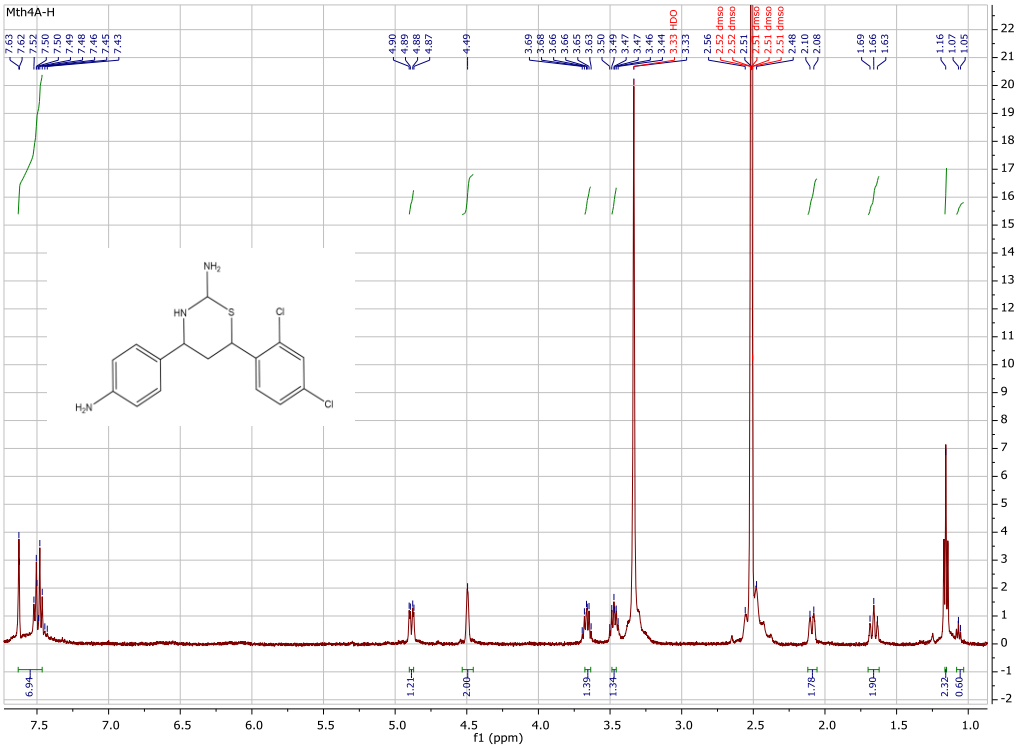


Figure 12: ^{1}H-NMR spectrum of $S_{p\text{-}NH2}$ compound.

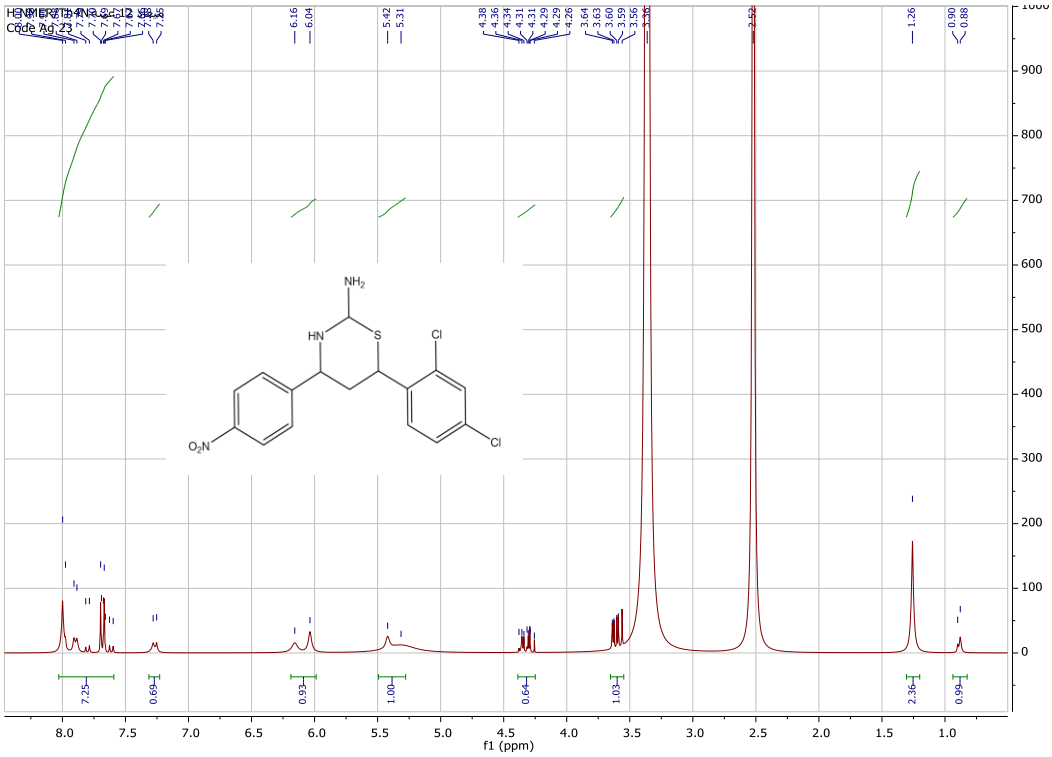
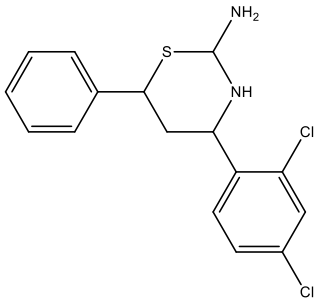
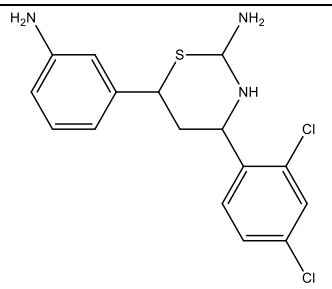
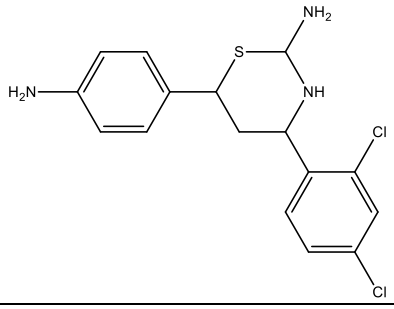
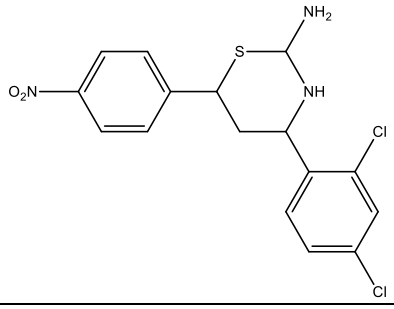


Figure 13: ^{1}H-NMR spectrum of $S_{m\text{-}NO2}$ compound.

3- Mass Spectrum of thiazinane derivative compounds:

The mass spectrum found that the peaks at (m/z = 339, 354, 354, and 384) for thiazinane derivative compounds ($S_{(H)}$, $S_{p\text{-}NH2}$, $S_{m\text{-}NH2}$, and $S_{P\text{-}NO2}$), respectively. These peaks support our study while the structures of the thiazinane derivative compounds that were synthesized are correct. See figures (14-17) The major fragmentation of thiazinane derivative compounds were shown in table 4:

Table(4): m/z of the main fragments of mass Spectra of the prepared compounds.

Name of sample	Structure	m/z of the main fragments
S_H		339, 324, 194, 118.
$S_{m\text{-}NH2}$		354, 339, 209, 263.
$S_{p\text{-}NH2}$		354, 339, 209, 263.
$S_{p\text{-}NO2}$		384, 147, 118, 123.

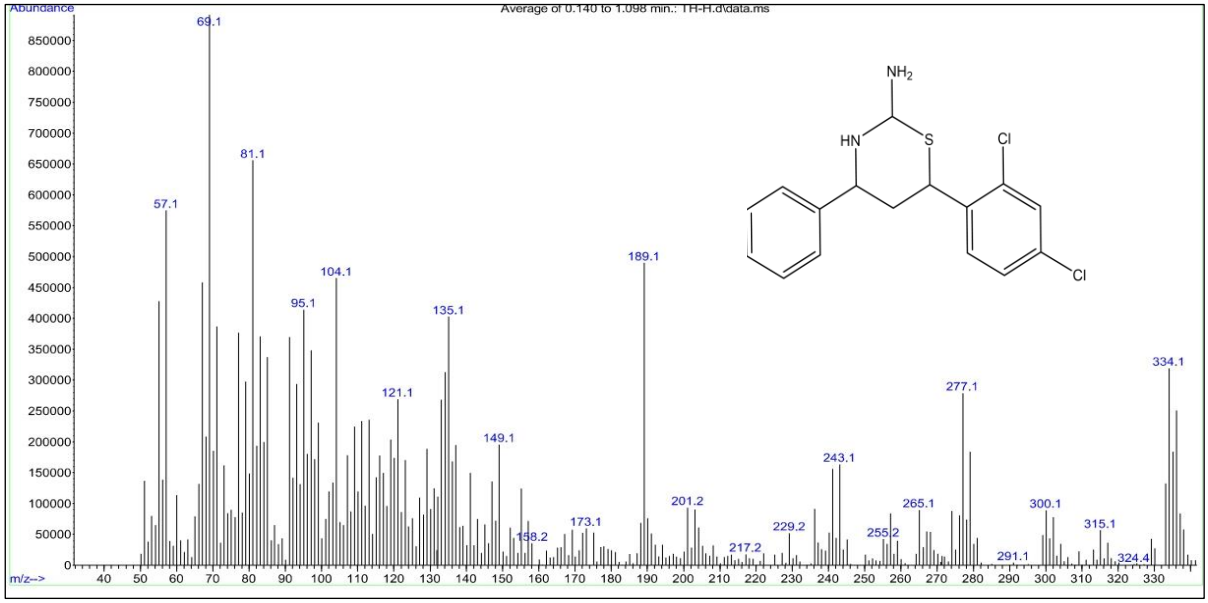


Figure 14: Mass spectrum of S_H compound.

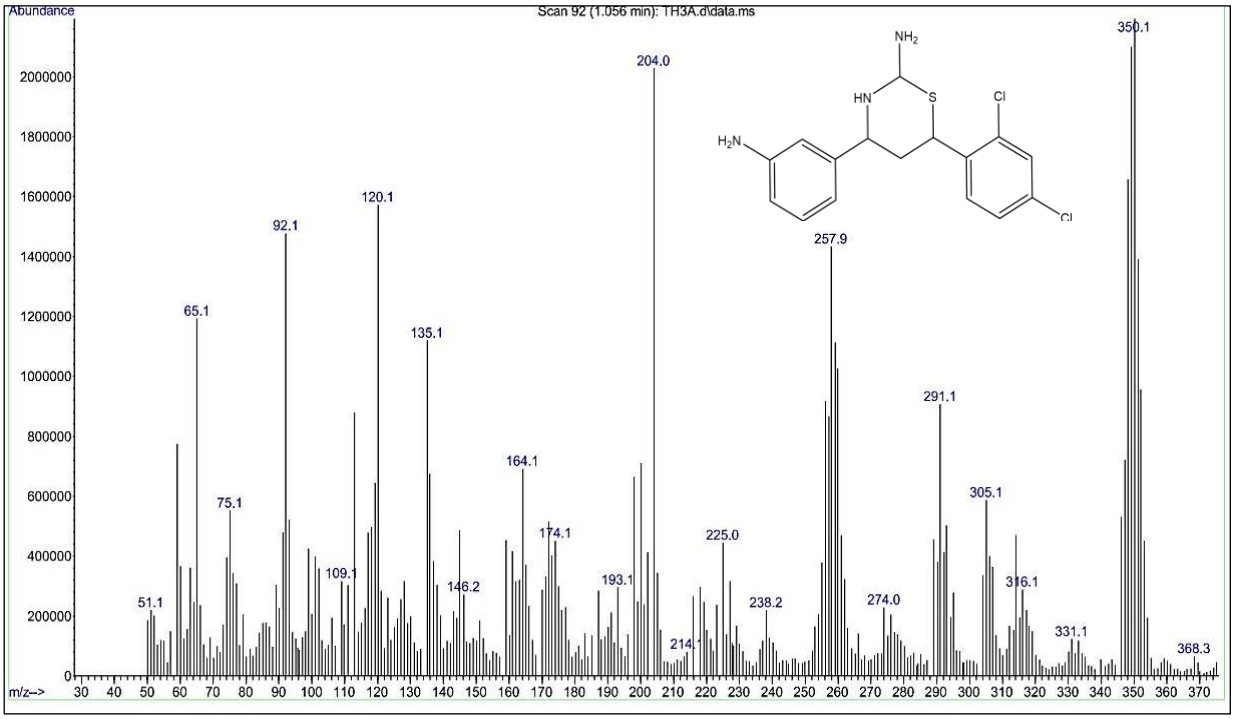


Figure 15: Mass spectrum of $S_{m\text{-}NH2}$ compound.

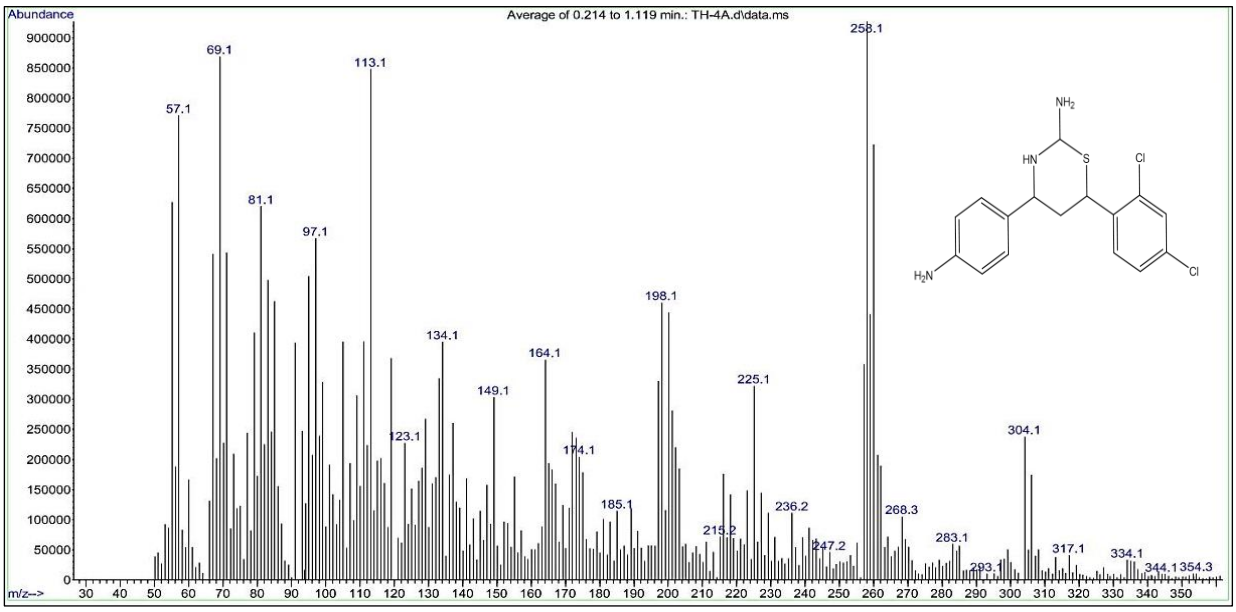


Figure 16: Mass spectrum of $S_{p\text{-}NH2}$ compound.

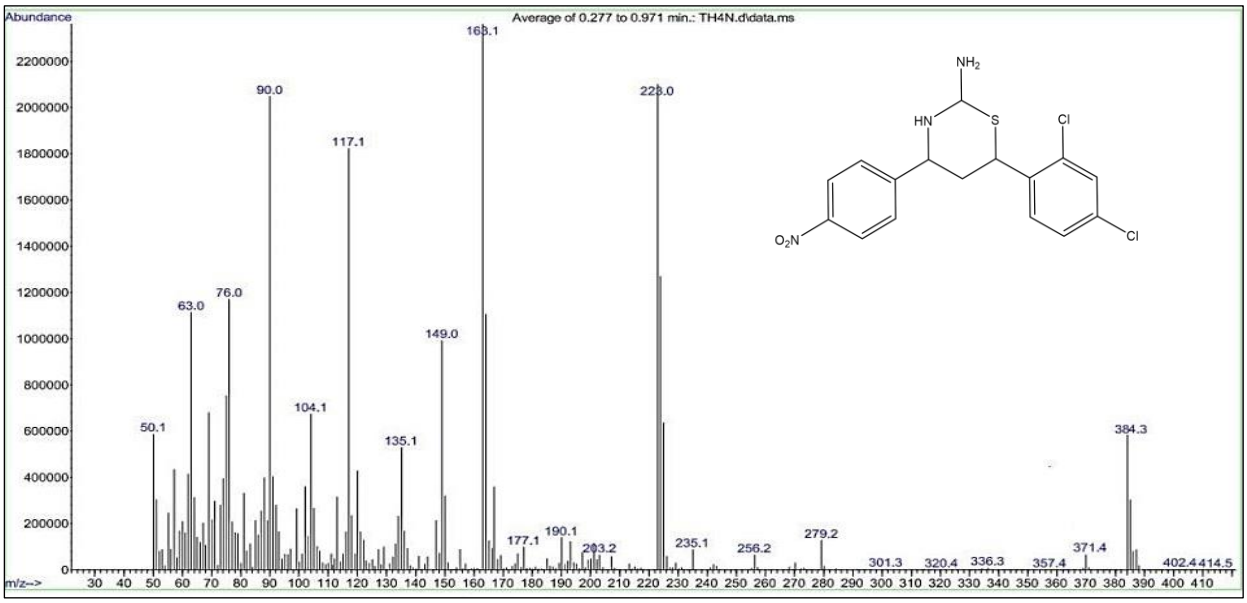


Figure 17: Mass spectrum of $S_{m\text{-}NO2}$ compound.

Results of Anticancer activity of thiazinane derivatives:

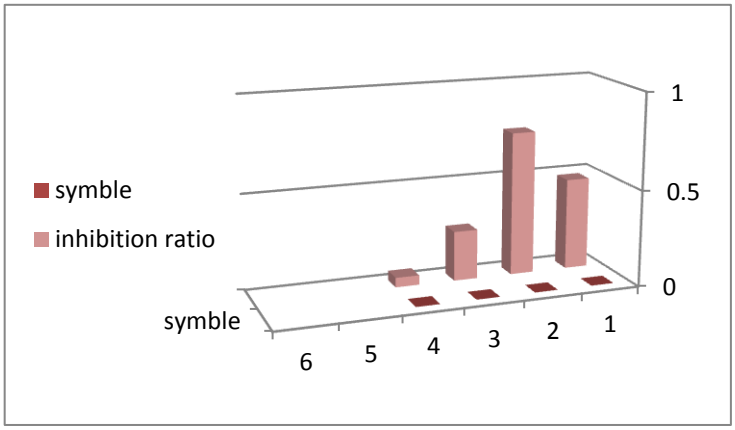
Cancer is one of the most diseases that trouble humans and finally leads to their death. For that, researchers began to grow and learn many effective anticancer therapeutics, such as treatment of cancer by chemotherapy, and heterocyclic compounds, such as thiazine derivatives containing anticancer drugs. Therefore, the chemical training of the thiazine core plays an important part in the synthesis of a range of heterocyclic compounds having a wide series of biological and pharmacological activities [17].

When the synthesized Thiazinans were applied to the breast cancer MCF-7 cell line, as shown in Table 5:

Compounds	Inhibition ratio in MCf-7
S_H	47.9%
$S_{m\text{-}NH2}$	75%
$S_{p\text{-}NH2}$	26%
$S_{p\text{-}NO2}$	5%

Table (5): inhibition ratio of thiazinane derivative.

The non-substituted compound ($S_{.H.}$) was given inhibition to breast cancer cells of 47.9%. When the amine group was interred into a meta-position, the inhibition ratio increased at 75%, but when replacing the amine group with the para-position , the inhibition ratio decreased from 75% to 26%. When the nitro group (withdrawing group) was replaced with the amine group, the inhibition ratio decreased to 5%. The discrepancy between the rates of inhibition was clarified in scheam 2:



Scheam (2): The percentage inhibition of breast cancer cells.

There are several reasons for interpreting these results, including;

1) the type of the group [17], whereas the withdrawing group was decreasing the electron density along the molecule leading to non-molecular capacity to capture cancer molecule.

2) They can attract electrostatic cancer cells [19], such as amino groups.

3) electronegativity for the substituted group [20].

Conclusion:

The compounds in meta substituted ($S_{m\text{-}NH2}$) had higher inhibition of cancer cells compared to the same substituted groups at the site of para ($S_{p\text{-}NH2}$), and the compounds containing a donating group($S_{m\text{-}NH2}$) gives much higher inhibition than the compounds containing withdrawing groups($S_{m\text{-}NO2}$).

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