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Synthesis and characterization of (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol 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 phenyl hydrazine in alkaline medium at controlled conditions which yields as corresponding hydrazone [(E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol]. The structure of synthesized [(E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol was elucidated by elemental analysis and spectroscopic techniques like Infrared spectroscopy, Ultraviolet–visible spectroscopy, High-performance liquid chromatography, Proton nuclear resonance and spectrum.

Keywords: phenyl hydrazine, 2-hydroxy-4, 5-dimethyl acetophenone, 3, 4-dimethyl phenyl acetate, hydrazone, mutagenicity

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–NHN=CH group and they are considered as derivatives of aldehydes and ketones during which the oxygen atom has been replaced by the Nitrogen-Nitrogen-Hydrogen functional group [2-4].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, 19], and anticonvulsant agents [19, 20].

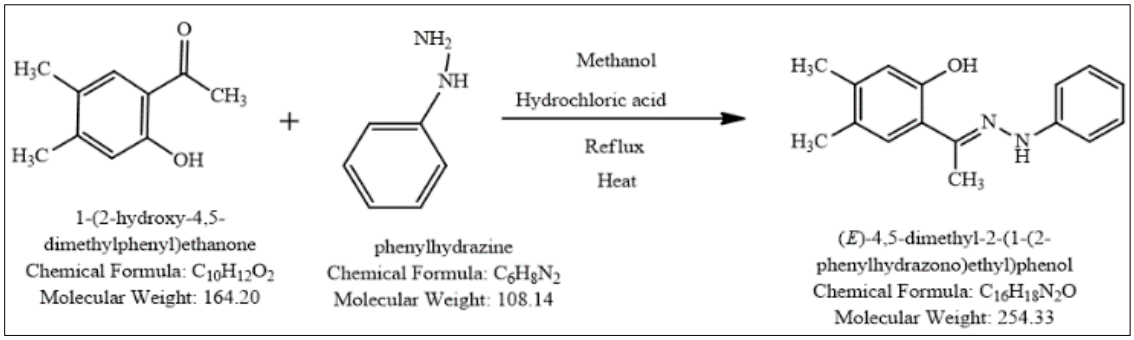
2. Experimental

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. 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 (1mole) and Phenyl Hydrazine (1mole) in 150 ml methanol, stirred for 25 min at room temperature and dissolved. The solution is refluxed for three hours in presence of hydrochloric acid at 65°C, then progress of reaction checked by TLC [10% Ethyl acetate : n-Hexane (10:90)]. After completion the mixture was cooled at room temperature. The off white color compound formed was filtered, washed with chilled methanol and dried at 50–55°C. The compound was re-crystallized in methanol for purification and improving description. Reaction scheme preparation of (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol is given below Scheme 1.



Scheme 1: Reaction Scheme preparation of (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol

3. Results and Discussion

The (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol was analyzed by Infrared spectroscopy, Ultraviolet–visible spectroscopy, Proton nuclear magnetic resonance, Carbon, Hydrogen, Nitrogen, Sulphur elemental analyzer, melting point, purity by High-performance liquid chromatography, water content and mass spectroscopy. Melting points were recorded using Veego Scientific Device (Model: VMP-AD) in open capillaries and were uncorrected. Some physical properties of (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol are mentioned in Table 1.

Table 1: Physical Properties

Hydrazone	Molecular Formula	Color	Solubility	Melting point (upper and lower range)	% Yield
(E)-4,5-dimethyl-2-(1-(2-phenyl hydrazono) ethyl) phenol	$C_{16}H_{18}N_2O$	Off white powder	▪ Freely soluble in Chloroform ▪ Soluble in Methanol, Ethanol, DMSO, Ethyl acetate ▪ Insoluble in Water	114°C to 118 °C	80 %

3.1 Mass Analysis: (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol

The mass spectrum of (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol was recorded on Waters Quattro Micro Triple Quad Spectrometer. The mass analysis of (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol shows molecular ion peak (M^+/e) correctly corresponding to molecular formula in Table 2 and the mass spectrum is shown in Figure 1.

Table 2: Mass Analysis

Hydrazone	Molecular Formula	Molecular Weight	Molecular ion Peak (M^+/e)
(E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol	$C_{16}H_{18}N_2O$	254.3	255.4

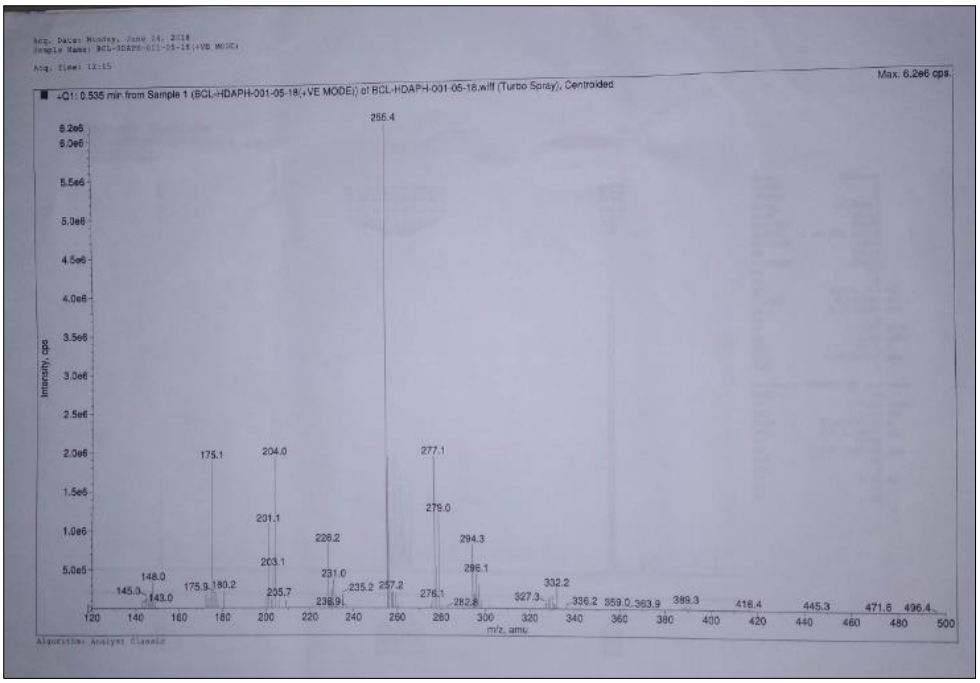


Fig 1: Mass Spectrum of (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol

3.2 1H NMR Analysis: (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol

Nuclear resonance spectra of the (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol was recorded in $CDCl_3$ solution and ^1H NMR, shown in Figure 2. The relevant data on observed chemical shifts together with their assignments are summarized in Table 3.

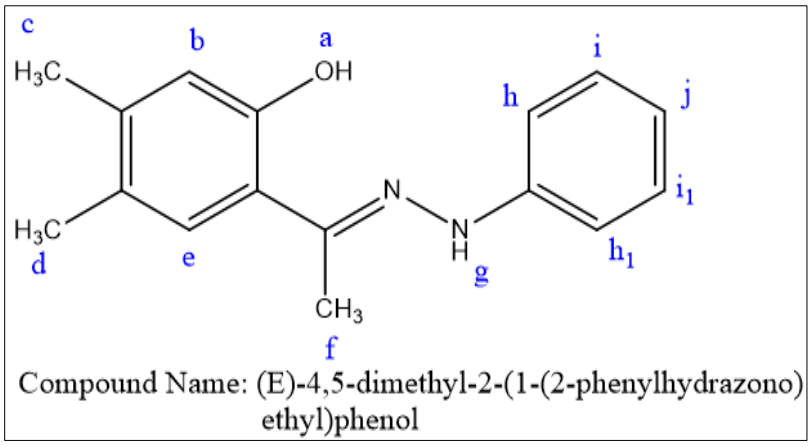


Fig 2

Table 3: ^1H NMR Analysis

Assignments	Chemical shift (ppm)	Functional group	No. of proton	Multiplicity
a	12.298	-OH	1	Broad, Singlet
b & e	7.144	-CH	2	Singlet
c & d	2.210-2.232	$-CH_3$	6	Multiplet
f	2.300	$-CH_3$	3	Singlet
g	6.785	-NH	1	Broad, Singlet
j	6.893-6.940	-CH	1	Triplet
h,h_1	6.6996-7.023	-CH	2	doublet

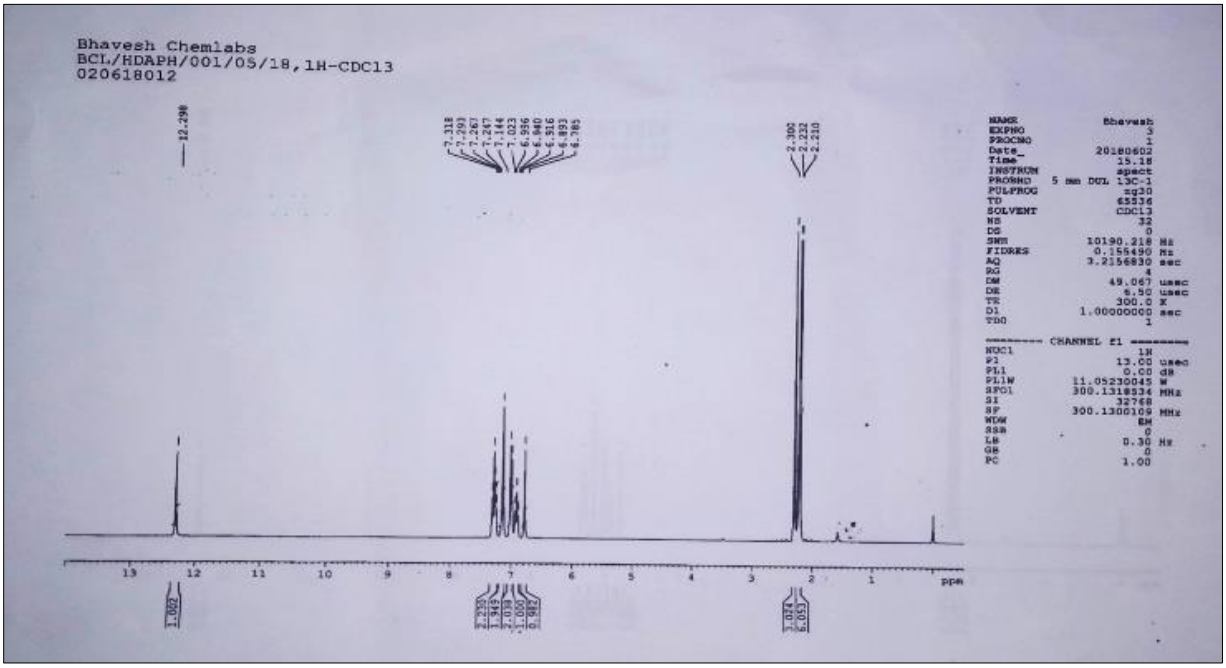


Fig 2: ^1H NMR spectrum for (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) Phenol

3.3 Infra-Red Analysis: (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol

The IR spectra of (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol compound was recorded in the region of 4000–600 cm^{-1} using FTIR spectrometer of model Agilent Resolutions Pro by direct sampling method.

The IR spectral data along with the possible assignments of (E)-4,5-dimethyl-2-(1-(2-phenylhydrazono)ethyl)phenol compound are provided in Table 4, followed by IR spectra performed by IR direct solid method, use of Agilent Resolutions Pro and IR spectrum are showed in Figure 3.

Table 4: IR Analysis

Bond/functional group	frequency, cm-1
C-O	1197
C=C (Aromatic Ring)	1454
C-C	1507
N-H	1601
C–H stretch alkenes	2921
C–H stretch aromatics	3030
O-H	3648

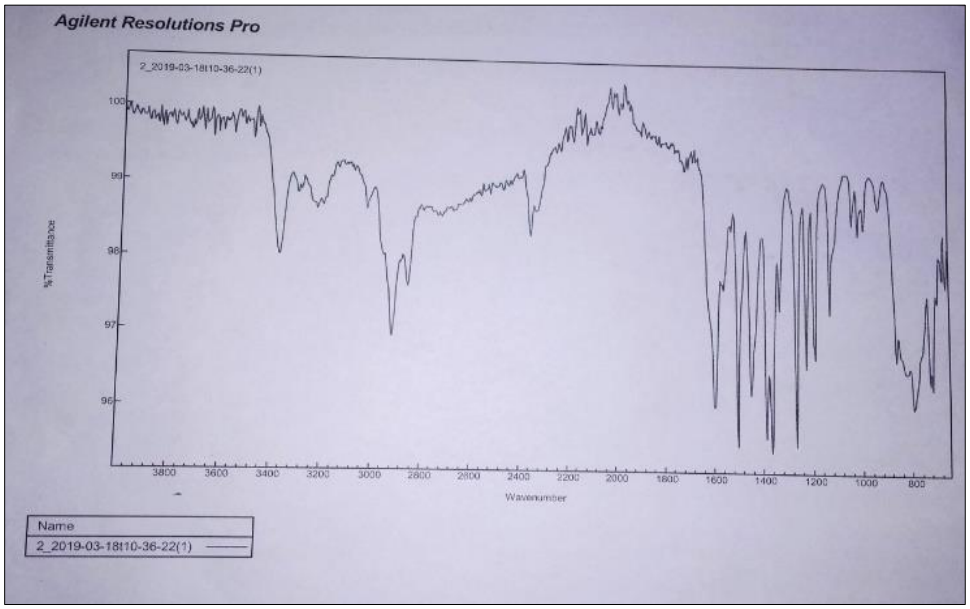


Fig 3: IR spectrum for (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol

3.4 UV Analysis: (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol

The UV spectra of the (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol compound in methanol was recorded on Systronics Double beam UV-Vis Spectrophotometer: 2202 using a Quartz Cell of 1 cm optical path where methanol was used as a blank.

The UV spectrum is shown in Figure 4. The spectra shows V (bands maximum in nm) in methanol: 254,325 and 375 nm

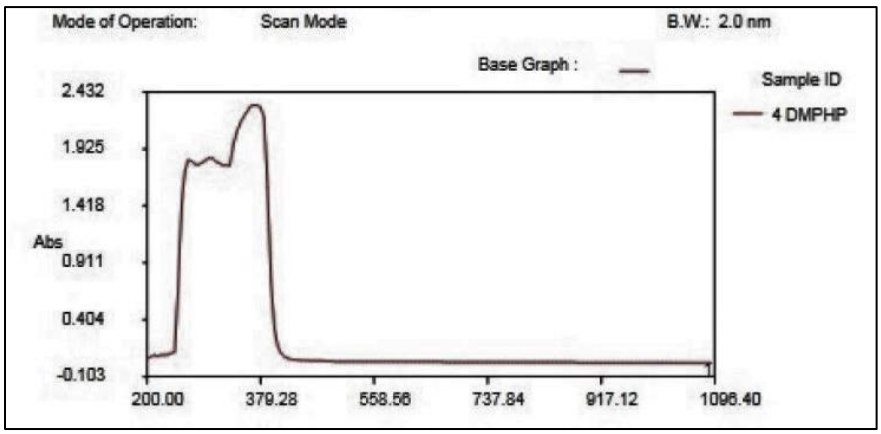


Fig 4: UV spectrum for (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol

3.6 Elemental Analysis (CHNS): (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol

Formation of (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol was further confirmed by elemental analysis which was recorded by Vario MICRO CHNS analyzer. The elemental analysis data of (E)-2-(1-hydrazonoethyl)-4, 5-dimethylphenol is summarized in Table 5.

Table 5: Carbon, Hydrogen, Nitrogen, Sulphur elemental analyzer

Hydrazone	Molecular Formula	Molecular weight	Elemental data (Required/Found)		
			Carbon	Hydrogen	Nitrogen
(E)-4,5-dimethyl 2-(1-(2-phenyl hydrazono) ethyl) phenol	$C_{16}H_{18}N_2O$	254.33	75.68 (75.56)	7.06 (7.15)	11.08 (11.02)

3.7 Water Content: (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol

The Water content of the (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol compound in methanol was recorded using Karl Fischer and was found to have 0.35 % water content.

3.8 Purity: (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol

The purity of the (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol compound was analyzed by Shimadzu high-performance liquid chromatography (HPLC) and purity was found to be 99.3% (99.307%) with retention time of 14.789 min using the parameters in Table 6. The Purity: HPLC chromatogram for (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol is shown in Figure 5.

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 1000 mL Mill-Q water	
Mobile Phase B	100 % Acetonitrile	
Diluent	Methanol	
Sample preparation	10 mg (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol transferred in 10 ml volumetric flask and dissolved in methanol.	

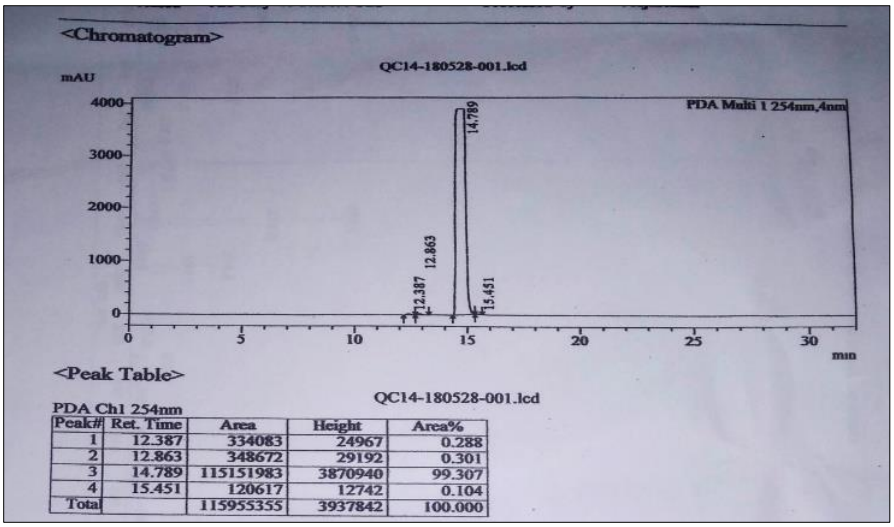


Fig 5: Purity HPLC Chromatogram for (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol

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

The evaluation of mutagenicity is carried out 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 software Toxicity Estimation Software Tool (TEST) version 4.2.1.

TEST estimates the toxicity values using several different advanced quantitative structure–activity relationship models (QSAR). The following structure was evaluated using "consensus method" and predicted mutagenicity was estimated by taking an average of the predicted toxicities. Theoretical evaluation of mutagenicity by T.E.S.T. software is shown in Table 7.

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

Chemical Name	Mutagenicity (Positive / Negative)
(E)-4,5-dimethyl-2-(1-(2-phenyl hydrazono) ethyl) phenol	Mutagenicity Positive
1-(2-hydroxy-4, 5-dimethylphenyl) ethanone	Mutagenicity Negative
Phenyl hydrazine	Mutagenicity Positive
3,4-dimethylphenyl acetate	Mutagenicity Negative
3,4-dimethylphenol	Mutagenicity Negative
Acetyl chloride	Mutagenicity Negative

4. Conclusion:

This study describes the synthesis of (E)-4, 5-dimethyl-2-(1-(2-phenylhydrazono) ethyl) phenol derivative from 2-hydroxy-4, 5-dimethylacetophenone. The structure of the synthesized hydrazone compound was well characterized by melting point, elemental analyses (CHNS), IR, UV, HPLC, ^{1}H NMR and mass spectral studies.

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