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Synthesis, Characterization and bioactivity Study of some new Trizole malimide Compounds.

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الخلاصة

حضر اثنين من المركبات الحلقية غير المتجانسة الجديدة المكونة من خمسة ذرات على التوالي (T1) 4-(4,6-dioxo-5-(p-tolyl)-4,5,6,6atetrahydropyrrolo[3,4-d][1,2,3] triazol-1(3aH)-yl)benzene sulfonamide و (T2) 4-(5-(4-chlorophenyl)-4,6-dioxo-4,5,6,6atetrahydropyrrolo[3,4-d][1,2,3]triazol-1(3aH)-yl) اللذين تم تصنيعهما بواسطة تفاعل الاضافة الحلقية ثنائية القطب من تفاعل (azidobenzenesulfonamide-4) مع 1-(p-tolyl)-1H-pyrrole-2,5-dione) (A_1) و (1-(4-) chlorophenyl)-1Hpyrrole-2,5-dione) (A2) باستخدام الحرارة. تم تصنيع مركبات T1 و T2 عند 56 درجة مئوية بوجود الأسيتون كمذيب. تمت إعادة بلورة المنتجات من الأسيتون والأثير (1: 2) ، وتجفيفها وتشخيصها باستخدام مطياف الأشعة تحت الحمراء ، الرنين النووي المغناطيسي ، وأطياف الكتلة. تمت دراسة النشاطات المضادة للبكتيريا والفطريات للتريازول (T1 & T2) حيث تم اختبار المركبات المحضرة كمضاد لنشاط البكتيريا والفطريات عن طريق إجراء المقايسة الحيوية للقرص والفتحة لتحديد نشاط المركبات المحضرة (T1 & T2) ضد *(Aspergillus Terreus* و *Psedomounus Aeroginosa* و *Kocuria Kristinie* و *Enterobactar Cloacae (Aeromonase veronii) and Staphylococcus Lentus),*

Abatract

New two compounds of a five-membered heterocyclic rings triazoles compounds (T_1) 4-(4,6-dioxo-5-(p-tolyl)-4,5,6,6atetrahydropyrrolo[3,4-d][1,2,3] triazol-1(3aH)-yl)benzene sulfonamide & (T_2) 4-(5-(4-chlorophenyl)-4,6-dioxo-4,5,6,6atetrahydropyrrolo[3,4-d][1,2,3]triazol-1(3aH)-yl) which were synthesized

by 1,3 di polar cycloaddition reaction of 4-azidobenzenesulfonamide with (A_1) (1-(p-tolyl)-1H-pyrrole-2,5-dione) & (A_2) (1-(4-chlorophenyl)-1Hpyrrole-2,5-dione) followed by thermal process. T_1 & T_2 compounds were synthesized at 56 °C in the present of acetone as a solvent. The products were recrystallized from acetone and ether (1:2), dried and characterized using Fourier transform infrared FT-IR, ^{1}HNMR, and mass spectra. The antibacterial activities of triazoles (T_1 & T_2) were studied. Antibacterial and anti-fungi activity, Hole-in-plate and disk bioassay procedure methods were used to determine activity of prepared compounds (T_1 & T_2) against (*Aspergillus Terreus,*fungus & *Psedomounus Aeroginosa, Kocuria Kristinie , Enterobactar Cloacae. , Aeromonase veronii and Staphylococcus Lentus)* bacteria.

Key words: triazole, malimides, azide, 1,3 di polar Cycloaddition, antibacterial

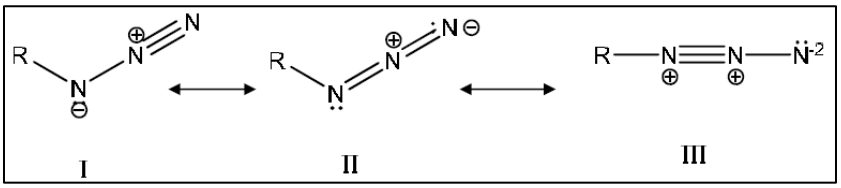
1. Introduction

Heterocyclic compounds tend to exhibit unique properties, formed by the changing of one or more carbon atoms with a heteroatom, such as nitrogen, oxygen, sulfur, or a combination of these atoms. Heterocyclic compounds can be classified into two main groups: aliphatic and aromatic [1]. Aliphatic heterocyclic compounds are saturated or partially saturated and have one or more sp3-hybridized atoms [2]. Aromatic heterocyclic molecules must be planar with a complete and uninterrupted cycle of p-orbitals and must obey Hückel's 4n + 2 rule with the variable n is equivalent to zero or a whole number. If one of these items were missing the molecule cannot be qualified as being aromatic [3].

1,3-dipolar cycloaddition is one of the important reactions to preparation five-member heterocyclic rings, by the reaction of two organic compounds, one is

called dipolar and the other dipolarophile [4,5]. Where it is a distinctive way to prepare the (five member –ring) heterocyclic compounds[6]. Dipolarophile could be alkenes ,alkynes ,carbonyls or nitriles whereas the 1,3-dipolar could be divided into two types ,the allyl anion type such as nitrones, azomethine imides , azomethine ylides, carbonylylides and thiocarbonyl ylides. The other type was linear propargyl 1,3-dipolar such as diazo compounds, nitrile oxides, nitrile imides, nitrile ylides, nitrile sulfides and azides [7]. The azide compounds contain three nitrogen atoms connected to each other where (N3) is the active group which has resonance hybrids between three structures as shown in (scheme 1), but structure (III) is not being a major contributor based on a consideration of the adjacent charge rule [8]. Two types of azides were reported, inorganic azides in which the active group (N3) is connect with metal such as sodium azide and lead azide were still widely used as an initiator in ammunition [9]. Azides has dual behavior during chemical reactions, so it can react with compounds which have electron deficiency (electrophiles) as well as with electronically rich compounds (nucleophiles) [10].

Triazoles are important five-member nitrogen heterocycles compounds containing three nitrogen atoms as well as two carbon atoms [11]. It has three positions (1, 4 and 5) could be substituted [12]. Triazoles compounds have several industrial applications such as, anti-weed [13] agrochemicals, dyes, optical brighteners [14], corrosion inhibitors [15] as well as biologically active agents, antifungal agents [16], anti-inflammatory [17], antimicrobial [18] and anti-cancer [19]. 1,3-dipolar Cycloaddition Thermal Reaction is the well-established method for the [1,2,3]-triazole ring systems synthesis which relies on the thermal 1,3-dipolar Huisgen Cycloaddition between alkynes or alkene and azides [20,21].



Scheme(1) resonance hybrids between three structures of azide

2- Experimental

2- 1 Synthesis of maleanic acid [22]

A solution of substituted aniline 10 m. mole in 10 ml of ether was added slowly over thirty minutes to a well stirred solution of 0.98 g. 10 m.mole of maleic anhydride in 30 ml of ether at room temperature. A white precipitate of N-phenyl maleanic acid was formed immediately upon the addition of the amine. The reaction mixture was stirred for one hour. The precipitate was collected, washed with ether, and recrystallized by using a (1:2) methanol-ether mixture.

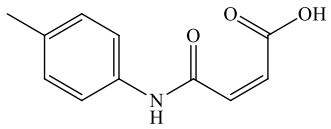
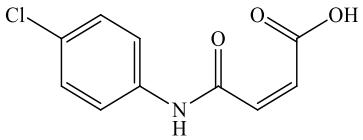
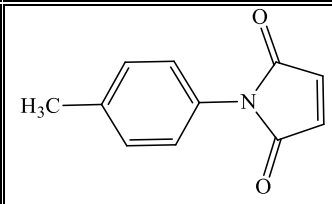
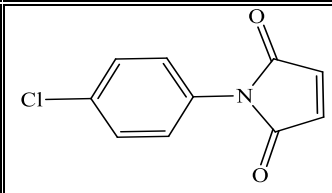
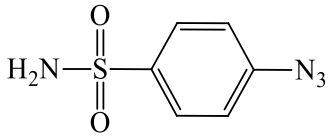
2- 2 Synthesis of N- substituted phenyl maleimide [22-24]

A mixture of 20 m. mole N- substituted phenyl maleanic acid in 10 ml of acetic anhydride, and 12 m. mole of sodium acetate were heated with stirring on a steam bath at for 1 hr. The reaction mixture was poured into 30 ml of ice water and stirred for 2 hrs. The aqueous mixture was extracted with ether. The ether layer was separated and dried over whith anhydrous sodium sulfate. The ether was evaporated in the hood and the residue was drayed and recrystallized from ethanol as shown in table (1).

2-3 Synthesis of the sulfonamide azide

To a well stirred solution of 0.001 mole sulfonamide amine in 1ml concentrated hydrochloric acid and 10 ml of distilled water at (0-2) ºC drop wise a solution of 0.07 g, 0.001 mole sodium nitrite in 5 ml distilled water was added. After 15 minutes a solution of 0.07 g, 0.001 mole sodium azide in 5 ml distilled water was added. The mixture was stirred at (0-2) °C for one hour and 20 ml ice water was added slowly with cooling. The precipitate was collected by suction filtration and recrystallized from chloroform and hexane(2:1) or butanol and hexane(2:1) [25].

Table (1): Synthesized Malenic acids, Maleamides and azide with their physical properties

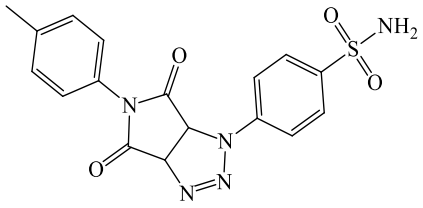
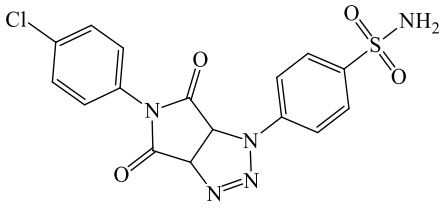
Name of compounds		Structure	Color	%	M.P°C
1	(p-methyl phenyl) maleanic acid		light-yellow	82	(200-201)
2	(p-chloro phenyl) maleanic acid		light-yellow	80	(205-206)
3	N-(p-methyl) phenyl maleimide		yellow	52	(152-153)
4	N-(p-chloro) phenyl maleimide		light-yellow	68	(116-117)
D_1	4-azido phenyl sulfonyl amide		White	82	116-118

2-4 Synthesis of 1, 2, 3- triazoline compounds (General procedure)

The mixture of 0.001 mole of 4-azido phenyl sulfonyl amide and 0.001 mole of malamide dissolved in 20 ml of acetone in 100 ml round bottom flask was heated under the reflux with stirring. The reaction mixture was followed by TLC using toluene and acetone (7:3) as a mobile phase. After monitoring for several hours, it was observed that a new spot was indicated in the reaction mixture [**22**].

The reaction mixture was cooled and the ether was added dropwise to the mixture, precipitate was collected using a filter paper, purified by acetone and ether (1:2) as shown in table (2).

Table (2) : Synthesized Triazoline Compounds (T_1 and T_2).

Symb.	**T_1**	**T_2**
Name of compounds	4-(4,6-dioxo-5-(p-tolyl)-4,5,6,6a-tetrahydropyrro lo [3,4-d][1,2,3]triazol-1(3aH)-yl)benzene sulfonamide	4-(5-(4-chlorophenyl)-4,6-dioxo-4,5,6,6a-tetra hydropyrrolo[3,4-d] [1,2,3]triazol-1(3aH)-yl) benzene sulfonamide
Structure		
Yield %	43	52
Reaction Time (hr.)	26	18
m.p. ^{o}C	180-181	159-160

2.5. IR spectra of synthesized compounds.

2-5-1 IR spectra of malimides compounds A_1 ,A_2:

The carbonyl group of malic anhydride should give a strong band approximately at (1750-1780) cm^{-1} whereas in the synthesized malimides A_1, A_2 its appears at (1708,1712) cm^{-1} respectively. Furthermore the other bands were given in table (3) [22,23,2**6**] as shown in figure (1&2).

Table (3) : The Important FTIR bands for A_1 and A_2.

Groups	C-H aromatic	C-H Aliphatic	C=O	C-N	C=C N=N
A_1	3039	2920	1708	1313	1517
A_2	3086	-	1712	1371	1583

2-5-2 IR spectrum of azide compound (D_1)

Generally azides have week stretching vibration bands in the range 2100-2250 cm^{-1} attributed to the group of N_3. As well as the absence of stretching vibration bands of NH_2 group which clearly appeared in infrared spectra of D_1, compound. In addition to the other packages shown in table (4). [2**2**,2**6**]

Table (4): The Important FTIR bands for D_1

Groups	NH_2	C-H aromatic	N_3	C-N	O=S=O	C=C N=N
D_1	3315	3051	2155	1259	1166-1342	1593

2-5-3 IR spectra of triazolene compounds:

The most important characteristic of the spectra of the triazoline compounds (T_1 and T_2) as shown in figure (3&4) is the disappearance of the peak belong to the azide group at 2150cm^{-1}, and the presents of the amine group peaks at 3255,3342 cm^{-1}, which belongs to the sulfonylamide that was contain the azide group before the reaction of cyclization addition.

Table (5): The Important FTIR bands for $T_1 – T_2$

Groups	NH_2	C-H triazole	C-H aromatic	C-H aliphatic	C=O	C-N	SO_2	C=C or N=N
T_1	3343	3256	3120	2920	1738	1334	1157	1597
T_2	3379	3269	3104	-	1724	1338	1163	1595

2-6 ^{1}HNMR nuclear magnetic resonance spectrum :

2-6-1 Nuclear Magnetic Resonance Spectrum of T_1:

Nuclear magnetic resonance spectrum of T_1which is shown in Figure (5) gaves the fallowing signals:

The signal at 2.34 ppm attributed to $3H^8$ belong to methyl group. Doublet signal at 5.35& 5.37 ppm refers to one proton H^1 with *J* coupling (10 Hz), while the Doublet signal at 6.00& 6.02 ppm is assigned to H^2 proton with *J* coupling (10 Hz). Both integral values for H^1 & H^2 were equal to integral of one proton exactly which improved the formation of T_1 with high purity. Doublets signals at 7.15&7.17.35 and 7.28&7.30 ppm refer to the aromatic protons $2H^6$ and $2H^5$ respectively , while the other Doublets signals at 7.74&7.76 and 7.86&7.88 ppm are assigned to the second aromatic protons $2H^3$ and $2H^4$ respectively .The signal at 7.33 ppm with integral value equal to tow protons exactly attributed to $2H^7$ belongs to amine group.

2-6-2 Nuclear Magnetic Resonance Spectrum of T_2:

Nuclear magnetic resonance spectrum of T_2 which is shown in Figures (6.&7) gives the following signals:

The doublet signal at 5.364, 5.391 ppm refer to one proton H^1 with J coupling (10.8Hz), while the doublet signal at 5.995, 6.021 ppm is assigned to H^2 proton with J coupling (10.8Hz). Doublets signals at 7.283, 7.305 and 7.538,7.559 ppm refer to the aromatic protons $2H^6$ and $2H^5$ respectively , while the other Doublets signals at 7.710,7.732 and 7.842,7.864 ppm are assigned to the second aromatic protons $2H^3$ and $2H^4$ respectively.The signal of 2 H^7 attributed to amine group was present approximately at 7.5 ppm which interacted with aromatic protons leading to increase of integrations values (0.51, 0.25, 0.43) belong to ten protons as in Figure (7) whereas the signal of amine was absent when deuterium oxide with DMSO used as a solvent which gave exact integrations values (0.28, 0.27, 0.25, 0.26) belong to eight protons of two AB system aromatic rings as in Figure (6).[22,23]

2-7 Mass spectrum:

Mass spectra of T_1 (Figure 8) shows molecular ion peaks at m/z = 385.2 and base ion peak at m/z = 357.2 whereas Mass spectra of T_2 (Figure 9) shows molecular ion peaks at m/z = 405.2 and base ion peak at m/z = 377.2.

2-8 Antibacterial and Anti fungi activity:

Hole-in-plate bioassay procedure method were used to determine activity of the prepared compounds (T_1&T_2) against ***Aspergillus terreus*** **fungus,** ***Psedomonus aeroginosa , Enterobactar cloacae***.(Gram-negative bacterium) and ***Kocuria kristinie.***(Gram-positive coccus)

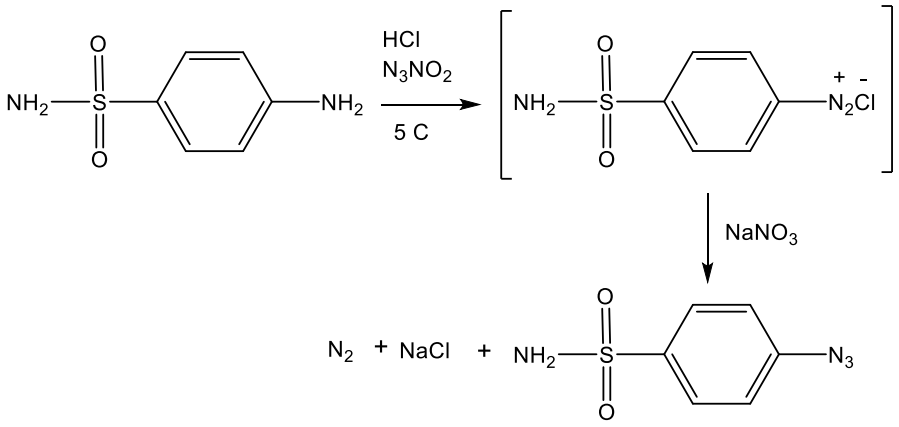
In order to purchase the efficiency of synthesized compounds towards bacteria, the hole –in- plate bioassay approach was applied [**27**]. The method was include twenty four hours incubation at 37^{o}C of pure culture of organisms which inoculated onto Muller-Hinton broth (MH) (Oxoid , England) , in order to gain a density of 10-6 cfu/ml equivalent to MC– Farland standard a sterile nutrient broth was used for dilution. The surface of (MH) agar plates were streaked by suspension, then a hole of 6mm in diameter into agar for each petri-dish containing bacterial culture were made by a sterile cork-borer. After initial setup steps, 500 mg/l of the synthesized compounds (T_1&T_2) were poured in to the wells followed with incubation step at 37^{o}C for (18-24 h, 24-72 h) for (*Psedomonus aeroginosa , Kocuria kristinie , Enterobactar cloacae)* and *(Aspergillus terreus*) respectively . Finally antibacterial activity was recorded according to the zone of inhibition.

Disc method also purchased for investigation of the efficiency of synthesized compounds (T_1&T_2) towards bacteria *(Aeromonase veronii)* is a Gram-negative and (*Staphylococcus Lentus*) is a Gram-positive, this approach applied by immersion a blank sterilized discs of size 6 mm into a solutions series containing 500mg/l of T_1&T_2 , after a disc absorption an adequate quantity, discs were stored in the refrigerator at 4°C till the use [**28**]. The streak of (MH) agar also streaked by suspension, then the plates then incubated at 37^{o}C for 18-24 h. Antibacterial activities were recorded according to the zone of inhibition.

3- Results and Discussion:

3-1 Synthesized Azide:

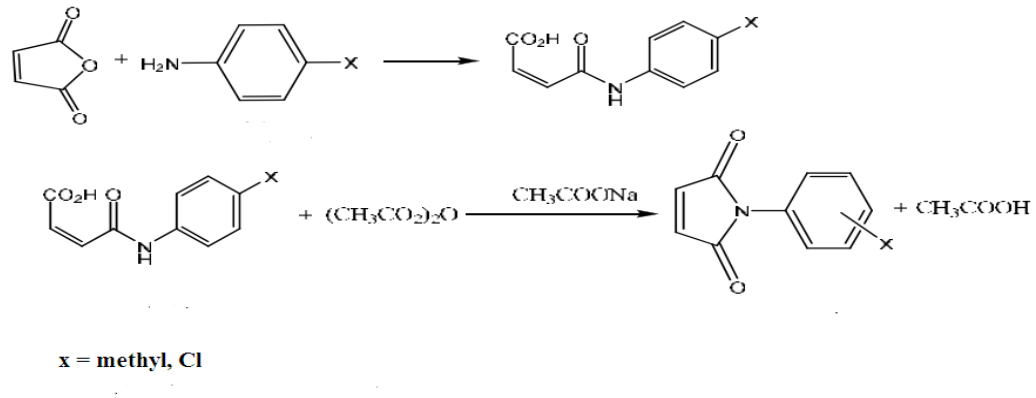
Azide were synthesized by the reaction of sodium azide with diazonium salts which prepared from the reaction of sulfonamide with HCl and sodium nitrite at 0 °C as shown in (scheme 2). The N_3 group where substituted onto aromatic ring fallowing the $S_{N\,Ar}$ mechanism (nucleophilic substitution).



Scheme (2) Synthesis of Azide compound

3-2 Synthesized malimid compounds:

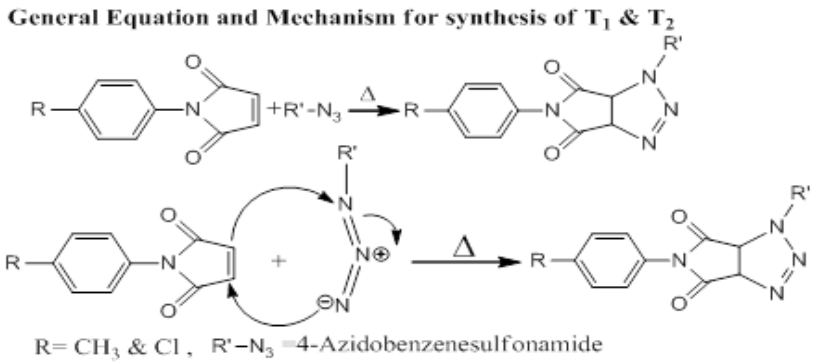
Malimides were synthesized in two steps, the first step including the reaction of malic anhydride with p-methyl aniline or p-chloroaniline in the present of ether as solvent at r.t. resulting malinic acids. In the second step malimides were formed by cyclization of malinic acids after losing H_2O in the present of acetic anhydride and sodium acetate as shown in scheme (3).



Scheme (3) Synthesis of malimids compounds

3-3 Synthesized triazole compounds.

Triazole compounds (T_1& T_2,) were formed by the reaction of unsaturated compounds (A_1& A_2) respectively with azide, according to 1,3 dipolar cycloadition reaction. the addition of azide to the double bonds compounds were fallow the Huisgen Cycloaddition thermal process which described below in scheme (4).



scheme (4): General equation and mechanism for synthesis of (T_1& T_2,)

The results showed the possibility of synthysizing the five-membered ring compounds containing three nitrogen atoms (triazoles) using the thermal 1,3-dipolar Huisgen Cycloaddition between the double bond in malimides and azide. With a yield that is not great, because the use of heat during the synthesis process for a long time may lead to the decomposition of the five- ring and its transformation into a triple ring with the loss of a nitrogen gas molecule. The results from the mass spectrum showed that the base ion is 28 less than the molecular ion.

For antimicrobial activity against both gram positive s and gram negative and antifungal activity the newly synthesized compounds were screened. The results showed that the compound T_2 has more activity against most of the bacteria and fungi under study, and this may be due to the presence of the chlorine group, which has greater activity than the methyl group in T_1 compound.

Table (6): Anti-Fugal activity and Anti-Bacterial activity of prepared compounds (T_1&T_2) at 500mg/l.

Comp.	***Aspergillus terreus***	***Psedomonus aeroginosa***	***Kocuria kristinie***	***Enterobactar cloacae***	***Staphylococcus Lentus***	***Aeromonse Hydrophila***
T_1	41	25	1	1	4	4
T_2	41	50	13	14	9	9

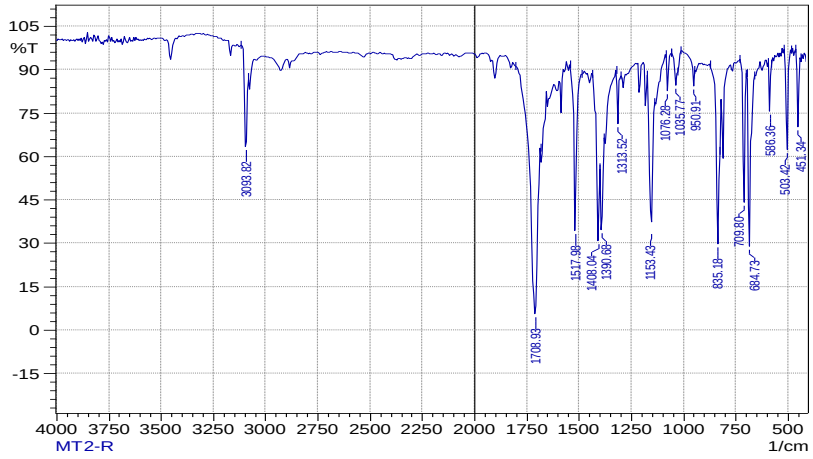


Figure (1): IR spectrum of malimide compound (A_1)

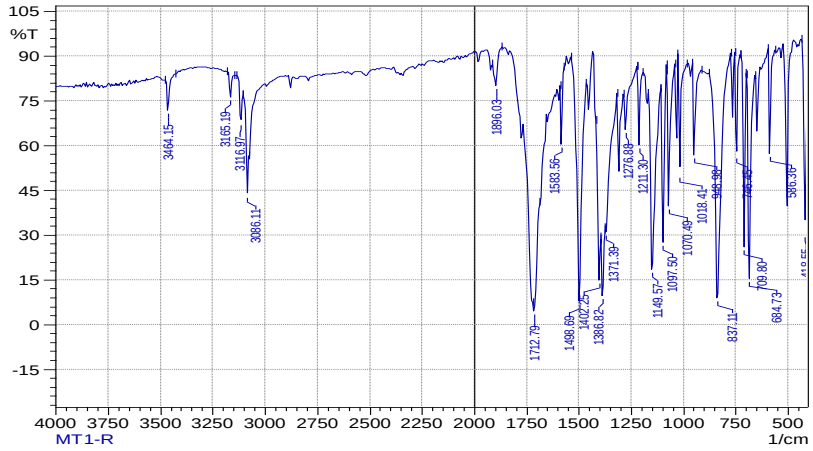


Figure (2):IR spectrum of malimide compound (A_2)

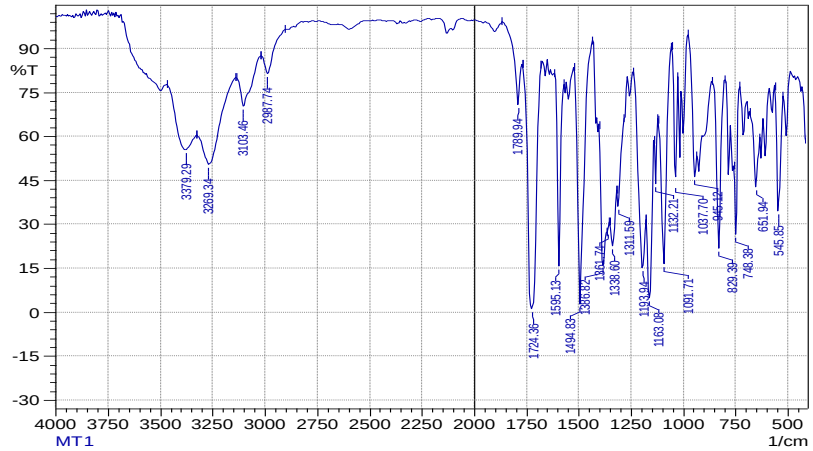


Figure (3): IR spectrum of T_1 compound.

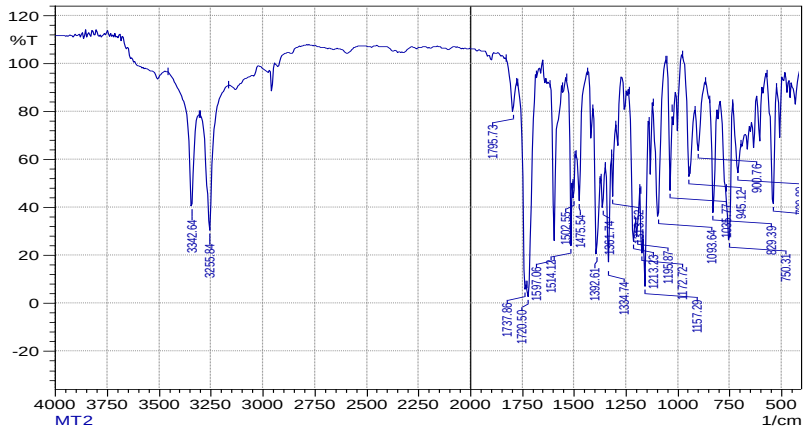


Figure (4): IR spectrum of T_2 compound.

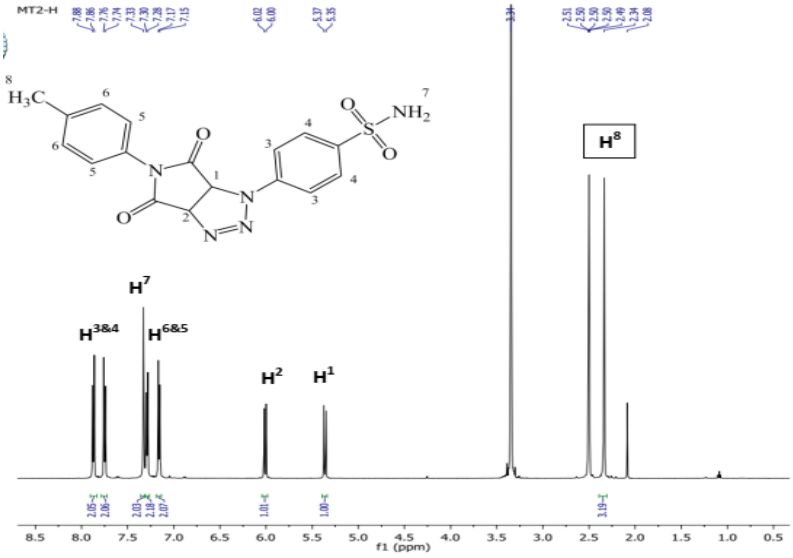


Figure (5): Nuclear Magnetic Resonance (H1-NMR) spectrum of T_1

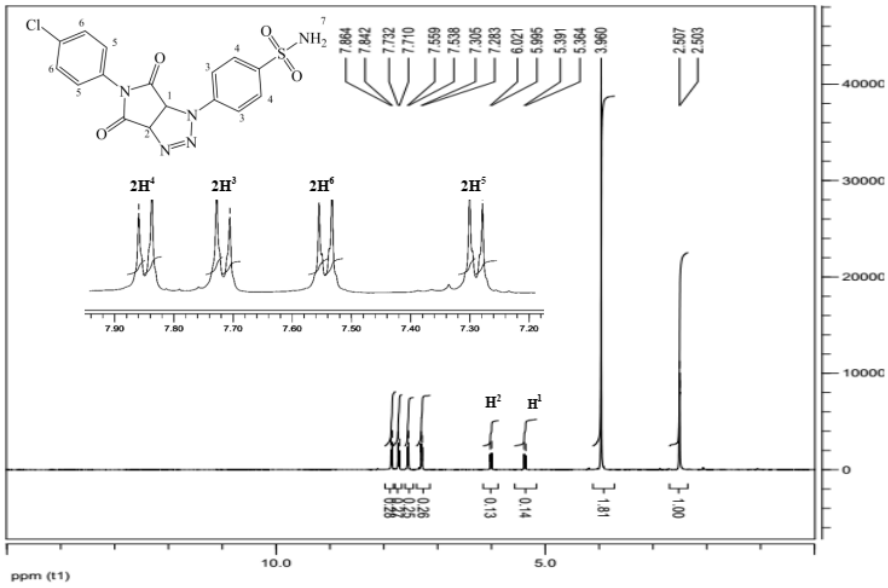


Figure (6): Nuclear Magnetic Resonance (H1-NMR) spectrum of T_2 in the present of D2O

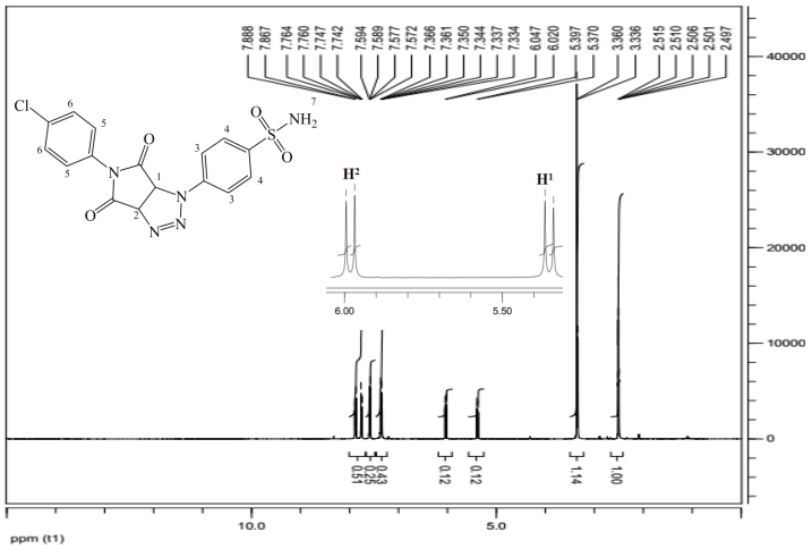


Figure (7): Nuclear Magnetic Resonance (H1-NMR) spectrum of T_2

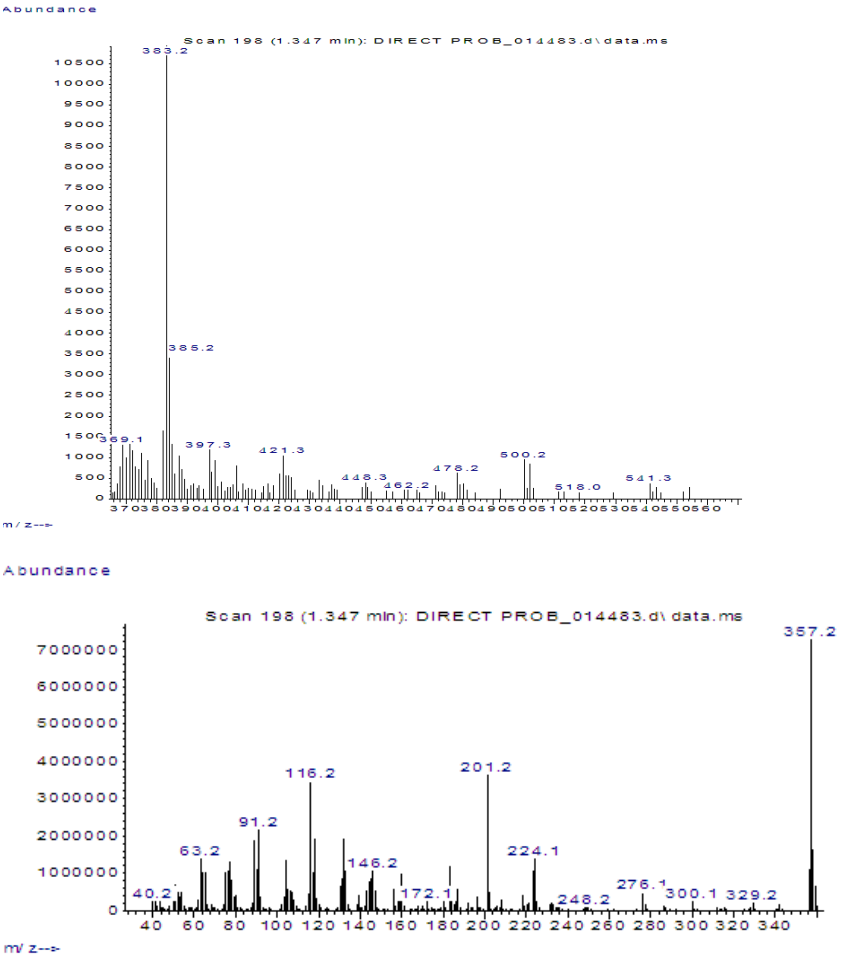


Figure (8): Mass Spectra of T_1

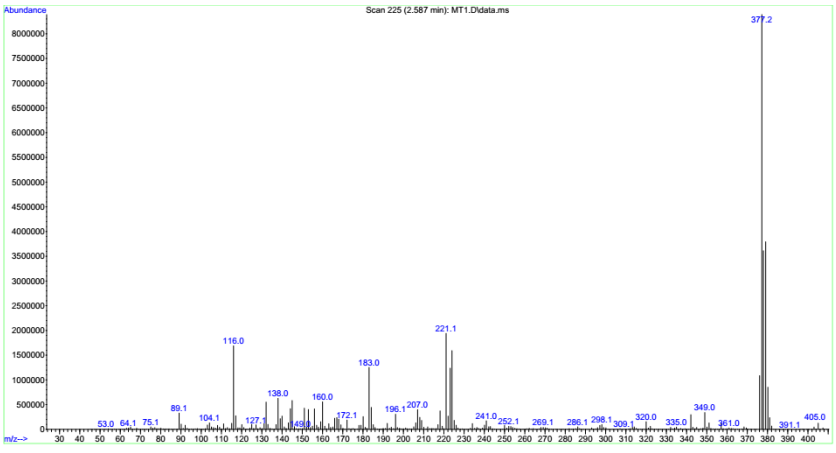


Figure (9): Mass Spectra of T_2

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