Article

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Synthesis , Characterization and Biological activity of New Heterocyclic (4-(pyridin-2-yl)phenyl)-1,2,4-triazolidine-3-thione Derivatives

Aseel A. Alsharefi, Faez A. Alrammahi

University of Kufa, Faculty of Education for Girls , Department of Chemistry

aseela.sharefi@student.uokufa.edu.iq . faez.alrammahi@uokufa.edu.iq

Abstract :

Benzoin is prepared by the condensation of two aldehyde molecules in the presence of potassium cyanide as a catalyst. The carbonyl group of the benzoin reacts with the primary amine groups of the phenylhydrazine derivative to give a hydrozone derivative (Schiff base). Sodium thiocyanide reacts with the imine group by (2+3) addition reaction in acidic media to produce a five-ring heterocyclic derivative of 1,2,4-triazolidine-3-thione Derivative. The product have biological activity with *Escherichia coli* and *Staphylococcus*. , The course of interaction was followed up with TLC, and the compounds prepared were diagnosed with infrared radiation spectrum FTIR and nuclear magnetic resonance spectrum ^{1}H-NMR.

Key word: Heterocyclic, 1,2,4-Triazolidine, benzoin , Schiff base, biological activity.

Introduction:

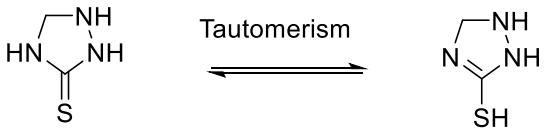
The name (benzoin) comes from a modification of the French word benjoin, from the Catalan word benjuí, from the Arabic lubān jāwi, literally meaning frankincense from Java (Merriam-Webster, n.d.). Chemically, benzoin is an alpha-hydroxyketone consisting of two phenyl groups linked by a carbonyl group with a hydroxyl at the alpha position. It is a milky-white crystalline substance with a camphor-like odor and a pungent taste (1,2). Justus von Liebig and Friedrich Wöhler were the first to create benzoin In 1832, while studying bitter almond oil, which contained benzaldehyde and traces of hydrocyanic acid And that the current literature has begun to include similar reactions containing thiamine or thiazole instead of cyanide(3). Compounds in the α-hydroxyketone functional category have numerous applications, including in antidepressant medications, astringent substances, anti-inflammatory drugs, perfumes, diuretics, expectorants, sedatives, anti-corrosive (4) chemicals and antibacterial compounds (5). When two aromatic aldehydes, especially benzaldehyde, react in the presence of a catalyst hey undergo a reaction known as benzoin condensation to yield benzoin, which is identified as homogeneous coupling (6).

Schiff bases:

The traditional condensation reaction between the carbonyl group of aldehydes or ketones and acid-etched primary amines remains a fundamental and effective approach for producing Schiff bases. Phenylhydrazine reacts with benzoin to form hydrazone containing an imine group (Schiff base) (7,8).

1,2,4-Triazolidin It is a pentacyclic compound consisting of two carbon atoms and three nitrogen atoms. However, it differs from triazole in that it is a saturated compound without double bonds within the ring. This arrangement makes it less stable than triazole due to its lack of aromatic properties. It is an important compound used in the manufacture of anti-inflammatory and antibacterial drugs. It is also important in the chemical industry and organic synthesis. (9,10) , also can be successfully synthesized by using microwave-assisted synthesis (11,12).

2.4.1 Isomers: 1,2,4-Triazolidin-3-thione has two isomers (13).



1,2,4-triazolidine-3-thione 2,5-dihydro-1*H*-1,2,4-triazole-3-thiol

Triazolidine exhibits similar properties to most heterocyclic compounds, such as pharmacological properties. It is used as a blood clotting inhibitor (14). Some studies have also shown that one of its derivatives is a general nervous system inhibitor (15). In another study, Masram, L. B., Salim, S. S., and their team utilized meglumine as a catalyst to create various derivatives of these compounds(16) .

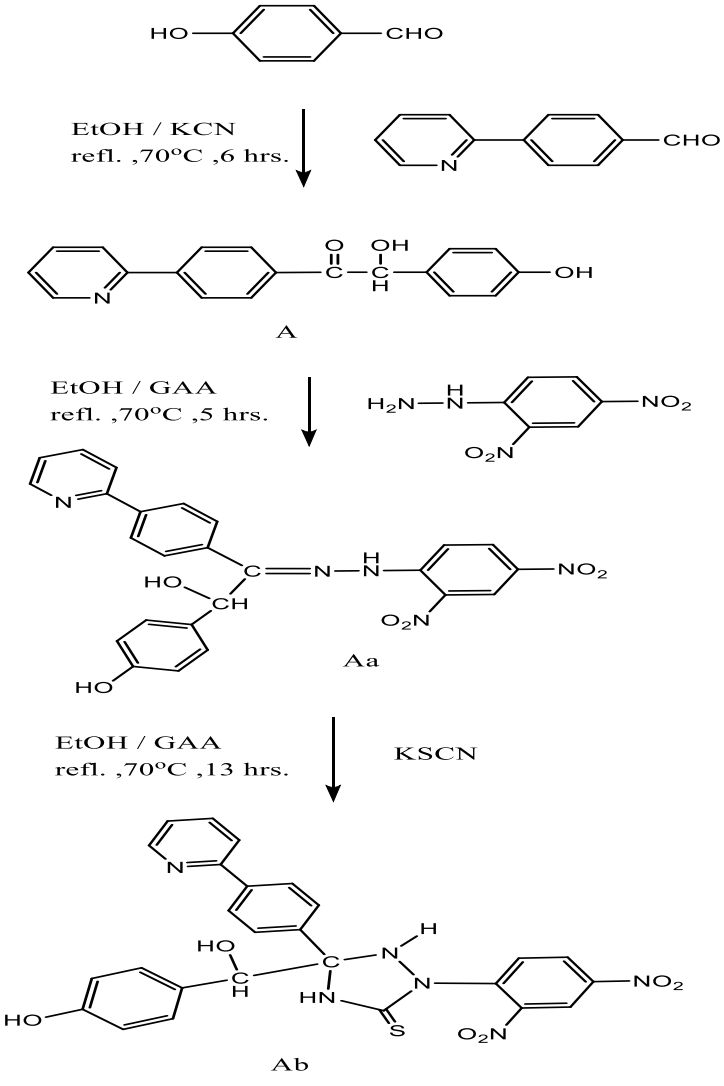


Figure (1) Scheme of synthesis compounds (A, Aa and Ab)

Materials and Synthesis Methods

Instruments Used

Distilled water was used to wash all glassware and homogenized with diluted ethanol (70%) before use. The reaction process was monitored using thin layer chromatography (TLC) using a mixture of solvents (ethanol: toluene). Iodine was used to visualize spots.

The following devices were used for spectral, physical, and biological measurements:

1:Infrared Spectrometer (FT-IR): A Shimadzu FTIR-Prestige Fourier transform infrared Spectrophotometer was used.

Registered at the University of Kufa / College of Pharmacy, within the range (400-4000 cm-1) using a KBr disk.

2: Proton Nuclear Magnetic Resonance (^{1}H-NMR) Spectrometer: A 1H-NMR-Bruker Spectrometer, 500MHz, with DMSO-d6 was used. This instrument was registered at Shahid Beheshti University, Islamic Republic of Iran.

3: Melting Point Meter: The melting points of the newly synthesized compounds were measured using a Stuart Scientific CoL TD Mode in Great Britain, 220-240 Hz Volts. These measurements were performed in the Graduate Studies Laboratory, College of Education for Girls, University of Kufa.

Chemicals Used

The following materials were used with a high degree of purity and from reputable international sources(Merck, Sigma Aldrich) :- (2,4-Dinitrophenylhydrazine , Ethanol absolute, Glacial acetic acid, P-Hydroxy benzaldehyde, Potassium cyanide, Potassium Thiocyanate, 4-(2-Pyridinyl)benzaldehyde, Toluene).

Synthesis of 2-hydroxy-2-(4-hydroxyphenyl)-1-(4-(pyridin-2-yl)phenyl)ethan-1-one (A).

The mixture of (1.22g , 0.01mol) of p-hydroxybenzaldehyde (1.83g , 0.01mol) of 4-(2-pyridinyl)benzaldehyde, and 6-methoxy-2-naphaldehyde was placed in a round-

bottom flask. Then (0.65g , 0.01mol) of KCN diluted in 3 mL of distilled water was added, and the mixture was heated and stirred for five hours at 70°C using 40 mL of absolute ethanol as the solvent(9). Thin layer chromatography (TLC) was used to monitor the reaction, and the mobile phase consisted of a volumetric combination of ethanol and toluene (2:5). Additionally, note how the mixture of products and reactants changed color over time. It began as an olive green tint, became red as the reaction progressed, and then dried to an earthy precipitate.

Synthesis of 4-(2-(2-(2,4-dinitrophenyl)hydrazineylidene)-1-hydroxy-2-(4-(pyridin-2-yl)phenyl)ethyl)phenol (Aa).

Result (0.005 mol, 0.99g) of 2,4-Dinitrophenylhydrazine and (1.525g , 0.005 mol) of compound (A) were mixed in a round-bottom flask in 30 ml of absolute ethanol were used as a solvent, and five drops of glacial acetic acid were used as a catalyst. The mixture was heated for five hours at 70^{o}C with constant magnetic stirring . Thin layer chromatography (TLC) was used to monitor the reaction, which included using 30 milliliters of absolute ethanol as the solvent and five drops of frozen acetic acid as a catalyst. The mobile phase consisted of a combination of ethanol and toluene in a volumetric ratio (1:4).

Synthesis of 2-(2,4-dinitrophenyl)-5-(hydroxy(4-hydroxyphenyl)methyl)-5-(4-(pyridin-2-yl)phenyl)-1,2,4-triazolidine-3-thione (Ab).

Add (0.001 mol, 0.097g) of KSCN potassium thiocyanate to(0.485g , 0.001 mol) of compound (Aa) , were mixed in a round-bottom flask in presence of Five drops of glacial acetic acid , and 30mL of absolut ethanol was used as the solvent. heated under continuous magnetic stirring. Five drops of glacial acetic acid were added to 30

milliliters of 100% ethanol, with constant magnetic stirring which was heated and stirred for 13 hours at 70^0C(9). Thin layer chromatography (TLC) was used to monitor the reaction, and a combination of ethanol and toluene served as the mobile phase. When the reaction started, the reaction mixture was red. When it dried, the precipitate became reddish brown and was recrystallized using pure ethanol. The table displays the physical attributes.

Table (1) Physical Properties of Compounds A,Aa, and Ab

	M.F	M.Wt g/mol	m.p˚C	Color	R_f	Yield%
A	$C_{19}H_{15}NO_3$	305.26	190-192	Beige	0.94	88
Aa	$C_{25}H_{19}N_5O_6$	485.26	170-175	Brown	0.83	89
A8	$C_{26}H_{20}N_6O_6S$	544	180-185	Reddish Brown	0.37	78

Results and Discussion

FTIR (cm^{-1}) :

The FTIR spectrum of compound (A) shows the presence of a carbonyl bond (C=O) appearing at the absorption band of 1700, while the O-H bond appeared in a broad band at 3365 and 3452. The aliphatic C-H bond appeared at 2816, while the aromatic bond appeared at 3053. The spectrum also shows the presence of C=C, belonging to the aromatic ring, at 1544 and 1591. The C=N bond also appeared within the ring at 1625, belonging to the pyridinyl ring.

It was observed from the spectrum of (Aa) that the carbonyl bond of the pyridinyl derivative and the NH triazolodine ring disappeared and were replaced by The C=N bond is shown at 1614. The spectrum also shows the NO_2 bond at 1510, the aliphatic C-H bond at 2918, and the aromatic bond at 3068. The N-H bond also appears at 3361.

The spectrum of (Ab) shows appearance of the imine C=N bond has disappeared and disappearance of carbonyl group within the aromatic ring.

Also, an absorption band appears at 1072 corresponding to the C=S bond, indicating the formation of a cyclic compound .

Biological testing was conducted on two types of bacteria (*Escherichia coli* and *Staphylococcus*). The results showed that the compounds inhibited the activity of the bacteria and destroyed their cell membrane, indicating their potential use as pharmaceutical compounds against these diseases.

Identification of the Compounds using ^{1}H NMR (ppm)

A: Weak signals appeared at position 2.5, attributed to the solvent used (DMSO-d6). A signal also appeared at position 4.36, attributed to the proton attached to the carbon atom bearing the hydroxyl. A signal also appeared at position 4.9, attributed to the benzoin OH (tautomerism). The spectrum also showed multiple signals at position 8.3-6.5, attributed to the protons of the aromatic rings. Their complex distribution indicates that they are asymmetric rings. A signal also appeared at position 9.9, attributed to the phenolic OH proton:

Aa:^{1}H- NMR showed a signal at position 2.5, which is due to the solvent used (DMSO-d6). The spectrum also showed a single signal at position (5.6), which is due to the aliphatic OH proton, while the aromatic OH appeared at 10.5. The spectrum also showed multiple signals at (7.7-8.8), which are due to the protons of the aromatic rings. From their complex distribution, it is concluded that they are asymmetric rings. A signal also appeared at 4.15, which is due to CH.

Ab: ^{1}H-NMR showed a signal at position 2.5 due to the solvent used (DMSO-d6), and the spectrum showed a signal at position 5 due to the aliphatic OH proton, while the

aromatic OH appeared at 10, and the spectrum also showed multiple signals at (8.8-7.7) due to the protons of the aromatic rings, and from their complex distribution it is concluded that they are asymmetric rings, and a weak signal appeared at 10.81 due to the NH of the triazole ring.

Table (2) Biological Activity of Compounds (A,Aa, Ab)

Bacterial / No of Comp.	*Escherichia coli*		*Staphylococcus*	
	con. 50%	**Con. 100%**	**con. 50%**	**Con. 100%**
A	**11**	**9**	**10**	**14**
Aa	**13**	**15**	**18**	**28**
Ab	**13**	**15**	**27**	**32**

Results

The results of the study of biological activity against bacteria showed that all compounds gave high biological activity. However, compound (Aa) was more effective against positive E. coli bacteria, and compound (Ab) was more effective against negative Staphylococcus bacteria.

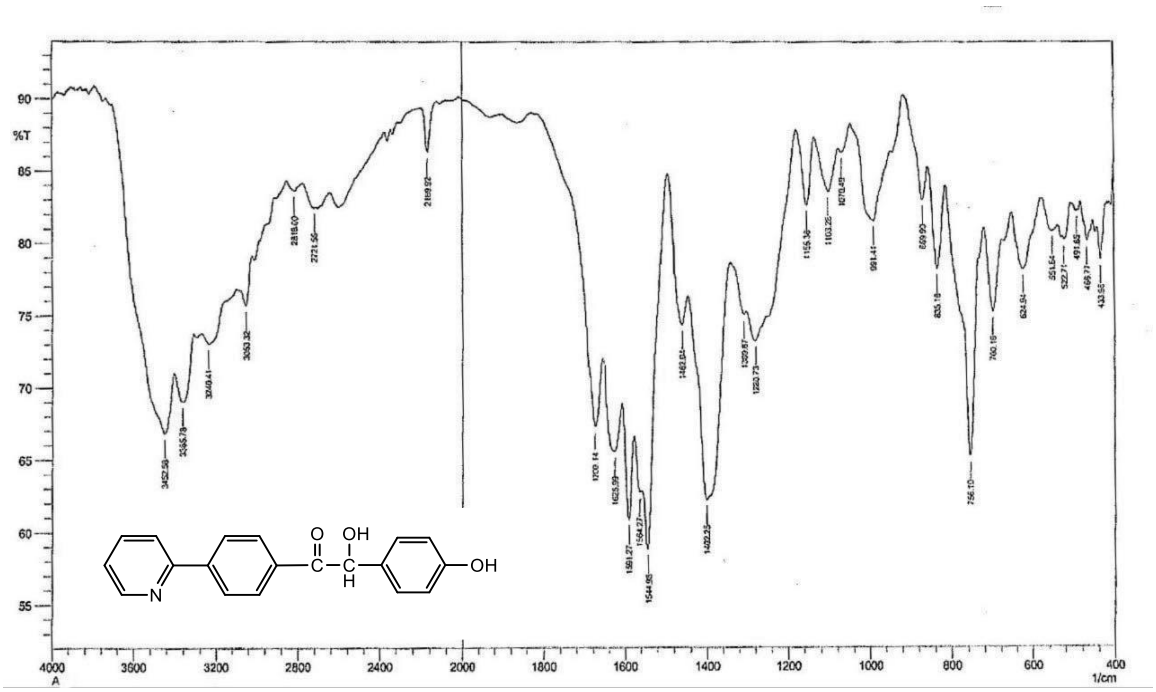


Figure (2) FTIR Spectrum of compound (A).

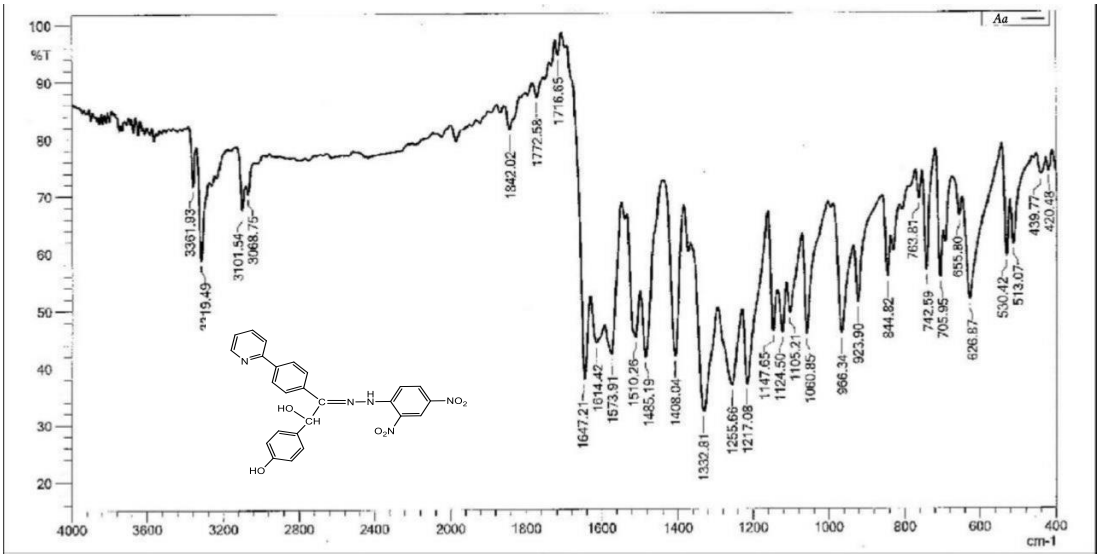


Figure (3) FTIR Spectrum of compound (Aa).

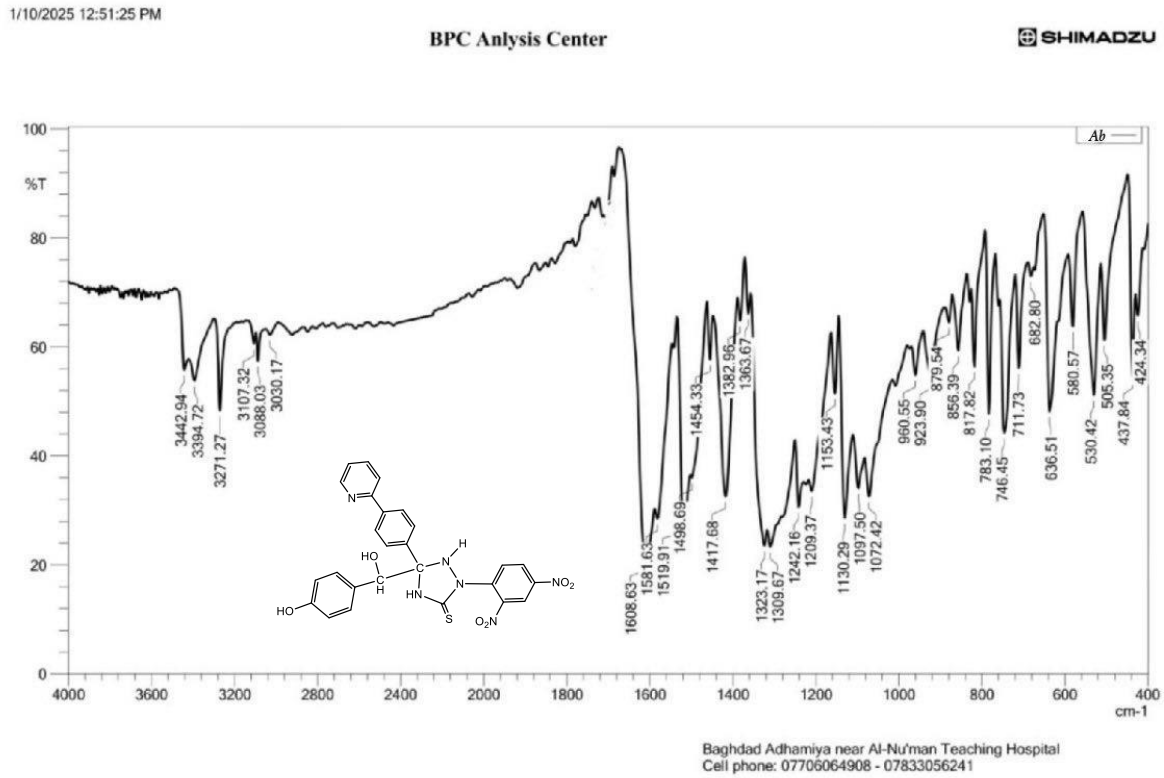


Figure (4) FTIR Spectrum of compound (Ab).

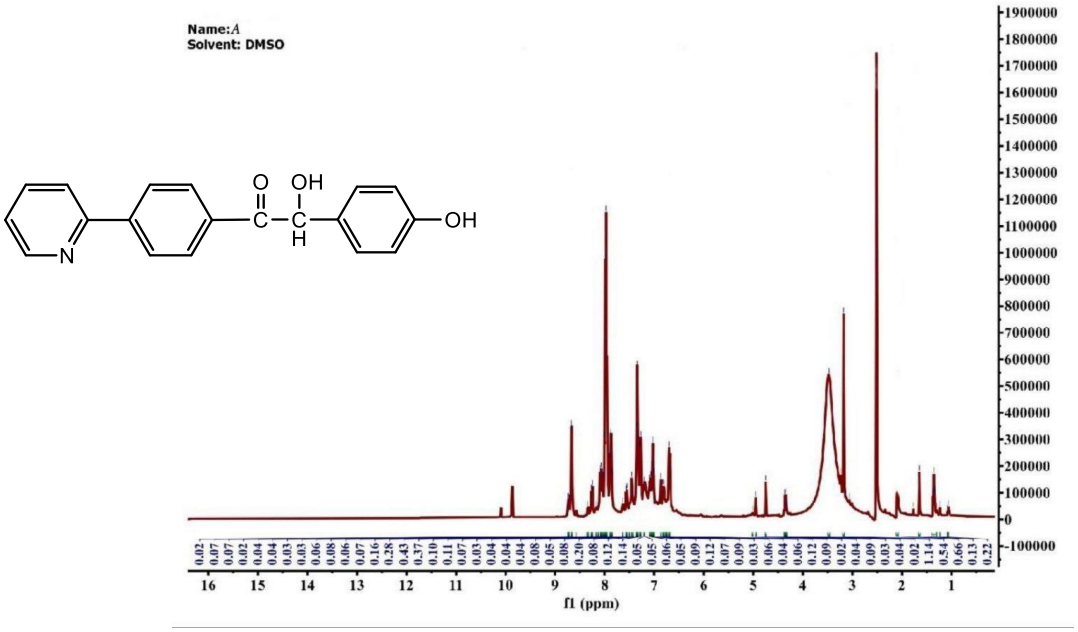


Figure (5) ^{1}H NMR Spectrum of compound (A).

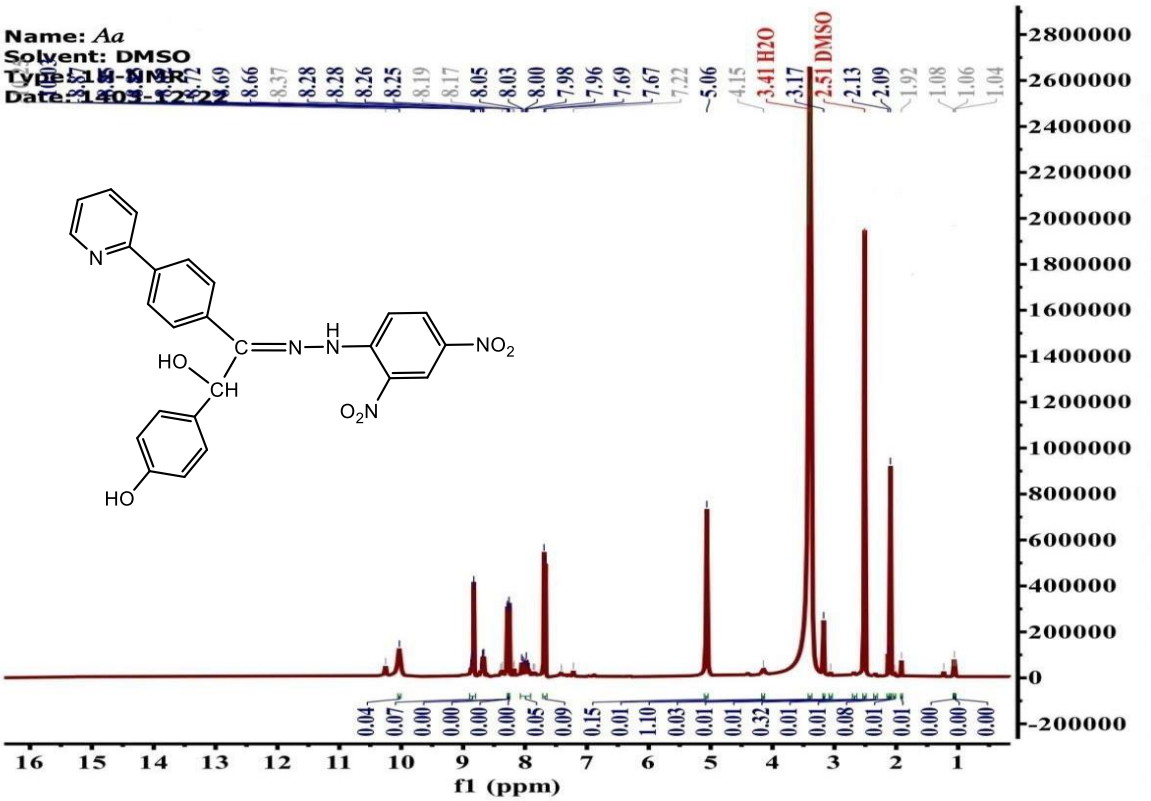


Figure (6) ^{1}H NMR Spectrum of compound (Aa)

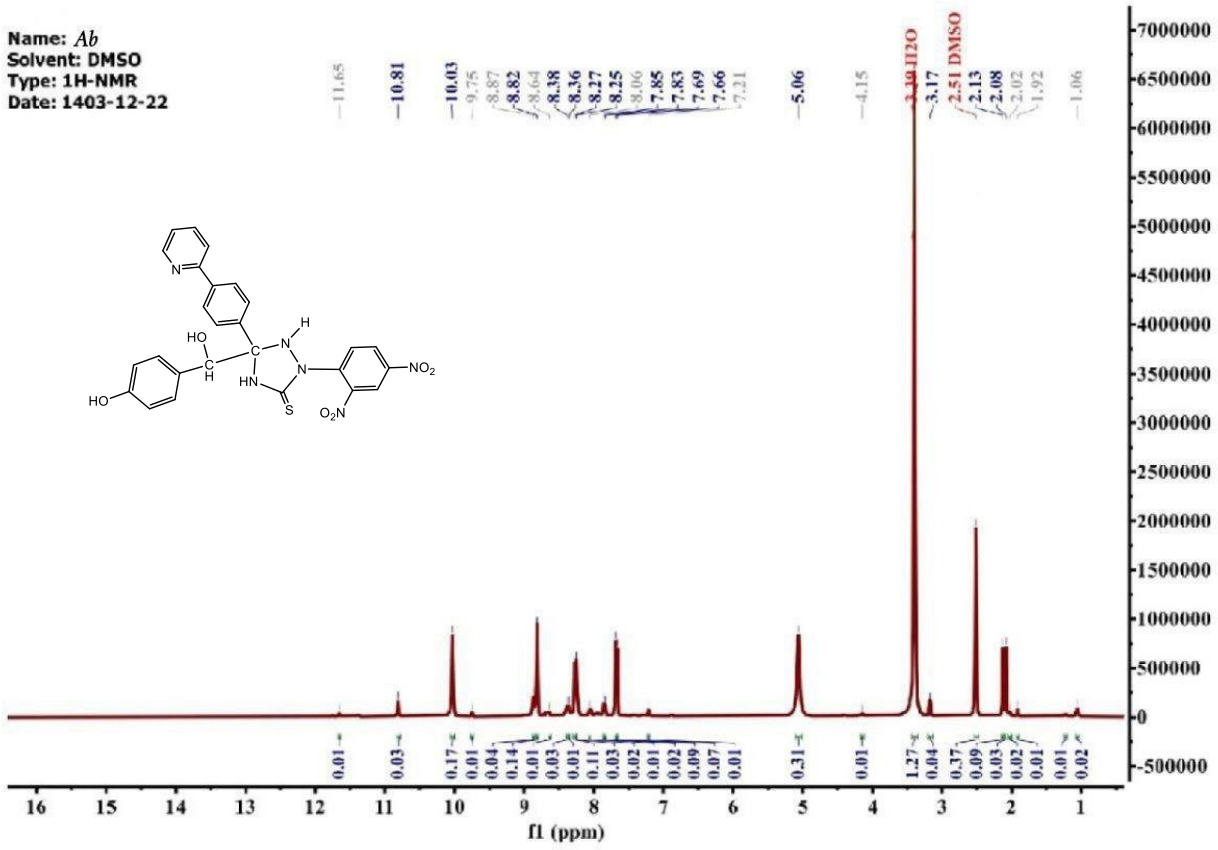


Figure (7) ^{1}H NMR Spectrum of compound (Ab)

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