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Synthesis and characterization of (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol from 2-hydroxy-4, 5-dimethyl acetophenone

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Abstract
This study reports the event of substituted hydrazone prepared from 1-(2-hydroxy-4,5-dimethylphenyl)ethanone and (2,4-dinitrophenyl)hydrazine in alkaline medium at controlled conditions which yields as corresponding hydrazone [(E)-2-(1-(2-(2,4-dinitrophenyl)hydrazono)ethyl)-4,5-dimethylphenol]. The structure of synthesized (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol was elucidated by elemental analysis and spectroscopic techniques like Infrared spectroscopy, Ultraviolet–visible spectroscopy, High-performance liquid chromatography, Proton nuclear magnetic resonance and Mass spectrum.

Keywords: 2-hydroxy-4, 5-dimethyl acetophenone, Hydrazone, (2, 4-dinitro phenyl) hydrazine

1. Introduction
Hydrazones are a category of organic compounds with the structure R1R2C=NNH2 [1]. Hydrazones are containing with ketones and aldehydes by the replacement of the oxygen with the Nitrogen-Nitrogen-Hydrogen functional group. Hydrazones containing an azomethine –NHN=CH group and they are considered as derivatives of aldehydes and ketones during which the oxygen atom has been replaced by the Nitrogen-Nitrogen-Hydrogen functional group [2, 4].
Hydrazone contains active center as carbon and nitrogen, both are major responsible for the physical and chemical properties of the hydrazones. Hydrazones shows reactivity toward electrophiles and nucleophiles due to the carbon and nitrogen. Hydrazones are heterocyclic compounds used for the synthesis of compound like with a spread of biological activities [5]. The IUPAC name of prepared hydrazone groups is (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol, in this it possess conjugate double bond and a delocalized π electron on both the benzene ring. Hydrazones are related to ketones and aldehydes by the replacement of oxygen with the Nitrogen-Nitrogen-Hydrogen functional group [6].
The C-atom in hydrazone has both electrophilic and nucleophilic characters and in both the N-atoms are nucleophilic although the amino type nitrogen is more reactive. Due to the electrophilic and nucleophilic properties, hydrazones are widely used in organic synthesis [7, 8].
The hydrazones show wide range of pharmaceutical activities, such as antibacterial [9], anti-malarial [10], analgesic agents [11, 12], antifungal [13, 14], antimicrobial [15, 18], and anticonvulsant agents [19, 20].

2. Experimental
2.1. Materials: This work were used following chemicals AR Grade and commercially available and used without further purification, 2-Hydroxy-4,5-dimethyl acetophenone, (2,4-dinitrophenyl)hydrazine, methanol, hydrochloric acid, sodium hydroxide, ethyl alcohol, ethyl acetate, n-Hexane, chloroform, DMSO, etc.,

2.2. Preparation of Substituted Hydrazone: (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol
1-(2-hydroxy-4, 5-dimethylphenyl) ethanone or 2-Hydroxy-4, 5-dimethyl acetophenone were synthesized following published procedure [21]. The substituted hydrazones prepared by stirring the equimolar concentration mixture of 2-Hydroxy-4, 5-dimethyl acetophenone (1 mole) and (2, 4-dinitrophenyl) hydrazine (1.2 mole) in 150 ml methanol, stirred for 25 min at room temperature and dissolved. The solution is refluxed for four hours in presence of Sulphuric acid at 65°C,then progress of reaction checked by TLC [10% Ethyl acetate : n-Hexane (10:90)]. After completion of reaction, the mixture cooled at room temperature. Thus Red colored compound formed was filtered, washed with chilled methanol and dried at 60°C to 65°C temperature. The compound was re-crystallized in methanol for purification and improving description.

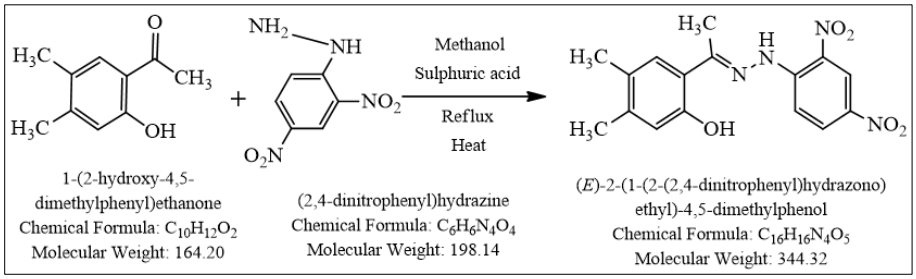

Fig 1: Reaction Scheme preparation of (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol

3. Results and Discussion

The (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol was analyzed by UV, CHNS, IR, ^{1}H-NMR, Melting Point, purity by HPLC, water content and Mass Spectroscopy.

Melting points was recorded in open capillaries and are uncorrected. Melting points were recorded on Veego Scientific Device (Model: VMP-AD). Some Physical properties of (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol is mentioned below table No. 1

Table 1: Physical Properties

Hydrazone	Molecular Formula	Color	Solubility	Melting point (upper and lower range)	% Yield
(E)-2-(1-(2-(2,4-dinitro phenyl)hydrazono)ethyl)-4,5-dimethylphenol	$C_{16}H_{16}N_4O_5$	Red powder	▪ Freely soluble in Dimethylformide ▪ Soluble in Methanol, Ethanol, Chloroform, DMSO, Ethyl acetate ▪ Insoluble in Water	251°C to 256 °C	80.00%

3.1 Mass Analysis: (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol

The Mass spectrum of (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol was recorded on Waters Quattro Micro Triple Quad Spectrometer. The mass analysis of (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol shows molecular ion peak (M^+ /e) correctly as corresponding to molecular Formula in Table No.2 and Mass spectrum show in the below Figure No. 2.

Table 2: Mass Analysis

Hydrazone	Molecular Formula	Molecular Weight	Molecular ion Peak (M^+/e)
(E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol	$C_{16}H_{16}N_4O_5$	344.32	343.2

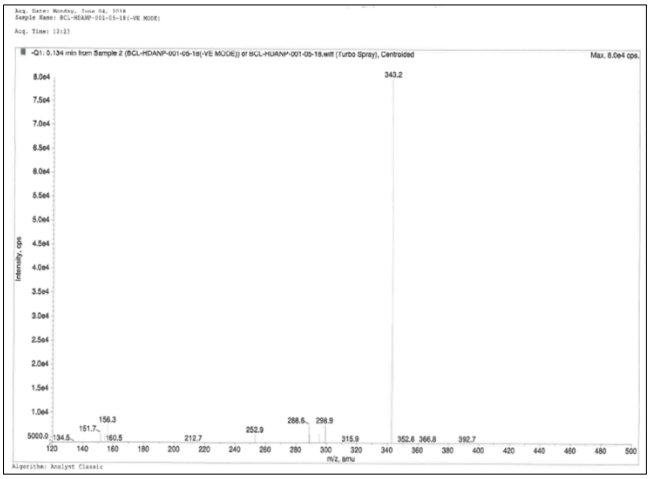

Fig 2: Mass Spectrum of (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol

3.2 1H NMR Analysis: (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol

Nuclear resonance spectra of the (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol was recorded in $CDCl_3$ Solution and ^{1}H NMR shown in figures 3. The relevant data on observed chemical shifts together with their assignments are summarized in table 3

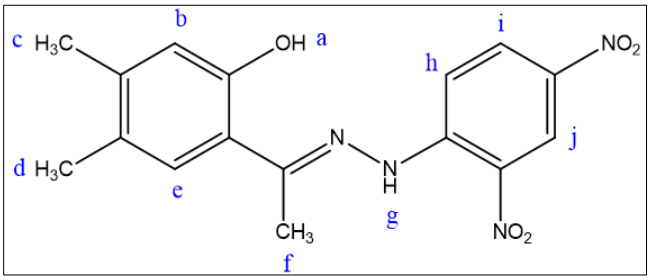

Fig 3

Table 3: ^{1}H NMR Analysis

Assignments	Chemical shift (ppm)	Functional group	No. of proton	Multiplicity
a & j	11.442-11.465	-OH& -CH	2	Multiplet
b	9.201	-CH	1	Singlet
e	7.283	-CH	1	Singlet
h	7.616-7.648	-CH	1	doublet
i	8.391-8.417	-CH	1	doublet
g	6.851	-NH	1	singlet
c, d	2.256-2.285	$-CH_3$	6	Multiplet
f	2.551	-CH3	3	singlet

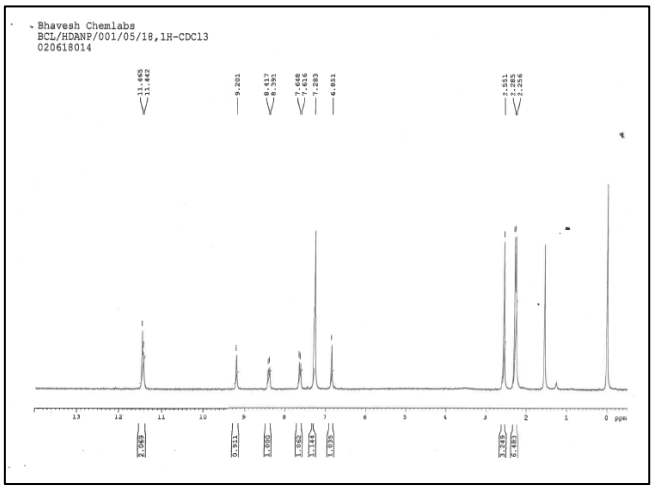

Fig 3: ^{1}H NMR spectrum for: (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol

3.3 Infra-Red Analysis: (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol

The IR spectra of (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol compound have been recorded in the region of 4000-600 cm^{-1} by using FTIR Spectrometer of model Agilent Resolutions Pro by direct sampling method.

The IR spectral data along with the possible assignments of (E)-2-(1-(2-(2,4-dinitrophenyl)hydrazono)ethyl)-4,5-dimethylphenol compound are present in table no.4 followed by IR spectra performed by IR direct Solid method use of Agilent Resolutions Pro and IR spectrum was showed in below figure 4.

Table 4: IR Analysis

Bond/functional group	frequency, cm-1
C-O	1113
C=C (Aromatic Ring)	1588
C-C	1506
N-H	1611
N-O	1506
C-N	1327
C–H stretch alkenes	2918
C–H stretch aromatics	3080
O-H	3297

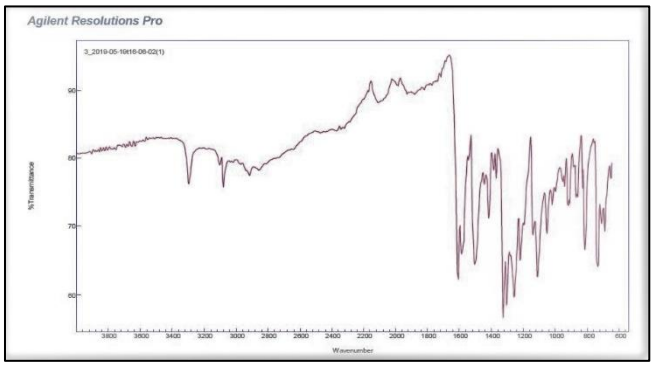

Fig 4: IR spectrum for (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol

3.5 UV Analysis: (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol

The UV spectra of the hydrazone compound in Dimethylformide were recorded on Systronics Double beam UV-Vis Spectrophotometer: 2202 using a Quartz Cell of 1 cm optical path. Dimethylformide was used as blank.

The UV spectrum was showed in below figure 5. The spectra shows V (bands Maximum in nm) in Dimethylformide : 254,325 and 370 nm

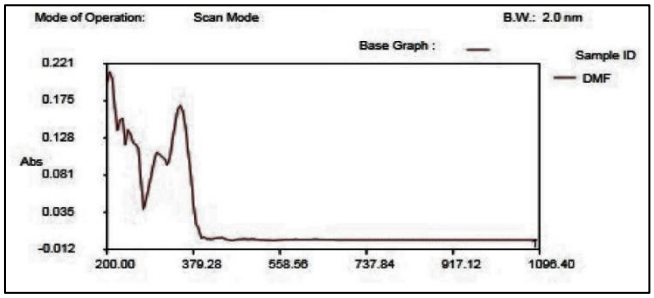

Fig 5: UV spectrum for (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol

3.6 Elemental Analysis (CHNS): (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol

Formation of the (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol was further confirmed by Elemental

analysis. Elemental analysis was recorded VarioMICRO CHNS analyzer. The elemental analysis data of (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol is summarized table No.5

Table 5: CHNS

Hydrazone	Molecular Formula	Molecular weight	Elemental data (Required/Found)		
			Carbon	Hydrogen	Nitrogen
(E)-2-(1-(2-(2,4-dinitro phenyl) hydrazono) ethyl)-4, 5-dimethylphenol	$C_{16}H_{16}N_4O_5$	344.32	55.96 (55.84)	4.59 (4.70)	16.18 (16.29)

3.7 Water Content: (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol

The Water content of the (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol compound in methanol was recorded on Karl Fischer and found 0.35 % water content.

3.8 Purity: (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol

The purity of the (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol compound was analysed by Shimadzu High performance liquid chromatography (HPLC) and Purity found 98.9 % (98.484%) at retention time 7.181 minute was showed in below figure 6 by using the following parameter in Table No 6.

Table 6: Purity HPLC analysis details

HPLC System	Shimadzu HPLC	
Column	Phenomenon C18,100A, 4.6 mm x 250 mm, 5µm	
Flow rate	1.0 ml/min	
Wavelength	254 nm	
Column Temperature	40 °C	
Sampler Temperature	25 °C	
Injection Volume	10 µl	
Run Time	32 minute	
Gradient		
Time in Minute	% of Mobile Phase A	% of Mobile Phase B
0	90	10
10	25	75
15	20	80
26	90	10
32	90	10
Mobile Phase A	1 ml of orthophosphoric acid dissolved in Mill-Q water	
Mobile Phase B	100 % Acetonitrile	
Diluent	Methanol	
Sample preparation	10 mg (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol transferred in 10 ml volumetric flask and dissolved in methanol.	

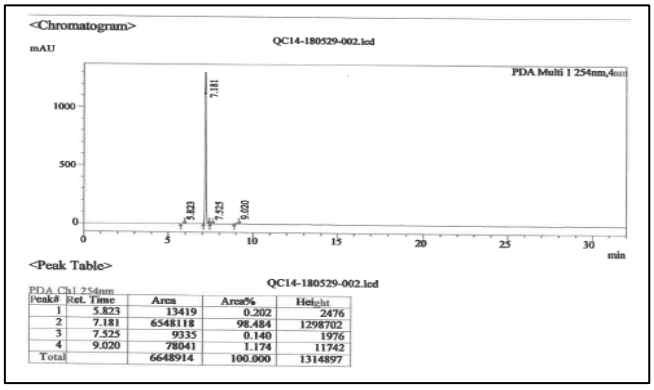

Fig 6: Purity HPLC Chromatogram for (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol

3.9 Theoretical Evaluation of Mutagenicity Study: (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol and Raw material used in preparation.

The evaluation of mutagenicity as per the International council for harmonization of technical requirements for pharmaceuticals for human use (ICH) "Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk M7 (R1)" by using the T.E.S.T software (Toxicity Estimation Software Tool) version 4.2.1.

T.E.S.T allows estimating toxicity values using several different advanced Quantitative structure–activity relationship models (QSAR) methodologies. The following structure was evaluated by use of "Consensus method" predicted Mutagenicity estimated by taking an average of the predicted toxicities,

Table 7: Theoretical Evaluation of Mutagenicity by T.E.S.T. software

Chemical Name	Mutagenicity (Positive / Negative)
(E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol	Mutagenicity Positive
1-(2-hydroxy-4, 5-dimethylphenyl) ethanone	Mutagenicity Negative
(2, 4-dinitrophenyl)hydrazine	Mutagenicity Positive
3, 4-dimethylphenyl acetate	Mutagenicity Negative
3, 4-dimethylphenol	Mutagenicity Negative

Acetyl chloride	Mutagenicity Negative

4. Conclusion

A synthesis of (E)-2-(1-(2-(2, 4-dinitrophenyl) hydrazono) ethyl)-4, 5-dimethylphenol derivative from 2-hydroxy-4, 5-dimethyl acetophenone is described. The structure of the synthesized hydrazone compound was well characterized by Melting point, Elemental analyses (CHNS), IR, UV, HPLC, 1H NMR, and Mass spectral studies.

5. Acknowledgement

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