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**Dr. Deepa D Parab**
Department of Chemistry, Guru Nanak Khalsa College of Arts, Science & Commerce, (Autonomous), Matunga, Mumbai, Maharashtra, India

**Dr. Harmeet Kaur Kohli**
Department of Chemistry, Guru Nanak Khalsa College of Arts, Science & Commerce, (Autonomous), Matunga, Mumbai, Maharashtra, India

**Corresponding Author:**
**Dr. Deepa D Parab**
Department of Chemistry, Guru Nanak Khalsa College of Arts, Science & Commerce, (Autonomous), Matunga, Mumbai, Maharashtra, India

# Synthesis and *in vitro* cytotoxic activity of novel Adamantane Carbohydrazide

**Deepa D Parab and Harmeet Kaur Kohli**

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**Abstract**
A new 1-adamantyl derivative was developed, starting with adamantane-1-carbohydrazide as the bioactive core. The newly synthesized adamantane derivative N'-(4-hydroxy-3-methoxy-5-nitro benzylidene) adamantane-1-carbohydrazide (5-NVAC) was characterised employing diverse spectroscopic methods. The cell viability/cytotoxicity was identified through [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] MTT assay. The sample's cytotoxicity was assessed using the MTT assay within the HeLa cell line. In comparison to the control Doxorubicin, 5-NVAC demonstrated dose-dependent activity against the tested HeLa cell line after 48 hours of exposure. The lipophilic character of the adamantane moiety in 5-NVAC facilitates extensive hydrophobic interactions within the active site residues, alongside other types of interactions.

**Keywords:** Adamantane-1-carbohydrazide, hydrazide-hydrazone, 5-nitrovanillin, computational, *in vitro* cytotoxicity

## Introduction
The adamantane cage is a notable core structure in several drugs with diverse pharmacological properties [1-3]. The chemotherapeutic potential of adamantane-based compounds was first investigated following the finding of amantadine [4] and rimantadine [5] as effective treatments for managing influenza, a viral infection. Tromantadine was also formulated as a powerful antiviral medication for addressing skin infections caused by the herpes simplex virus [6]. The adamantane cage is a key component in many currently used anticancer drugs. Adaphostin is a tyrosine kinase inhibitor which shows anti-proliferative activity against leukemia, non-small cell lung carcinoma, and prostate cancer [7]. Adarotene, an IκB kinase-β inhibitor, has also been developed as a potent anticancer agent for treating lymphoma, leukemia, and prostate cancer [8]. The adamantane-based synthetic retinoid CD437 is a promising anticancer agent, with its mechanism involving the suppression of DNA polymerase [9], while Opaganib is a newly approved adamantane-based anticancer drug used for advanced solid tumors Fig.1 [10]. Opaganib exerts its anticancer effects by inhibiting sphingosine kinase (SK). Recently, Opaganib has been classified as a prospective agent for the treatment of severe COVID-19 pneumonia [11]. Other adamantane based derivatives have demonstrated substantial activity against pathogenic bacteria, mycobacteria and fungi. The adamantane diamine hybrid derivative SQ109 was identified for the therapy of drug-resistant tuberculosis [12], meanwhile, the structurally related dipiperidine analogue SQ609 was developed as a lead compound with strong activity against Mycobacterium tuberculosis [13].
Additionally, hydrazones are important compounds because of their versatility and structural similarities to various biologically significant natural compounds [14, 15]. Hydrazone derivatives contain an imine (-N=C-) group, play an essential role in the mechanisms of transformation and racemization reactions within biological systems [16-18]. Considering these factors and continuing our efforts to develop new bioactive compounds [19-22], we havedeveloped and synthesized a novel hydrazide hydrazone derivative of 5-nitrovanillin (4-hydroxy-3-methoxy-5-nitro benzaldehyde) incorporating an adamantane bioactive core as a promising heterocyclic scaffold and assessed for anticancer activity.

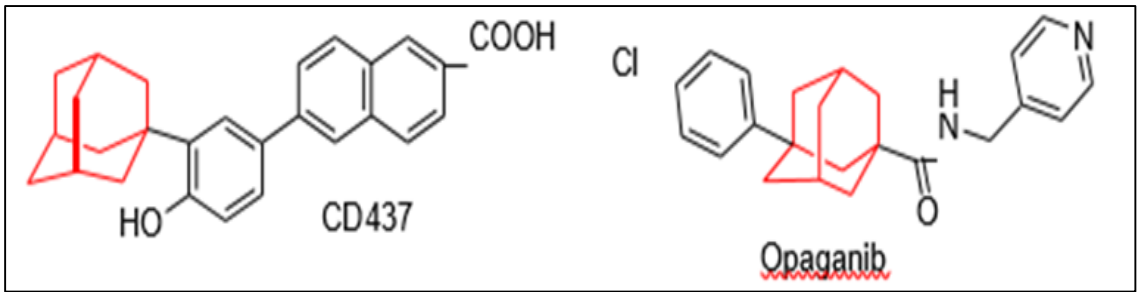



**Fig 1:** Adamantane based anticancer drugs

## Materials and Methods

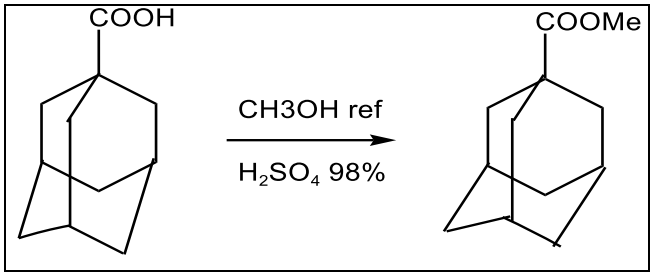
Chemicals were sourced from SBL, Loba Chemie, and Ottokemi. The structural verification of adamantane derivative was done using FTIR and NMR spectroscopy. FTIR was obtained using the KBr pellet technique on a Bruker 3000 Hyperion Spectroscopic Microscope with a Vertex 80 FTIR spectrometer (Germany). $^1$H NMR spectra was acquired in deuterated dimethyl sulfoxide (DMSO-$d_6$) at 600 MHz with a JEOL ECZR Series 600 mega Hertz NMR Spectrometer (Japan), which used TMS as an internal standard, and chemical shifts are recorded in δ ppm. Elemental analysis was carried out using ThermoFisher Scientific Flash smart V CHNS/O analyser. Anticancer study was performed by measuring the cell viability/cytotoxicity using MTT assay in HeLa cell line.

## Results

### Synthesis of Methyl Adamantane-1-Carboxylate

5 g (27 mmol) of adamantane-1-carboxylic acid was combined with 50 ml of methanol (1235 mmol) and 9.2 g of 98% sulphuric acid (5.11 ml). This mixture was stirred and heated with reflux for 4 h. After this, the mixture was neutralized to pH 7–8 using a 10% aqueous sodium bicarbonate ($NaHCO_3$) solution. The solution was subsequently allowed to reach room temperature. Following this, 200 ml of ice and water was added, and then subjected to recrystallization with absolute ethanol yielding 4.92 g of white, needle-shaped crystals of Methyl Adamantane-1-Carboxylate [23, 24] with an 88.2% yield. (Scheme 1).

The melting point of the final product was established as 37 °C. The compound was identified by comparing its melting point with the published value [25].

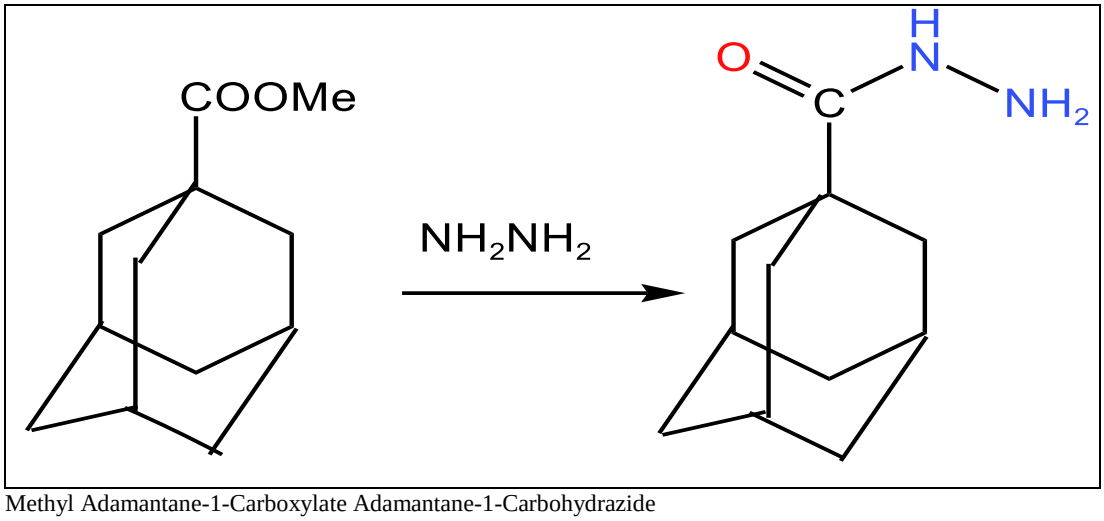



Adamantane-1-carboxylic acid Methyl Adamantane-1-Carboxylate

**Scheme 1:** Synthesis of Methyl Adamantane-1-Carboxylate

### Synthesis of Adamantane-1-Carbohydrazide

4 g (20 mmol) of compound and 25 ml (412 mmol) of 80% hydrazine hydrate solution in 18 ml of ethanol was refluxed for 15 h. Upon completion, 200 ml of cold water was introduced in the reaction. The formed precipitate was than filtered and given washings with ice water, and dried to yield 3.82 g of an opalescent, scaly solid identified as Adamantane-1-Carbohydrazide, with an 88.99% yield. Melting point observed was 148 °C. (Scheme 2).




Methyl Adamantane-1-Carboxylate Adamantane-1-Carbohydrazide

**Scheme 2:** Synthesis of Adamantane-1-Carbohydrazide

FTIR $\upsilon_{max}$ ($cm^{-1}$): 3332.47, 3278.23 (N-H), 2912.48, 2894.13, 2849.49 (C-H), 1613.79 (C=O), 1523.69 (N-H), 1452.70, 1368.80 (C-H) (23, 25) (Figure 2).

$^1$H NMR (600 mHz, DMSO-$d_6$, δ-ppm): 1.93 (3H, adamantane), 1.74 (6H, adamantane), 1.63 (6H, adamantane), 4.12 (2H, -$NH_2$), 8.68 (H, NH-C) [24], (Figure 3)

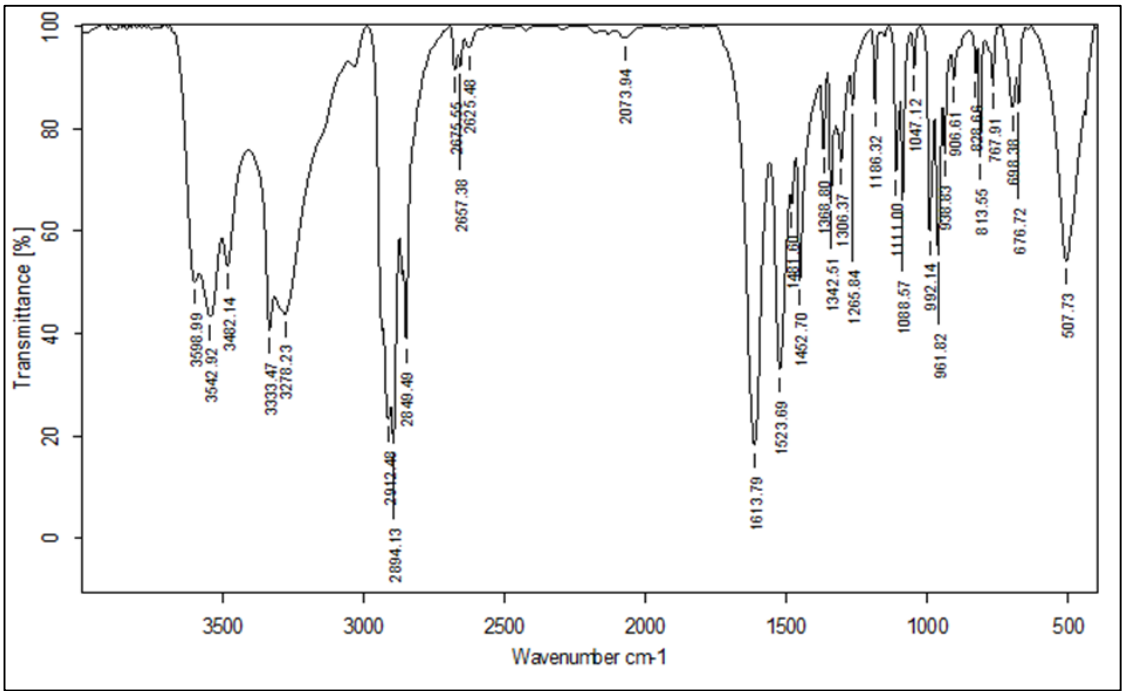



**Fig 2:** FTIR spectra of Adamantane-1-Carbohydrazide

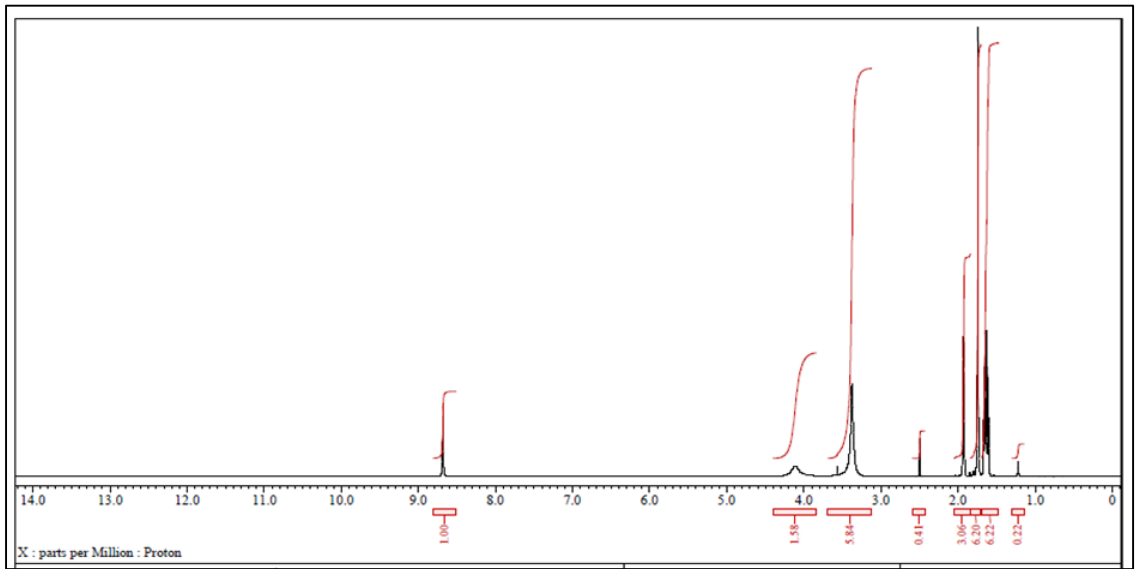
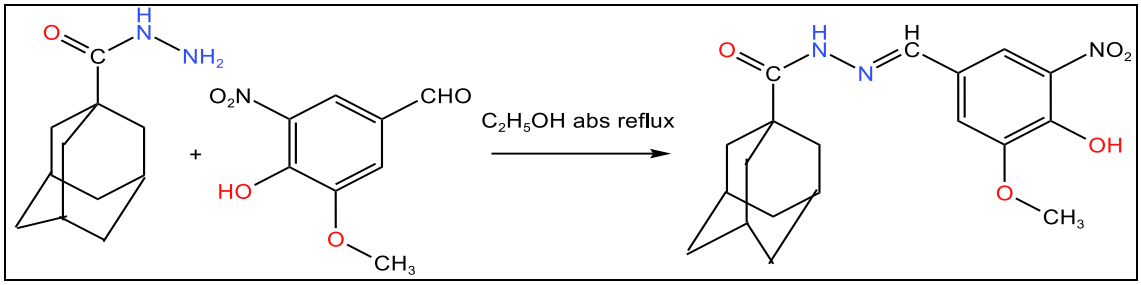



**Fig 3:** NMR spectra of Adamantane-1-Carbohydrazide

**Synthesis of 5-Nitrovanillin Adamantane Carbohydrazide (5-NVAC)**

A mixture of 2 mmol of adamantane-1-carbohydrazide and 2 mmol of 5-nitrovanillin in 15 ml of ethanol was kept on stirring and refluxing for 4 h. Upon completion of the reaction, ethanol was evaporated. The resulting mixture was allowed to crystallize at 0–5 °C. The resulting crystals were filtered and given washings with ethanol, and air-dried, yielding N'-(4-hydroxy-3-methoxy-5-nitro benzylidene) adamantane-1-carbohydrazide (5-NVAC) with a 92.98% yield. The melting point was determined to be 190 °C. (Scheme 3)




Adamantane-5-Nitro Vanillin N'-(4-hydroxy-3-methoxy-5-nitro 1-Carbohydrazide benzylidene) adamantane-1-carbohydrazide

**Scheme 3:** Synthesis of N'-(4-hydroxy-3-methoxy-5-nitro benzylidene) adamantane-1-carbohydrazide (5-NVAC)

**Elemental analysis CHN: Found (Calculated): C, 61.33 (61.15); H, 5.95 (6.16); N, 10.97 (11.25) (Fig. 4)**

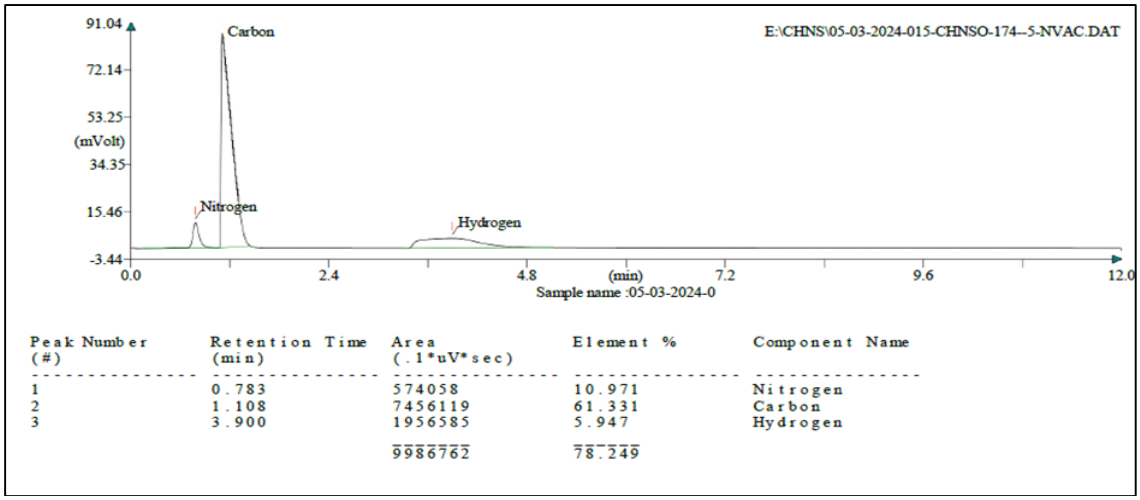



**Fig 4:** Elemental analysis of N'-(4-hydroxy-3-methoxy-5-nitro benzylidene) adamantane-1-carbohydrazide (5-NVAC)

The Molecular formula was confirmed to be $C_{19}H_{23}O_5N_3$, Molecular wt =373.19 g/mol.

FTIR $\upsilon_{max}$ $cm^{-1}$: 3243.02 (N-H), 3084.15 (C-H aromatic), 2904.07, 2850.52 (C-H aliphatic), 1654.60 (C=O), 1617 (C=N), 1531.91 (N-H), 1453.53, 1422.95, 1369.42 (C-H). (Fig. 5)

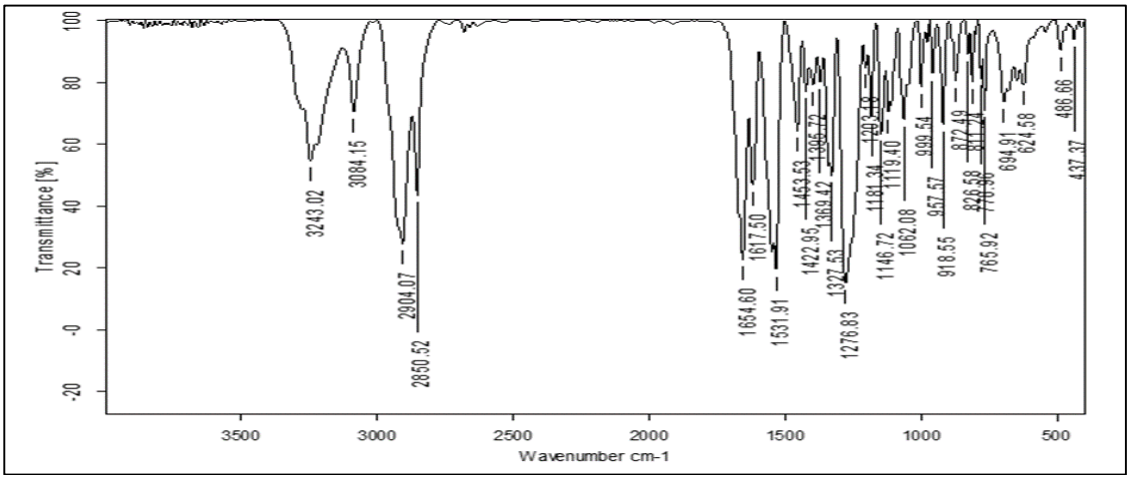



**Fig 5:** FTIR spectra of N'-(4-hydroxy-3-methoxy-5-nitro benzylidene) adamantane-1-carbohydrazide (5-NVAC)

$^1$H NMR (600 MHz, DMSO-$d_6$, δ ppm): 2.00 (3H, adamantane), 1.86 (6H, adamantane), 1.69 (6H, adamantane), 3.92 (3H, $OCH_3$), 7.67, 7.53 (2H, Ar-H), 8.32 (H, -N=CH), 9.80 (H, -NH-C), 10.89 (H, -OH). (Fig. 6)

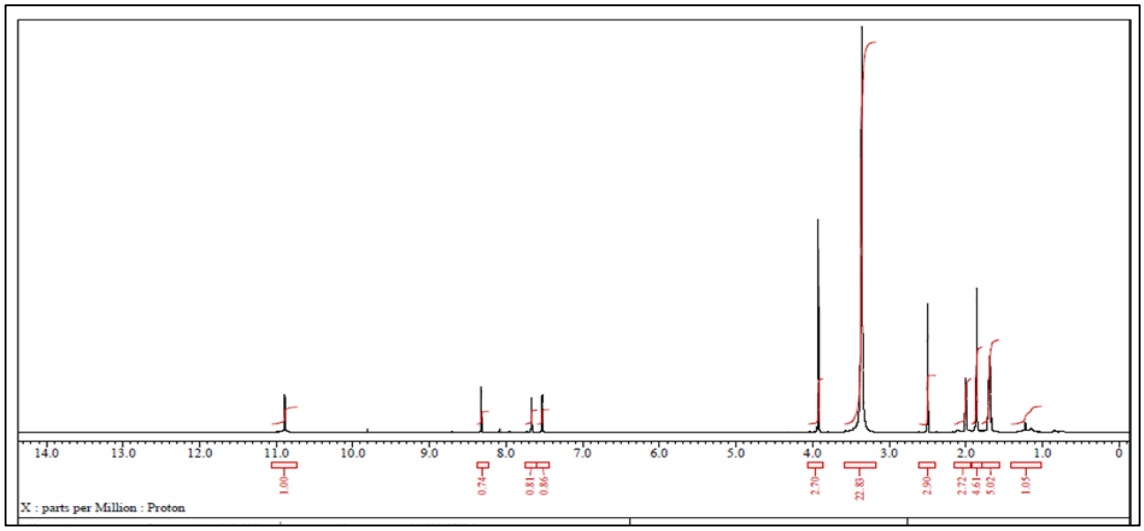



**Fig 6:** $^1$H NMR spectra of N'-(4-hydroxy-3-methoxy-5-nitro benzylidene) adamantane-1-carbohydrazide (5-NVAC)

**Cytotoxic activity**

The cell toxicity of the synthesized compound 5-NVAC on HeLa cell line, developed from human cervical cancer was tested using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method, as described in a previous publication [26]. The study was conducted by measuring cell viability (Figure 7).

The solid sample received was dissolved and then sterilized by filtering through a 0.22 µm membrane filter. The MTT-based cytotoxicity assay for the sample was conducted using the HeLa cell line. 5 X $10^3$ cells per well were seeded in a 96-well plate and allowed to incubate for 48 h prior to drug exposure. After drug treatment, 5 mg/ml MTT reagent was introduced and allowed to incubate for 4 hours at 37 °C. 100 µl of acidified isopropanol was introduced to each well to dissolve the formazan crystals, followed by gentle shaking for 20 mins and the optical density (OD) was recorded at 570 nm. (Fig. 8) Doxorubicin was taken as the control.

Cell viability (%) was found using the formula-

Cell Viability% = [OD (Treated) / OD (Control)] X 100

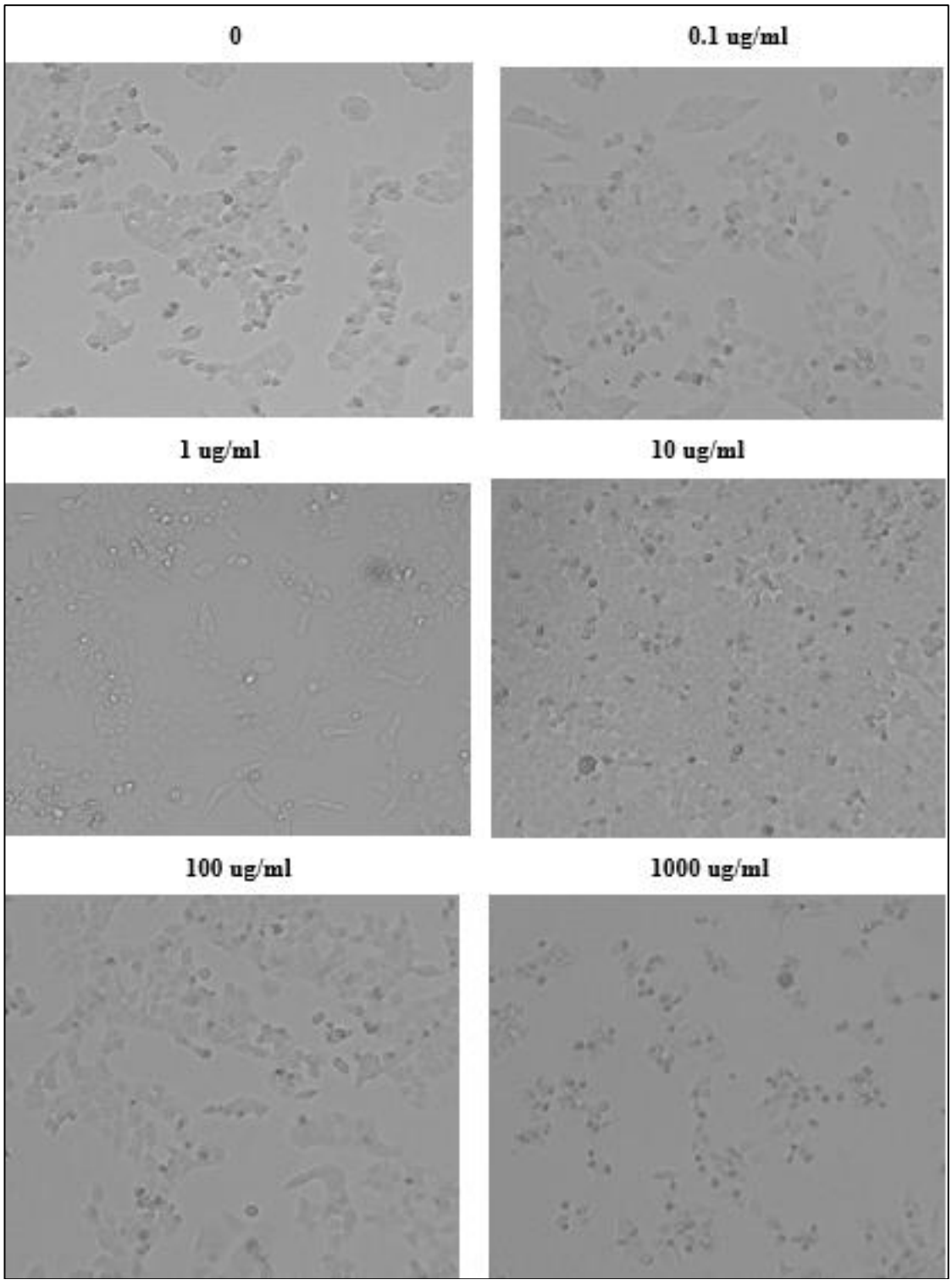



**Fig 7:** 5-NVAC Bright Field Images

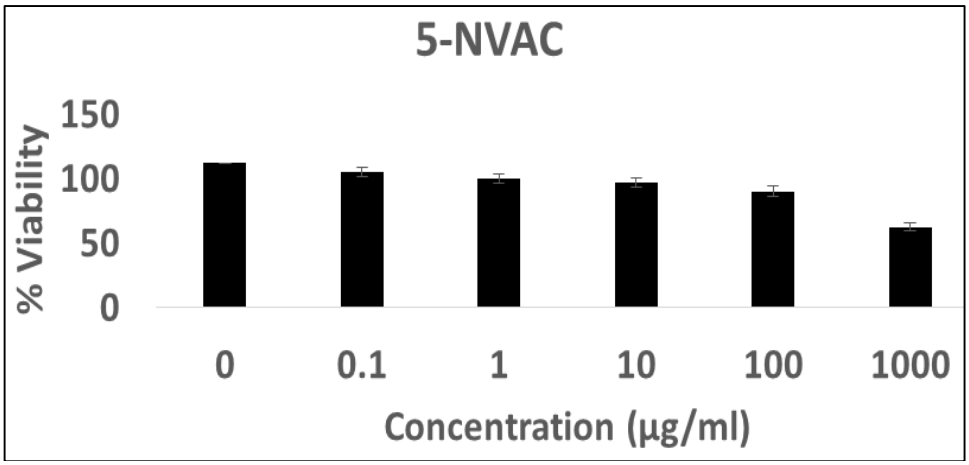



**Fig 8:** O.D. Measurement at 570 nm

## Discussion

The structure of hydrazide hydrazone was verified through elemental analysis, FTIR and $^1$H NMR. The hydrazone formation was proved with IR studies; following the detection of hydrazide C=O and azomethine –CH=N-peak in IR spectra.

$^1$H proton NMR spectra also proved the hydrazide formation. $^1$H NMR signals from the adamantane moiety appeared in the range of δ 1.69-2.00 ppm. The protons in the aromatic ring appeared as expected at δ 7.53 and 7.67 ppm. The signal from the methyne proton (CH=N) was observed as a singlet at δ 8.32 ppm, whereas the amide proton appeared as a singlet at δ 9.80 ppm. The –OH proton was detected at δ 10.89 ppm, whereas the $–OCH_3$ protons was detected at δ 3.92 ppm. All spectra were in full agreement with the proposed structure.

The synthesized hydrazide-hydrazone 5-NVAC was evaluated for its cytotoxicity against the HeLa cells, derived from human cervical cancer. The synthesized hydrazide-hydrazones demonstrated antiproliferative activity against the tested cancer cell lines.

Structure-cytotoxicity relationship analysis indicated that compound 5-NVAC, featuring $5\text{-}NO_2$ and $3\text{-}OCH_3$ groups on the benzyl ring, shows substantial cytotoxicity against the tested cancer cell lines. Based on the observations, it can be inferred that the $5\text{-}NO_2$ and $3\text{-}OCH_3$ groups is optimal for enhancing anti-proliferative activity. Additionally, the lipophilic nature of adamantane core strengthens the hydrophobic interactions with the active site.

## Conclusion

The preparation and analysis of new hydrazone of 5-nitro vanillin with Adamantane -1-carbohydrazide was achieved. Based only on our experimental observation we conclude that, in comparison with the control Doxorubicin, the sample 5-NVAC demonstrated dose-dependent activity after 48 h of exposure. The synthesized compound 5-NVAC demonstrated significant antiproliferative activity against HeLa cells, derived from human cervical cancer. The lipophilic nature of the adamantane moiety in the compound promotes extensive non polar interactions with the binding site residues, along with other interactions.

## Acknowledgement

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