Nat. Volatiles & Essent. Oils, 2021; 8(4): 15373-15389

NVEO
Natural Volatiles &
Essential Oils

Characterization, Antioxidant And Biological Activity Of 1,3-Thiazolidine-4-One Derivatives Synthesized From Azomethine Compounds

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Abstract: The most common method was used to prepare azomethine compounds, which is the method of thermal condensation of the amino group in primary aromatic amines containing one amino group or two amine groups, with a carbonyl group or two carbonyl groups in the aromatic aldehydes, through refluxing process in absolute ethanol with the addition of drops from glacial acetic acid as a catalyst. By cycloaddition of thioglyocolic acid with various azomethine compounds in anhydrous 1,4-dioxane under reflux conditions, new compounds of 2,3-disubstituted-1,3-thiazolidine-4-one were synthesized. The end points of all reactions are checked by TLC. The products were identified using FT-IR and ^{1}H-NMR spectroscopy and mass spectra. After confirming the processes for preparing the final compounds, their effectiveness anti-oxidant and against specific types of pathogenic bacteria and pathogenic fungi was evaluated, and the results were good at some concentrations.

Key words : Thiazolidine-4-one , azomethine , thioglyocolic acid, antioxidant, biological activity.

1. Introduction

Schiff bases, named after the German Scientist (Hugo Schiff) prepared it in 1864 [1], which contain the azomethine (-CH=N-) group, these compounds are formed by condensation reaction of aldehydes or ketones with primary amines [2-4]. Because of their importance in analytical chemistry, organic synthesis, metal refining, electroplating, and photography, the derivatives complexes from Schiff bases compounds have attracted much interest [5-8]. Due to a wide biological activities of such as anti-cancer, anti-tuberculosis, anti-bacterial, anti-microbial, anti-spasmodic, and anti-oxidants, azomethine have gained great importance in the medicinal and pharmaceutical fields [9]. Heterocyclic compounds are abundant in nature and have a significant impact on human health since structural subunits of these compounds can be found in many natural products such as vitamins, hormones, and medicines [10]. Thiazolidine-4-one is a five-membered heterocyclic ring containing one carbonyl group and N and S heteroatoms, it was prepared using several methods including reaction aldehyde derivatives and amine derivatives with thioglyocolic acid [11], and prepared by cyclic addition (3+2) α-halohydroxamates and synthetically significant and useful isothiocyanides, resulting in the formation of novel potentially bioactive 2-iminothiazolidin-4-ones [12], thiazolidine derivatives are a group of heterocyclic compounds that have anticancer, anti-infectious and antibacterial [13], anti-seizure, anti-tubercular, anti-fungal and anti-amoebic [14].

2. Materials and methods.

2.1. Chemistry

Thioglyocolic acid, methylene di-aniline, 1H-1,2,4-triazole-3,5-diamine, 2,6-diamin–pyridine 2,4-dichlorobenzaldehyde and substitutions for benzaldehyde in para sit are used in reactions. The melting points were determined in uncorrected open capillary tubes, an Infrared Spectrophotometer Model Tensor 27 , Bruker Co., Germany, was used to record FT-IR spectra, Using denuterated DMSO-d^6. as a solvent ^{1}H-NMR spectra were recorded on a Bruker ultrashield 500 MHz spectrometer, MS 5973 Agilent Technology was used to measure mass spectrometry. TLC eluent methanol : cyclohexane was used at the percent ratio (2:7) in the synthesis of azomethine compounds and the eluent of EtOAc : benzene was used at the percent ratio (2:4) in the synthesis of 1,3-thiazolidine-4-one derivatives, spots were determined by iodine

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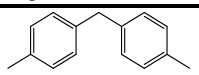
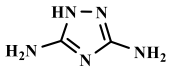
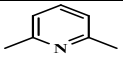
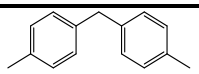
2.1.1. General Procedure for Synthesis of Azomethine compounds. A_1-A_4

A mixture of 1H-1,2,4-triazole-3,5-diamine (0.018 mol, 1.8 g) and 4-nitro benzaldehyde (0.036 mol, 5.4 g) in absolute ethanol 40 mL was placed in a round-bottom flask 100 mL with stirring and 3-4 drops of glacial acetic acid as a catalyst for 25 minutes to 3 hr., the mixture was allowed to react at reflux temperature, the end reaction of the synthesized compounds is monitored using TLC as the stationary phase and methanol : cyclohexane solvents at the percent ratio (2:7) as the mobile phase, and then allowed to cool to room temperature, where a crystalline solid of azomethine A_4 segregates out, the ethanol was used to recrystallize the solid product twice [15-17] The same process was used to synthesis other [A_1-A_3], chemical and physical properties of azomethine compounds are showed in table 1.

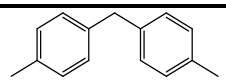
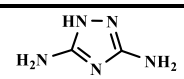
Chemical and physical properties of the prepared azomethine compounds [A_1-A_4]

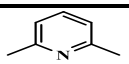
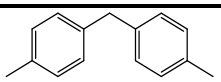
92	0.76	212-215	464.48	$C_{27}H_{20}N_4O_4$	NO_2		A_1
74	0.75	247-251	375.10	$C_{16}H_{11}Cl_2N_5$	Cl		A_2
90	0.74	229-232	344.20	$C_{19}H_{13}N_5O_4$	NO_2		A_3
80	0.85	185-188	512.26	$C_{27}H_{18}C_{l4}N_2$	2,4-Cl		A_4

2.1.2. General procedure for synthesis of 1,3- Thiazolidine-4-one Derivatives (T_1-T_4).

In a well dried 100 mL round bottom flask equipped with condenser and anhydrous calcium chloride tube guard, a mixture 0.0015 mol, 0.77 g) of azomethine A_4 and (0.003 mol, 0.2 mL) of thioglygolic acid, and uses of anhydrous Zinc chloride (0.003 mol, 0.41 g) as a catalyst [18], the mixture is refluxed for 11-20 hr. the end product is monitored by TLC using silica gel as stationary phase and checked by (benzene: EtOAc at the percent ratio (4:2)) then cooled down to the room temperature and re-washed in water and then recrystallized with chloroform the separated solid product T_4, the table 2 below lists some of the physical properties of compounds as well as their yield %.

.For the synthesized 1,3-thiazolidine-4-one compounds molecular weight, m.p and yield%.

58	0.71	128-130	612.68	$C_{31}H_{24}N_4O_6S_2$	NO_2		T_1
53	0.57	252-255	492.39	$C_{20}H1_5Cl_2N_5O_2S_2$	Cl		T_2

77	0.74	134-137	523.54	$C_{23}H_{17}N_5O_6S_2$	NO_2		T_3
63	0.73	264-266	660.45	$C_{31}H_{22}Cl_4N_2O_2S_2$	2,4-Cl		T_4

2.2. Antioxidant

2.2.1. Free radical scavenging activity of DPPH: The antioxidant activity of synthesized compounds is measured in vitro using the free radical scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH). which allows measuring the ability of the compounds to displace free radicals scavenging action, 1 mg of each sample was dissolved in 1 mL of DMSO to obtain the concentrations of the sample solutions 0.5, 0.2, 0.15, 0.1, & 0.05 Mm and the standard solution was also prepared at different concentration, by dissolving 4mg of ascorbic acid in 100ml of methanol to prepare 400µg/ml of the (Standard) solution, and from it different concentrations of ascorbic acid 0.5, 0.2, 0.15, 0.1, & 0.05 Mm were prepared and used as a standard solution, the concentrations of the samples solutions 0.5, 0.2, 0.15, 0.1, & 0.05 Mm were prepared by dissolving 1mg of each sample in 1 mL of (DMSO), the above concentrations were prepared, 4 mg of 1,1-Diphényl-2- Picrylhydrazyle (DPPH) was dissolved in 100 mL methanol, which was protected from light by a dark aluminum foil canister [19].

2.2.2. Protocol for assessment of DPPH scavenging activity: For the control reading, absorbance reagent (DPPH) was taken immediately at 517nm, Upon completing the concentration preparation, 1.5 ml of reagent solution (DPPH) was added to each test tube containing the sample and standard solution concentrations both alone, After completing the reagent addition, the samples are incubated in the dark for 30 minutes. After 30 minutes, the absorbance was taken in a UV-visible spectrometer at 517nm using methanol as Planck's solution ,Free radical anti-radical activity (% scavenging activity) is calculated by using the following equation. Anti-radical activity% $= A_{Control} - A_{Test} / A_{Control} * 100$ Where:
A Control = is the control reaction's absorbance (containing all reagents unless sample extract).
A Test = the sample's absorbance (prepared compounds).

2.3. Biological activity:

2.4.1. Evaluation of antibacterial activity:

1- Two compounds for each type of heterocyclic five heterocyclic derivatives was evaluated on two different types of pathogenic bacteria, one of which is Gram negative which is (**Escherichi coli**) and the other is Gram positive which is (**Staphylococcus aureus**).
2- Both nutrients were used. By heating and stirring with a magnetic stirrer until completely dissolved, 28gm of it was dissolved in 1 L of distilled water. The flask was sealed with cotton, and the medium was sterilized for a period of time in an autoclave at (121C0). Mix (1.5 mL) of used and active bacterial isolates in the nutrient medium in an Erlenmeyer flask with (250 mL) of culture medium in its liquid state at 40 ^oC) for 30 minutes under pressure (15bound/inch), then stir well to ensure complete contamination, and place in a Petri dish about (15 mL) for each dish and left to solidify at room temperature.
3- For some of the produced compounds, test solutions were made in dimethyl sulfoxide (DMSO) solvent at concentrations of 150, 100, and 50 mg/mL for each of these derivatives, as follows:
1- To get a concentration of (150 mg/mL), dissolve (150mg) in (1ml) of (DMSO).
2- A solution of concentration (100 mg/mL) was prepared by withdrawing (1 mL) of the solution of concentration (150 mg/mL) and adding (0.5ml) of solvent (DMSO) to it.

3- (1 mL) of the solution with a concentration of 150 mg/mL was withdrawn and replaced with (2 mL) of solvent (DMSO), yielding a solution with a concentration of 50 mg/mL.

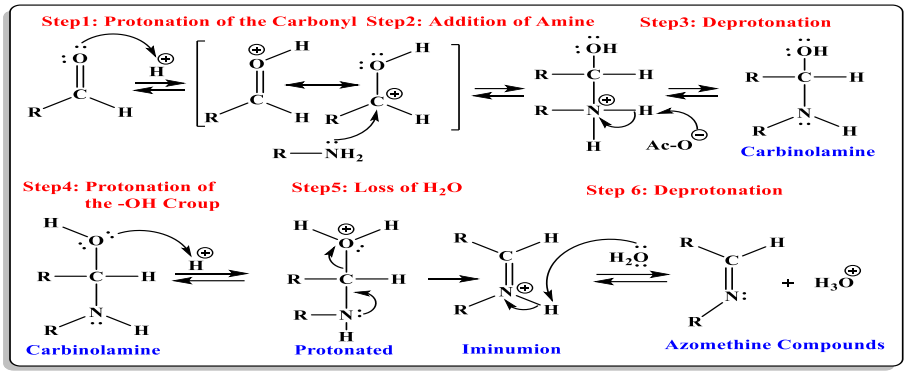
4- The agar-well diffusion method was used to test the sensitivity of bacteria to the selected chemicals against two species of bacteria. In each hole of the pits, The dishes were incubated in the incubator at a temperature of (37 ^{o}C) for a period of (24hr.) for the control materials, and the diameters of the areas of inhibition of microbial growth around the holes used were measured by the device (Zone Reader) the next day, as an increase in the diameter of the inhibition means an increase in the biological activity of the prepared compounds[20]

2.4.2. Evaluation of antifungal activity: The agricultural media are being prepared. Culture and the Media The culture medium (agar) was made using the diffusion method by digging on the medium (Muller-Hinton agar), which has a dark yellow hue and is used to assess microorganisms' antibiotic sensitivity[21]. It has an animal infusion that helps most bacteria and other microorganisms grow. The manufacturer's instructions (Biomark Laboratories) were to dissolve (38g) of culture media in (1000ml) of distilled water in a glass beaker and heat to complete dissolution, then sterilize the prepared media by (autoclave) for (20min) at a temperature of (115 ^{o}C) under Pressure (1.5 bar), then cool to a temperature of (45 ^{o}C), then add 2 percent glucose sugar, where (0.5 g/L) of (Methylene blue) dye, and The dishes were placed in the incubator for (24 hours) at room temperature to solidify the agricultural medium.

2.4.3. Antifungal activity test for some of the prepared compounds: The fungi were disseminated in the dishes using the diffusion method by etching on a medium in concentrations of (10,15,30 mg/mL) of the solutions to be assessed for their biological effectiveness (Muller-Hinton agar). Where colonies of pure fungal isolates were transferred by smearing (0.3 mL) of the fungal solution on the agar medium with a sterile cotton swab and drilling (6mm) holes in each dish with a Cork. The inhibitory substance was added to the pure sterilized perforator by heating on flame and alcohol, (0.1 mL) of solutions were poured in the fungus-grown agar pits, and the plates were incubated at a temperature of (37 ^{o}C) for (42 hrs. ,then, using a millimeter ruler, the area of inhibition diameters resulting from the influence of the compounds surrounding the hole was measured to determine the sensitivity of the compounds[22] Because increasing the width of inhibition increases the biological activity of some drugs.

3. Results and Discussion

3.1. Characterizations of azomethine compounds (A_1-A_4): The infrared spectrum (FT-IR) was used to characterize the active groups in prepared azomethine compounds, adding greater preliminary evidence of product formation, Bond v-N-H in the amine group with a range of 3200-3500 cm^{-1} [23], showed the disappearance of asymmetric and asymmetric vibration absorption bands, and starch band absorption at the rang 1720-1740 cm^{-1} due to aromatic carbonyl v-C=O vibrations and new absorption bands of the starched vibration of the characteristic vC=N of synthesized amine compounds extending from the wavelength range 1596 - 1724 cm^{-1} [24], six steps nucleophilic addition mechanisms were used in the preparation of the azomethine compounds. as given in scheme 1, see table 3 of FT-IR spectral of prepared azomethine compounds A_1-A_4.



Scheme 1. Mechanism of the synthesis process of azomethine compounds

Table 3. FT-IR spectral data azomethine compounds A1-A4

Comp.	C-H arom.	C-H ali.	=C-H alk	C=N azom.	C=C arom.	Others
A_1	3051	2869-2927	3051	1625	1588	1337 C-NO_2 sy 1522 C-NO_2 ays
A_2	3113	-----	3013	1661	1489 1520	1085-C-Cl
A_3	3088	------	3043	1631	1484 1579	1087 -C-Cl
A_4	3090	2924	3020	1614	1468 1577	1095-C-Cl

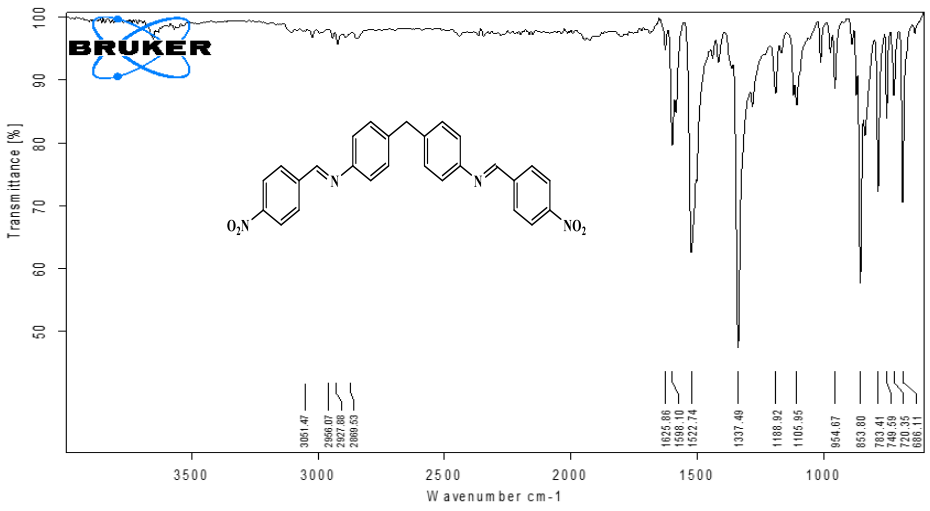
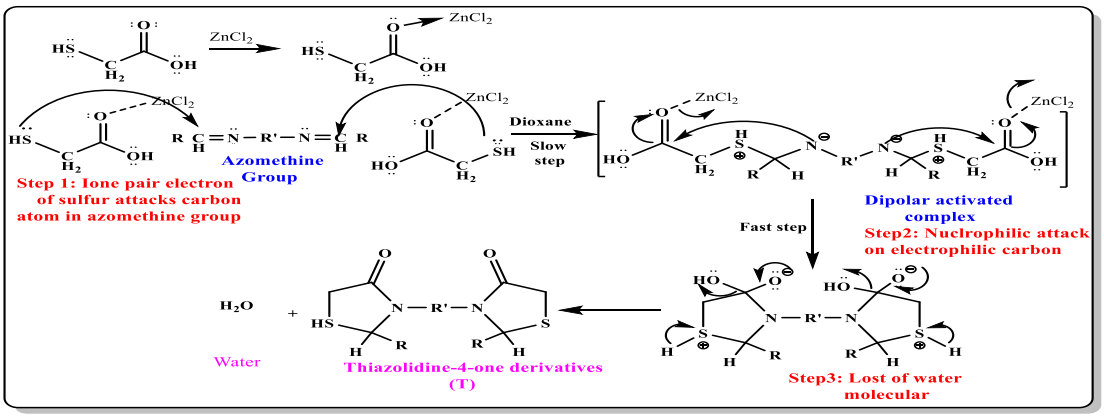


Figure 1. FT-IR spectra of A_1

3.2. Characterizations of 2,3-disubstiuted 1,3- Thiazolidine-4-one (T_1-T_4):

1,3- Thiazolidine-4-one were synthesized by reaction of azomethine with 2-mercaptoacetic acid using Dioxane as a solvent, FT-IR spectra for 2,3-disubstiuted 1,3- Thiazolidine-4-one (T1-T4) showed the disappearance of the absorption bands of the $_{\nu}$C=N and ν C=O groups of azomethine and 2-mercaptoacetic acid, respectively, and the appearance of the stretching absorption, i.e. aromatic bands C-H at 3055-3068

cm^{-1}, lactam C=O at 1672-1694 cm^{-1} [25], C-S at 664-702 cm^{-1} [26-29], table (4) shows that and (Figures 2 and 3). The electron-double nucleophile sulfur atom in 2-mercaptoacetic acid attacks the electrophilic carbon atom of the azomethine group, followed by the electron-double nucleophile nitrogen atom of the Schiff base attacking the electrophilic carbon atom of the carboxylic group in the aforementioned reaction. The reaction is thought to happen as a result of the mechanism shown below [30]. See scheme 2.



Scheme 2. Mechanism of synthesis of 2,3-disubstiuted 1,3- thiazolidine-4-one with Zinc chloride as a catalyst

FT-IR spectral data 2,3-disubstiuted 1,3- thiazolidine-4-one (T_1-T_4)

Comp.	C-H arom.	C-H ali.	C=O lactam	C=C arom.	C-S
T_1	3103	2971	1684	1598	664
T_2	3059	2965	1697	1590	702
T_3	3017	2931	1672	1551	692
T_4	3012	2915	1693	1568	692

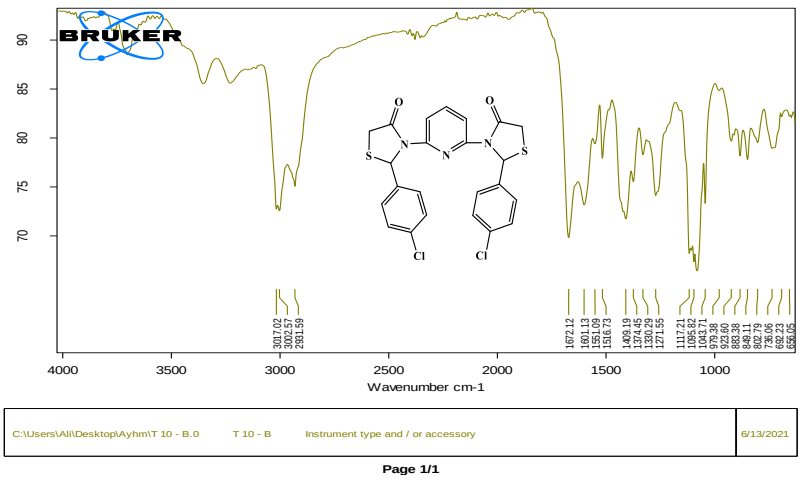
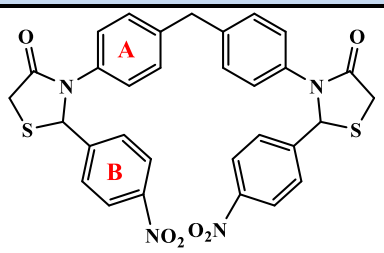
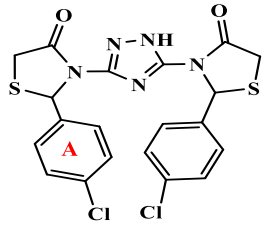
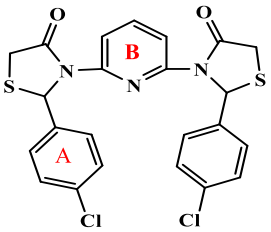
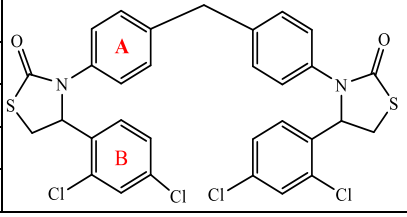


Figure 2. FT-IR spectra of T_3

3.3. Proton spectrum ^{1}H-NMR: The ^{1}H-NMR spectra for 2,3-disubstiuted 1,3- thiazolidine-4-one showed singlet signal within the range of δ 3.64-4.05 ppm assign to (s4H, CH_2-Het.) in five ring, 6.38-6.53 ppm (s2H, -N-CH-S- in five ring), while the signal for aromatic protons observed as multiplet-signal [31], ^{1}H-NMR data are presented in Table 4.

Table 4. Values of chemical displacements ^{1}H-NMR for some 2,3-disubstiuted 1,3- Thiazolidine-4-one**.**

Group	Type of single	No. of Protons	Chemical Shift (ppm)	Structure	Comp.
Ar-CH_2-Ar	s	2	3.69		T1
-CH_2-Hetero	s	4	4.05		
-N-CH-S-	s	2	6.53		
Aro.Proton[A]	d,d	8	7.01-7.19		
Aro.Proton[B]	d , d	8	7.66-8.16		
-CH_2-Hetero	s	4	3.64		T2
-N-CH-S-	s	2	6.39		
=N-NH-C-	s	1	9.53		
Aro.Proton[A]	d,d	8	7.12-7.41		
-CH_2-Hetero	s	4	3.96		T3
-N-CH-S-	s	2	6.38		
Aro.Proton[A]	d,d	8	7.16-7.30		
Aro.Proton[B]	t , d	3	7.75-7.94		
Ar-CH2-Ar	s	2	3.77		T4
-CH_2-Hetero	s	4	3.99		
-N-CH-S-	s	1	6.44		
Aro.Proton[A]	d,d	8	6.88-7.01		
Aro.Proton[B]	S, d, d	6	7.11-7.51		

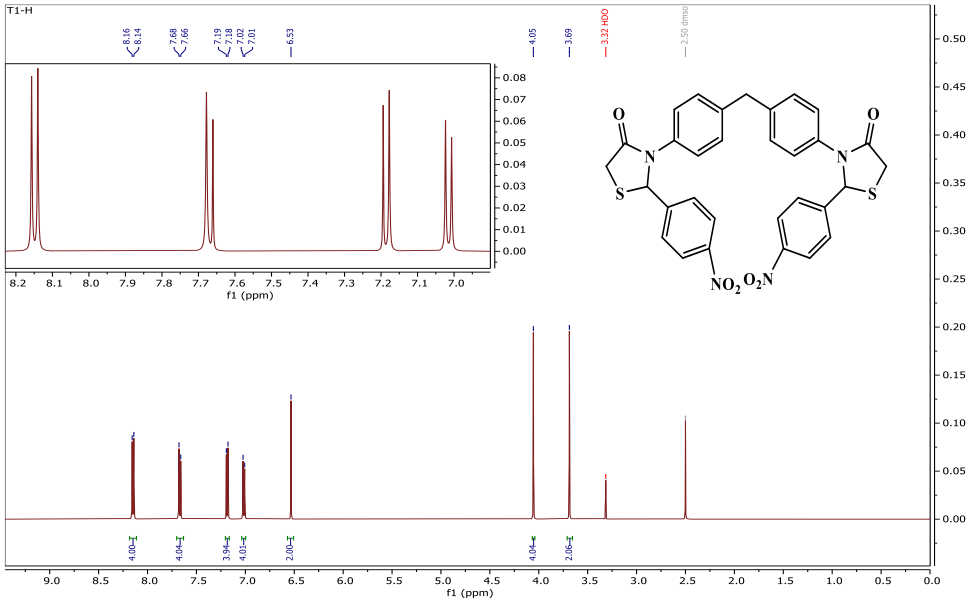


Figure 3. Expanded and condensed ^{1}H-NMR spectrum for compound T1

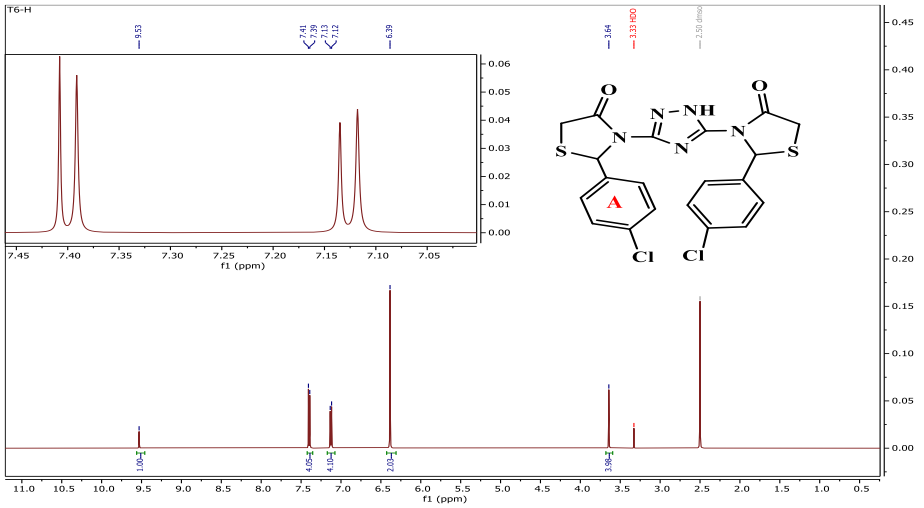


Figure 4. Expanded and condensed ^{1}H-NMR spectrum for compound T_2

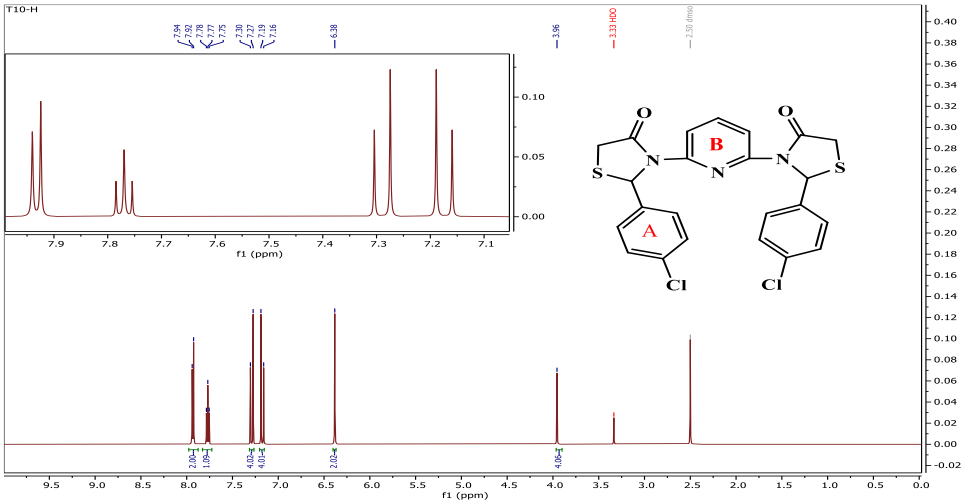


Figure 5. Expanded and condensed ^{1}H-NMR spectrum for compound T_3

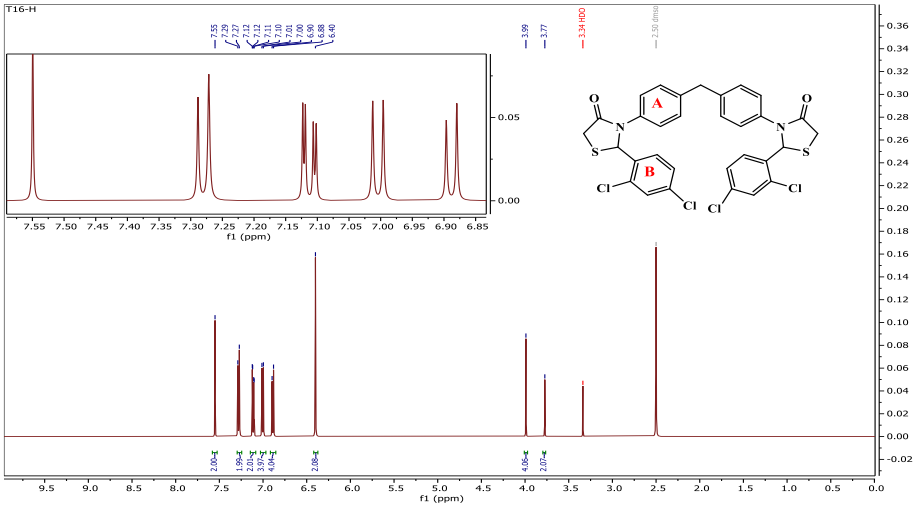


Figure 6. Expanded and condensed ^{1}H-NMR spectrum for compound T_4

3.4. Mass spectrum

One of prepared oxazepine derivatives showed the value of it's fragments in figure (8) and in scheme (3).

Table 5. Values of m/z for the molecular ion M^+,

Fragment	Mass / Charge m/z))
M^+: $C_{31}H_{24}N_4O_6S_2$	612.0
$C_{31}H_{24}N_3O_4S_2^+$	567.0
$C_{30}H_{26}N_3O_4S_2^+$	557.0
$\bullet+ C_{31}H_{24}N_2O_2S_2$	522.0
$C_{25}H_{20}N_3O_4S_2^+$	491.1

$C_{25}H_{22}N_3O_4S^+$	461.2
•+ $C_{21}H_{19}NOS$	334.1
•+ $C_{17}H_{15}NOS$	282.1
•+ $C_{13}H_{10}$	167.1
•+ $C_{12}H_{12}$	157.0
+ C_7H_7	91.1 Base Peak
•+ C_5H_6	67.1
+ C3H5	42.2

File :C:\MSDCHEM\3\DATA\Snapshot\7G996D.D
Operator :
Acquired : 18 Jun 2021 13:10 using AcqMethod test000414.M
Instrument : MSD
Sample Name: T1
Misc Info

Scan 106 (1.533 min): 7G004D.D\data.ms

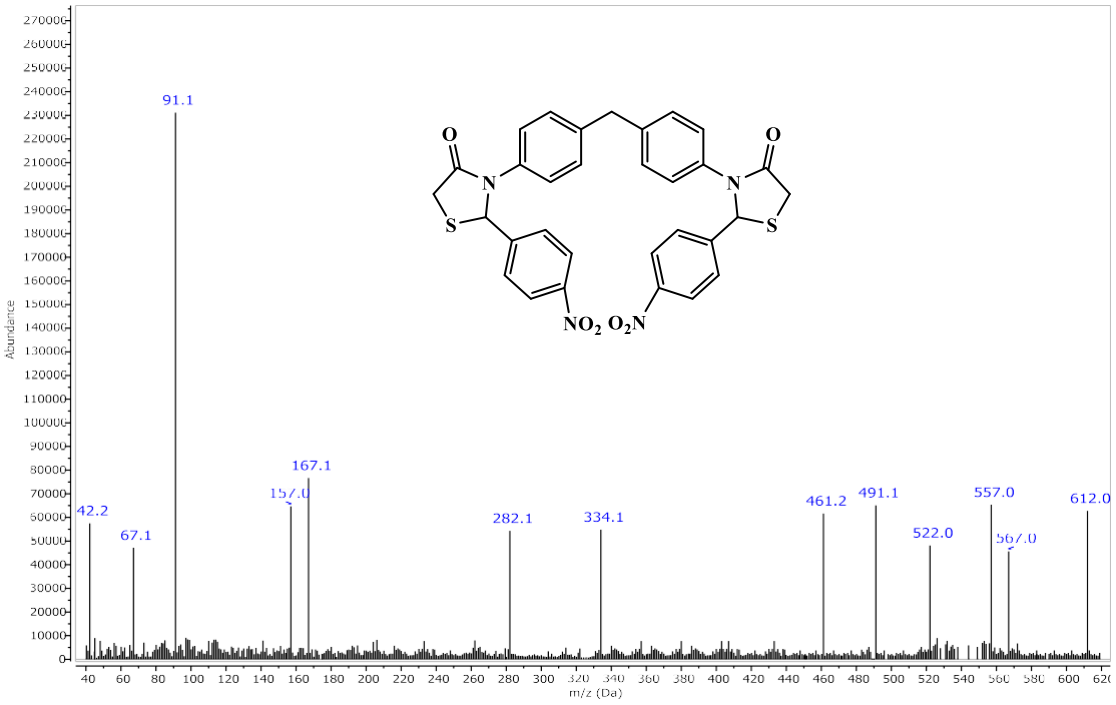
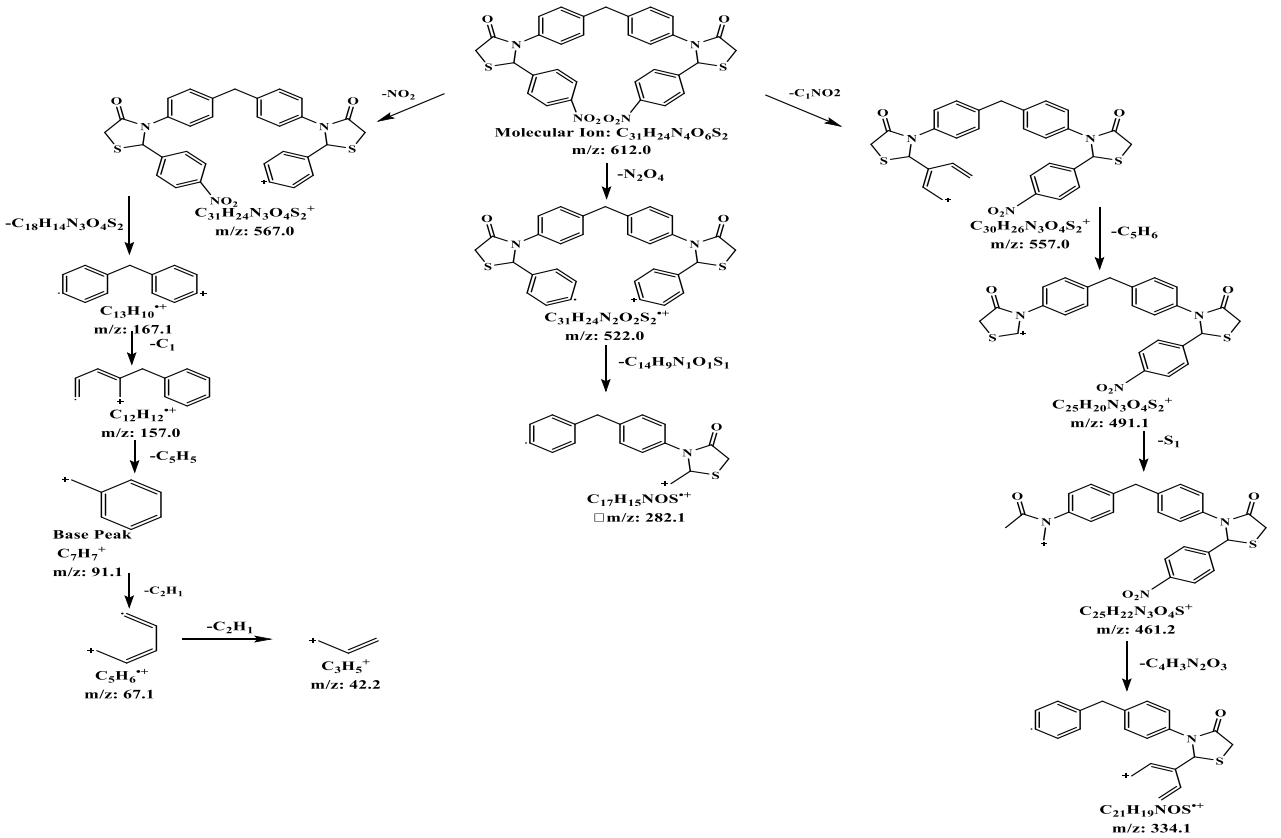


Figure 7. Mass spectra of compound T_1



Scheme 3. Fragments structure of T_1

3.3. Antioxidant activity

The activity of any molecule is seen when the purple hue of DPPH turns yellow, and the concentrated color is an indication of effectiveness, and the compounds (A_1, T_1 and T_2) are a better source of anti-oxidant than ascorbic acid (as standard) [32]. Table 6 show it. The efficiency compounds' free radical scavenging ability was compared to that of ascorbic acid, which served as a control. It is obvious from the results that the compounds under investigation's free radical scavenging activity increased as their concentration increased.

showing. It is a dose dependent nature. Therefore, the highest free radical scavenging was given with 0.5 Mm of T_2 of 33%, where the A_1 had the lowest percentage of 24.5% compared with standard with 87.6%. This result confirms the result in figure (8) above that's made ensure the T_2 has the best ability of free radical scavenging.

Percentage of antioxidant activity of (A_1,T_1-T_2) compounds.

Concentration (Mm)	Percentage of antioxidant activity			
0.05	11.5	9	14.6	63
0.1	13	13	19	65.7

0.15	17.4	15.3	23	69
0.2	20	17	28.6	76.8
0.5	24.5	21.8	33	87.6

*Vitamin C

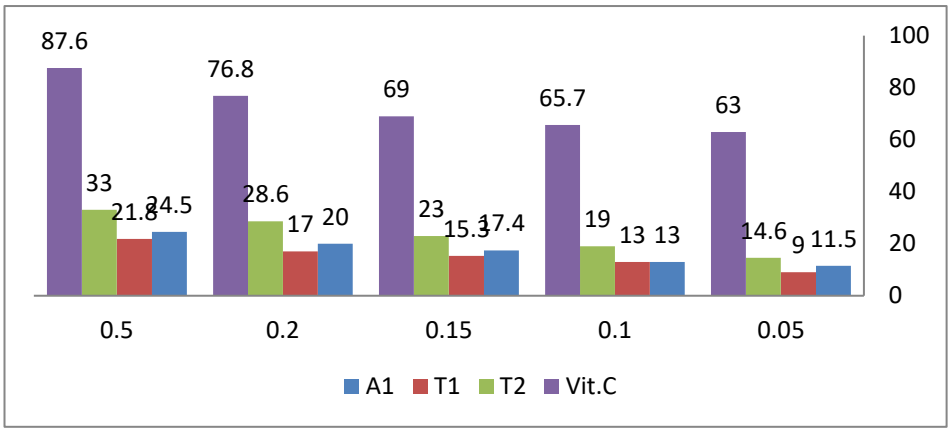


Figure 8. antioxidant activity of (A_1,T1-T_3) compounds.

The IC_{50} for each compound were particular and display in the table (7) and figures (9-11).

Table 7. IC_{50} values of antioxidant activity of (A_1, T_1-T_2) compounds .

Compound Name	IC_{50} (Mm)
A1	0.783
T1	0.885
T2	0.574
Vitamin C	0.205

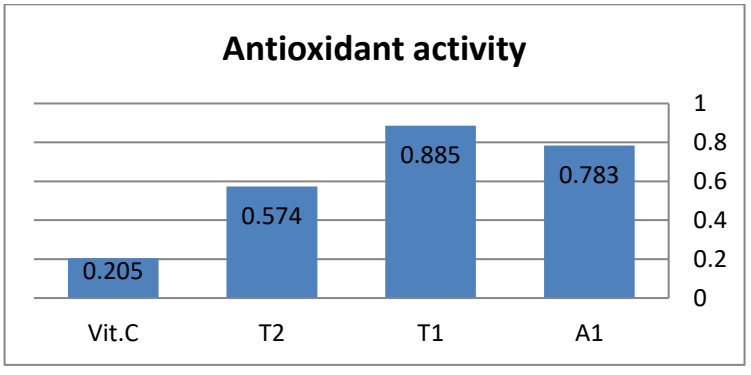


Figure 9. IC_{50} anti-oxidant activity of (A_1,T_1, T_2) compounds.

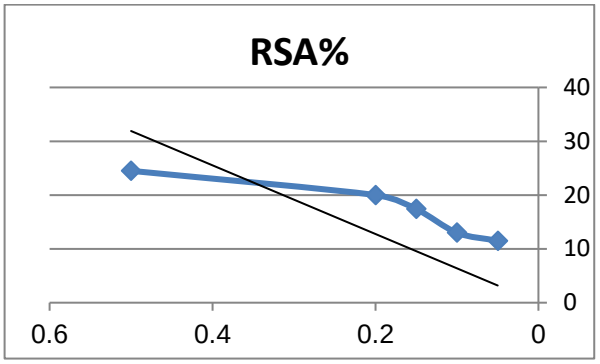


Figure 10. The IC_{50} for A_1

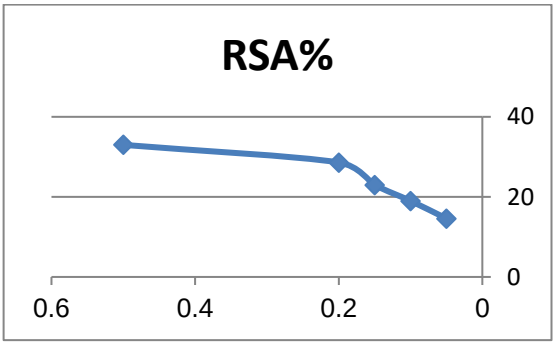


Figure 11. The IC_{50} for T_2 derivative

3.4. Antibacterial Activity

Because of the differences in the compensation groups, concentration, and type of bacteria under examination, the findings of the antibacterial activity test of the five rings derivatives revealed a clear variation in the results. We can see that the derivative (T_1) had a good inhibition rate against the bacterium (Staphylococcus aureus), whereas the derivative (T_2) had a good inhibition rate and a good percentage against the bacteria (Escherichia coli) (Staphylococcus aureus) ,table (8) showed that**.**

Table 8. Biological activity of T_1, T_2, against pathogenic bacteria

Compound	*Escherichia coli*		*Staphylococcus aureus*	
	50	100	50	100
T1	17	22	14	21
T2	14	20	22	22
Cefotaxime sodium	22	26	24	30

3. 5. Anti-fungi Activity

By measuring the damping diameters of the fungal colony and measuring the percentage of inhibition, the antifungal activity of some compounds synthesized using two types of fungus (Candida albicans, Aspergillus niger) was examined. [33]. Antifungal antibiotics (Itraconazol and Fluoconazol) were used to compare the inhibitory results of the produced compounds. The percentages of inhibition vary significantly depending

on the type of derivative, the form of the compensation, the concentration, and the type of fungi under examination, as shown in table (9) of the test findings. When compared to the control treatments, the heterocyclic five rings derivatives showed differences in the percentage of fungus suppression and demonstrated good efficacy [34]. In all of the prepared concentrations, the derivative (T_2) had the highest inhibition rate against Candida albicans. In all concentrations tested, the derivative (T_1) had the highest inhibitory value for Aspergillus niger growth. Tables 9 and 10 illustrate the antifungal percentages of derivatives with five rings, as well as the biological action of antibiotics on fungal growth. [35-36].

Table 9. Biological activity of T_1, T_2 against pathogenic fungi (mm)

	Candida albicans			***Aspergillus Niger***		
Compound	10 mg/mL	15mg/mL	30mg/mL	10mg/mL	15mg/mL	30mg/mL
	Inhibition diameter			**Inhibition diameter**		
T_1	8mm	12mm	13mm	0mm	9mm	12mm
T_2	9mm	11mm	14mm	0mm	0mm	11mm
Control DMSO	*	*	*	*	*	*

* No effect on fungal growth was observed.

Table 10. Biological activity of antibiotics against pathogenic fungi (mm)

	Candida albicans			***Aspergillus Niger***		
Anti-Fungi	10 mg/mL	15mg/mL	30mg/mL	10mg/mL	15mg/mL	30mg/mL
	Inhibition diameter			**Inhibition diameter**		
Itraconazole	8mm	13mm	22mm	0mm	10mm	14mm
Fluoconazole	15mm	18mm	27mm	9mm	15mm	21mm

4. Conclusion: According to FT-IR, 1H-NMR, and mass spectra data, the reaction of azomethine with thioglyocolic acid to 1,3-thiazolidine-4-one derivatives was successfully completed, and the expected probable reaction mechanism was provided, as well as evaluation of its antioxidant and biological properties. The formation of a charged linear intermediate in the transition state that eventually collapses via an internal cyclization reaction to give the target molecule has been found to have excellent antioxidant activity. The reaction of azomethine with carboxylic acid derivatives to produce 1,3-thiazolidine-4-one derivatives was successful. Based on FT-IR, ^{1}H-NMR, and mass spectra, as well as an assessment of its antioxidant and biological activity, a feasible reaction mechanism was proposed.

5. Acknowledgments: Thanks to everyone who help us to complete this research at the best form. Special thanks to the President of Tikrit University and the Dean of the College of Sciences for their continuous support to the researchers .

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