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Synthesis, characterization and pharmacological studies of some novel N-acyl hydrazones of 1,2,3-triazole as potent cytotoxic agents

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ABSTRACT

*A novel series of5-methyl-1-(p-nitrophenyl)-N'-[(aryl substituted)methylidene]-1H-1,2,3-triazole-4-carbohydrazone **(4)**,was prepared from 5-methyl-1-(p-nitrophenyl)-1H-1,2,3-triazole-4-carbohydrazide **(3)** by reaction with appropriate aldehydes.The structures ofnew hydrazone derivatives**(4)** were characterized on the basis of IR, NMR, mass spectra1 data and elemental analysis. The newly synthesized compounds were assayed for their cytotoxicstudy and antioxidant activities.*

Keywords: 1,2,3-Triazole, Nitrofuran, N-acyl Hydrazones,*In-Vitro* Cytotoxic Study, Antioxidant studies.

INTRODUCTION

1,2,3-Triazoles are attractive constructs, because of their unique chemical properties and they find many applications in organic and medicinal chemistry[1,2]. Not present in natural products, they are remarkably stable to metabolic transformations, such as oxidation, reduction, and both basic and acidic hydrolysis.

The 1,2,3-triazole based derivatives have received much attention due to their wide coverage of biological properties including antiviral[3], anti-HIV[4,5], anticonvulsants[6], anti-allergic[7], antimicrobial[8,9], analgesic[10], anti-inflammatory[10], antioxidant and anticancer properties[11].

Hydrazones are a class of organic compound formed by condensation of substituted hydrazines with aldehydes and ketones. These compounds exhibit various optical properties, complex formation with metal ions and pharmacological properties[12-16].

Prompted by these observations and in an attempt to synthesize a series of hydrazone derivatives possessing potent 1,2,3-triazole entity with improved biological activity, a new series of hydrazone derivativescarrying 1,2,3-triazole moiety were synthesized and evaluated for their cytotoxic and antioxidant property.

EXPERIMENTAL SECTION

Melting points were determined in open capillary tubes in Innovative DTC-967A digital melting point apparatus and are uncorrected. IR spectra were recorded by dispersing the compounds in KBr pellets on a Schimadzu FT-IR 157 spectrophotometer. ^{1}H NMR spectra were recorded on a 400 MHz BrukerAvance II NMR spectrometer and all the chemical shift values were reported as δ (ppm), downfield from TMS and proton signals are indicated as s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Mass spectra were recorded either on a Waters UPLC-MS spectrometer or LCMS (API 3000, Applied Bio Systems) operating at 70eV. Elemental analysis was carried out on a SchimadzuElementarVario EL III model. The X-ray diffraction measurements were carried out in Bruker SMART APEXII CCD area-detector diffractometer.

General procedure for the preparation of 1-azido-4-nitrobenzene(1)
p-Nitroaniline (0.1 mol) was dissolved in 1:1 ratio of HCl: water and taken in a round bottom flask equipped with stirrer. The reaction was agitated at 0-5 ^{0}C; sodium nitrite (0.12 mol) was dissolved in water and added drop wise, sodium azide (0.1 mol) dissolved in water and added drop wise, then reaction is allowed to continue for 30 min. The resultant precipitate was extracted with chloroform and washed successively with water. The organic layer was dried over anhydrous sodium sulphate, and the solvent stripped out in rotary evaporator to get 4-nitroazidobenzene, yield 93.5% ; m.p. 65-68 °C. (lit.[17] 63-66 ^{o}C).

General procedure for the preparation of ethyl 5-methyl-1-(p-nitrophenyl)-1H-1,2,3-triazole-4-carboxylate (2)
1-Azido-4-nitrobenzene (0.01mol) was treated with ethyl acetoacetate (0.01mol) in methanol (75 ml) and the mixture was cooled to 0 °C. Sodium ethoxide (0.015mol) was added under inert atmosphere to the above mixture and stirred at ambient temperature for 2 h. Progress of the reaction was monitored by TLC (ethyl acetate/petroleum ether, 2:3, v/v). After completion of the reaction, the mixture was poured on to ice cold water. The precipitated solid was filtered, washed with water and recrystallized from ethanol and the structure was confirmed by single crystal XRD study[18], Yield 75%, m.p. 165-170 ^{o}C; Mol. Formula: $C_{12}H_{12}N_4O_4$; Elemental analysis (Found): C, 52.14; H, 4.34; N, 20.28; (calculated) C, 52.17; H, 4.38; N, 20.28.

General procedure for the preparation of 5-methyl-1-(p-nitrophenyl)-1H-1,2,3-triazole-4-carbohydrazide (3)
The ethyl 5-methyl-1-(*p*-nitrophenyl)-1H-1,2,3-triazole-4-carboxylate (0.01 mol) in dimethyl formamide and hydrazine hydrate (99 %, 0.01 mol) were taken in a round bottomed flask equipped with reflux condenser. The contents were refluxed for 1 hour. Solid separatedwas filtered, dried and recrystallised using ethanol. Yield 80%; m.p. 210-214 °C; Mol. Formula: $C_{10}H_{10}N_6O_3$; Elemental analysis (Found): C, 45.82; H, 3.86; N, 32.06; (calculated) C, 45.80; H, 3.84; N, 32.05; IR (KBr) γ/cm^{-1}: 3338 (N-H), 2985 (C-H), 1660 (C=O), 1500 (Asym. NO_2), 1346 (Sym. NO_2); ^{1}H NMR (DMSO-d$_6$) δ: 2.61 (s, 3H, CH_3), 4.51 (s, 2H, NH_2), 8.0 (d, 2H, J = 9.0 Hz, meta protons of p-nitrophenyl), 8.49 (d, 2H, J = 8.96 Hz, ortho protons of p-nitrophenyl), 9.84 (s, 1H, NH); LC-MS (m/z, %) 263.2 (M^++1, 98), (M.F. $C_{10}H_{10}N_6O_3$).

General procedure for the synthesis 5-methyl-1-(p-nitrophenyl)-N'-[(aryl substituted)methylidene]-1H-1,2,3-triazole-4-carbohydrazone(4a-k)
To a solution of 5-methyl-1-(*p*-nitrophenyl)-1H-1,2,3-triazole-4-carbohydrazide**(3)** (0.01mol) in a mixture of DMF and ethanol (25ml), was added substituted aryl aldehydes (0.01mol). Concentrated sulphuric acid (0.5ml) was added to this reaction mixture. The contents were refluxed for about 1-2 hours. The solid product separated was collected by filtration. It was dried and recrystallised from ethanol.

5-Methyl-1-(p-nitrophenyl)-N'-[(5-nitrofuran-2-yl)methylidene]-1H-1,2,3-triazole-4-carbohydrazone 4a
IR (KBr) γ/cm^{-1}: 3309.6 (N-H), 3096.2 (C-H), 1682.9 (C=O), 1577.5 (C=N stretch), 1501.4 (asym. NO_2), 1343.6 (sym. NO_2); ^{1}H NMR(DMSO-*d$_6$*): δ2.64 (s, 3H, CH_3), 7.25 (d, 1H, J = 3.9 Hz, Nitrofuran-3H), 7.79 (d, 1H, J = 3.9 Hz, Nitrofuran-4H), 8.02 (d, 2H, J = 8.9 Hz, meta protons of *p*-nitrophenyl), 8.49 (d, 2H, J = 8.9 Hz, ortho protons of *p*-nitrophenyl), 8.53 (s, 1H, N=CH), 12.30 (s, 1H, NH); UPLC Mass (m/z, %) 386.3 (M^++1, 79), (M.F. $C_{15}H_{11}N_7O_6$).

5-Methyl-1-(p-nitrophenyl)-N'-[(5-nitrothiophene-2-yl)methylidene]-1H-1,2,3-triazole-4-carbohydrazone 4b
IR (KBr) γ/cm^{-1}: 3318.6 (N-H), 2975.8 (C-H), 1676.2 (C=O), 1565 (C=N stretch), 1567.3 (asym. NO_2), 1334.9 (sym. NO_2);^{1}H NMR(DMSO-*d$_6$*): δ2.65 (s, 3H, CH_3), 7.28 (d, 1H, J = 3.9 Hz, Nitrothiphene-3H), 7.82 (d, 1H, J = 4 Hz, Nitrothiphene-4H), 8.06 (d, 2H, J = 9 Hz, meta protons of *p*-nitrophenyl), 8.50 (s, 1H, N=CH), 8.56 (d, 2H, J = 9 Hz, ortho protons of *p*-nitrophenyl), 12.32 (s, 1H, NH); UPLC (m/z, %) 401.74 (M^++, 86), (M.F.- $C_{15}H_{11}N_7O_5S$).

5-Methyl-1-(p-nitrophenyl)-N'-(phenylmethylidene)-1H-1,2,3-triazole-4-carbohydrazone 4c
IR (KBr) γ/cm^{-1}: 3335.2 (N-H), 3001.6 (C-H), 1666.2 (C=O), 1597.5 (C=N stretch), 1508.9 (asym. NO_2), 1370.7 (sym. NO_2); ^{1}H NMR(DMSO-d$_6$): δ 2.65 (s, 3H, CH_3), 7.43-7.46 (m, 3H, 3',4',5'Ar-H), 7.71 (d, 2H, J = 7.71 Hz, 2',6'Ar-H), 8.02 (d, 2H, J = 8.97 Hz, meta protons of p-nitrophenyl), 8.49 (d, 2H, J = 8.94 Hz, ortho protons of p-nitrophenyl), 8.58 (s, 1H, N=CH), 12.20 (s, 1H, NH); UPLC (m/z, %) 351.2 (M^++1, 100), (M.F.- $C_{17}H_{14}N_6O_3$).

5-Methyl-1-(p-nitrophenyl)-N'-[(4-fluorophenyl)methylidene]-1H-1,2,3-triazole-4-carbohydrazone 4e
IR (KBr) γ/cm^{-1}: 3345 (N-H), 2985.8 (C-H), 1695 (C=O), 1588 (C=N stretch), 1565.3 (asym. NO_2), 1329.7 (sym. NO_2); ^{1}H NMR(DMSO-d$_6$): δ 2.64 (s, 3H, CH_3), 7.70-7.74 (m, 4H, aromatic protons of p-flurophenyl), 7.92 (d, 2H, J = 9.0 Hz, meta protons of p-nitrophenyl), 8.49 (d, 2H, J = 9.0 Hz, ortho protons of p-nitrophenyl), 8.58 (s, 1H, N=CH), 12.20 (s, 1H, NH); LC-MS (m/z, %) 368.2 (M^+, 90), (M.F.- $C_{17}H_{13}FN_6O_3$).

5-Methyl-1-(p-nitrophenyl)-N'-[(3-bromophenyl)methylidene]-1H-1,2,3-triazole-4-carbohydrazone 4h
IR (KBr) γ/cm^{-1}: 3335.2 (N-H), 2985.8 (C-H), 1666.2 (C=O), 1588 (C=N stretch), 1561 (asym. NO_2), 1327.2 (sym. NO_2); ^{1}H NMR(DMSO-d_6): δ2.66 (s, 3H, CH_3), 7.45 (m, 1H, 5'Ar-H of *m*-bromophenyl), 7.64 (d, 1H, J = 8.0 Hz, 6'Ar-H of *m*-bromophenyl), 7.70 (d, 1H, J = 7.7 Hz, 4'Ar-H of *m*-bromophenyl), 7.90 (s, 1H, 2'Ar-H of *m*-bromophenyl), 8.03 (d, 2H, J = 8.8 Hz, meta protons of *p*-nitrophenyl), 8.50 (d, 2H, J = 8.9 Hz, ortho protons of *p*-nitrophenyl), 8.55 (s, 1H, N=CH), 12.40 (s, 1H, NH); LC-MS (m/z, %) 430.5 (M^++1, 94), (M.F.- $C_{17}H_{13}BrN_6O_3$).

5-Methyl-1-(p-nitrophenyl)-N'-[(3,4-dichlorophenyl)methylidene]-1H-1,2,3-triazole-4-carbohydrazone 4i
IR (KBr) γ/cm^{-1}:3325.2 (N-H), 2975.8 (C-H), 1670.2 (C=O), 1562.8 (C=N stretch), 1565.3 (asym. NO_2), 1329.7 (sym. NO_2);^{1}H NMR(DMSO-d_6): δ2.65 (s, 3H, CH_3), 7.72-7.80 (m, 3H, aromatic protons of 3,4-dichlorophenyl),8.02 (d, 2H, J = 9.0 Hz, meta protons of p-nitrophenyl),8.49 (d, 2H, J = 9.0 Hz, ortho protons of p-nitrophenyl), 8.58 (s, 1H, N=CH), 12.20 (s, 1H, NH); LC-MS (m/z, %) 419.5 (M^+, 100), (M.F.- $C_{17}H_{12}Cl_2N_6O_3$).

5-Methyl-1-(p-nitrophenyl)-N'-[(3,4-dimethoxyphenyl)methylidene]-1H-1,2,3-triazole-4-carbohydrazone 4j
IR (KBr) γ/cm^{-1}: 3360 (N-H), 2949.1 (C-H), 1672.2 (C=O), 1597 (C=N stretch), 1517.9 (asym. NO_2), 1338.6 (sym. NO_2); ^{1}H NMR(DMSO-d_6): δ2.65 (s, 3H, CH_3), 3.89 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 7.04 (d, 1H, J = 8.2 Hz, 3,4-dimethoxyphenyl Ar-H), 7.65 (d, 1H, J = 8.0 Hz, 3,4-dimethoxyphenyl Ar-H), 7.91 (d, 2H, J = 9.0 Hz, meta protons of p-nitrophenyl), 8.26 (s, 1H, 3,4-dimethoxyphenyl Ar-H), 8.49 (d, 2H, J = 9.0 Hz, ortho protons of p-nitrophenyl), 8.52 (s, 1H, N=CH), 12.10 (s, 1H, NH); LC-MS (m/z, %) 410.3 (M^+, 96), (M.F.- $C_{19}H_{18}N_6O_5$).

5-Methyl-1-(p-nitrophenyl)-N'-[(4-hydroxy-3-methoxyphenyl)methylidene]-1H-1,2,3-triazole-4-carbohydrazone 4k
IR (KBr) γ/cm^{-1}: 3329.2 (N-H), 2982.4 (C-H), 1668.6 (C=O), 1582.4 (C=N stretch), 1564.3 (asym. NO_2), 1330.7 (sym. NO_2); ^{1}H NMR(DMSO-d_6): δ2.66 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 6.86 (d, 1H, J = 8.04 Hz, 5'Ar-H), 7.07 (d, 1H, J = 8.2 Hz, 6'Ar-H), 7.31 (s, 1H, 2'Ar-H), 8.03 (d, 2H, J = 9.0 Hz, meta protons of *p*-nitrophenyl), 8.46 (s, 1H, N=CH), 8.51 (d, 2H, J = 8.96 Hz, ortho protons of *p*-nitrophenyl), 9.41 (s, 1H, OH), 12.04 (s, 1H, NH); UPLC (m/z, %) 397.1 (M^++1, 100), (M.F.- $C_{18}H_{16}N_6O_5$).

Biological Evaluation
The newly synthesized 5-methyl-1-(*p*-nitrophenyl)-N'-[(aryl substituted)methylidene]-1H-1,2,3-triazole-4-carbohydrazone **(4)**, were evaluated for the cytotoxic and antioxidant property.

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] cell assay is a safe, sensitive, *in vitro* assay for the measurement of cell proliferation and cell viability. MTT assay, first described by Mosmann in 1983, is based on the ability of a mitochondrial dehydrogenase enzyme from viable cells to cleave the tetrazolium rings of the pale yellow MTT and form a dark blue formazan crystals which is largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. Addition of a detergent results in the liberation of the crystals from the cells which get solubilized. The number of surviving cells is directly proportional to the level of the formazan product created. The color is quantified using a simple colorimetric assay. Samples are read directly in the wells. The optimal wavelength for absorbance is 540 nm.

The free radical scavenging activity of test samples **(4a-k)** was measured by DPPH according to the method of Brand-Williams et al.[19] The antioxidant screening revealed that, some of the tested compounds showed good free radical scavenging capacity by comparison with the standard ButylatedHydroxytoulene (BHT).

In vitro Cytotoxicactivity (MTT Assay)
Human cancer cell lines (HeLa cells), procured from National Centre for Cell Sciences, Pune, India, were cultured in MEM medium supplemented with 10% FBS, 1% L glutamine and 50 μg/ml gentamicin sulphate in a CO_2 incubator in a humidified atmosphere of 5% CO_2 and 95% air.

In vitro cytotoxicity was determined using a standard MTT assay[20] with protocol appropriate for the individual test system. In brief, exponentially growing cells were plated in 96-well plates (10^4 cells/well in 100 μl of medium) and incubated for 24 h for attachment.

Test compounds were prepared prior to the experiment by dissolving 0.1% DMSO and diluted with medium. The cells were then exposed to different concentrations of the compounds (25, 50, 100 and 200 μg) in the volume of 100 μl/well. Cells in the control wells received the same volume of medium containing 0.1% DMSO**.** After 48 h, the medium was removed and cell cultures were incubated with 100 μl MTT reagent (1 mg/ml) for 4 h at 37 ^{o}C. The formazan produced by the viable cells was solubilized by addition of 100 μl DMSO. The suspension was placed on micro-vibrator for 5 min and absorbance was recorded at 540 nm by ELISA reader. The experiment was performed in triplicate. The percentage cytotoxicity was calculated using the formula

$$\% \text{ Cytotoxicity} = \frac{(\text{Control abs - Blank abs}) - (\text{Test abs - Blank abs})}{(\text{Control abs - Blank abs})} * 100$$

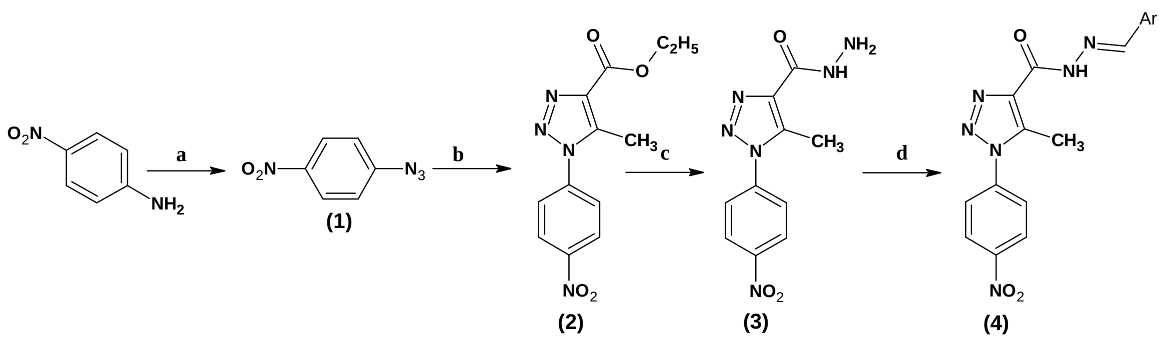
The percentage cytotoxicity and IC_{50} values were determined and results are tabulated in **Table 1**.

Antioxidant activity
Free radical scavenging activity of the test compounds **(4a-k)** were carried based on the scavenging activity of stable DPPH. 100 mg/mL of each test sample and standard BHT was taken in different test tubes and the volume was adjusted to 1 mL using MeOH. Freshly prepared 3 mL of 0.1 mM DPPH solution was mixed and vortexed thoroughly and left in dark for 30 min. The absorbance of stable DPPHradical was measured at 517 nm. The DPPH control (containing no sample) was prepared using the same procedure. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the equation.

$$\text{DPPH radical scavenging activiy (\%)} = \frac{(\text{Abs Control - Abs Sample})}{(\text{Abs Control})} * 100$$

Where Abs Control is the absorbance of DPPH radical + methanol; Abs Sample is the absorbance of DPPH radical + test sample/standard BHT.

The antioxidant study results are tabulated in **Table 1**.



Scheme 1. Reagents and condition : (a) HCl, $NaNO_2$, NaN_3, 0-5^{0}C; (b) Ethylacetoacetate, NaOEt,0-5^{0}C; (c) $N_2H_4.H_2O$, Reflux; (d) ArCHO, DMF, Reflux

Scheme 1. Synthetic pathway for the prepaertion of 5-methyl-1-(4-nitrophenyl)-N'-[(aryl substituted)methylidene]-1*H*-1,2,3-triazole-4-carbohydrazide derivatives **4**

RESULTS AND DISCUSSION

Chemistry
The ethyl 5-methyl-1-(p-nitrophenyl)-1H-1,2,3-triazole-4-carboxylate **(2)** was prepared by the reaction of 1-azido-4-nitrobenzene **(1)** with ethyl acetoacetate in presence of sodium ethoxide. Ethyl-5-methyl-1-(p-nitrophenyl)-1H-1,2,3-triazole-4-carboxylate **(2)** when treated with hydrazine hydrate gave 5-methyl-1-(p-nitrophenyl)-1H-1,2,3-triazole-4-carbohydrazide **(3)(Scheme 1)**.

Condensation of 5-methyl-1-(p-nitrophenyl)-1H-1,2,3-triazole-4-carbohydrazide **(3)** with substituted aryl aldehydes in the presence of catalytic amount of conc. sulphuric acid gave hydrazones**(4a-k)** in good yield **(Scheme 1)**.

The structures of the newly synthesized compounds have been established by analytical and spectral data.Characterization data of 5-methyl-1-(*p*-nitrophenyl)-N'-[(aryl substituted)methylidene]-1H-1,2,3-triazole-4-carbohydrazone**(4a-k)**were established on the basis of elemental analyses, IR, ^{1}H NMR and Mass spectral data.

In the IR spectra of 5-methyl-1-(p-nitrophenyl)-1H-1,2,3-triazole-4-carbohydrazide **(3)**the NH absorption band was observed in the range of 3338 cm^{-1} and the C-H stretching at 2985 cm^{-1}. Characteristic C=O stretching frequency was observed at 1660 cm^{-1} and the asymmetric and symmetric stretching frequency of the nitro group appeared at 1500 cm^{-1} and 1346 cm^{-1} respectively.

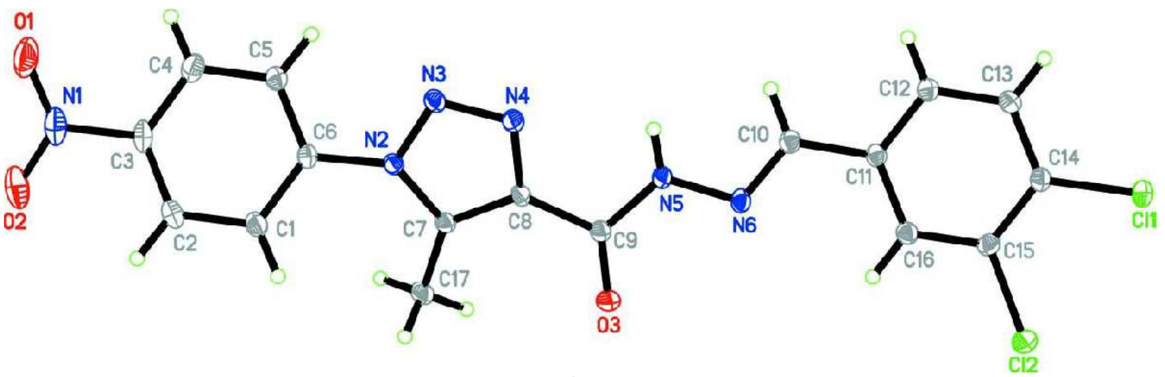
However in the IR spectra of hydrazones**(4a-k)** the absorption band due to C=N stretching was seen in the range of 1597-1562 cm^{-1}there by indicating the formation of hydrazones from hydrazide.

In the ^{1}H NMR spectra of **(3)** the signal due to methyl protons of 1,2,3-triazole appeared as a singlet at δ 2.6178 integrating for three protons. The NH_2 protons appeared as a singlet at δ 4.5167 integrating for two protons. Meta and ortho protons of *p*-nitrophenyl appeared as two doublets in the region δ 7.9816-8.0041 (J = 9 Hz) and δ 8.4677-8.4901 (J = 8.96 Hz) integrating for two protons each. The signal due to NH proton appeared as a singlet at δ 9.8498 integrating for one proton, whereas in hydrazones**(4a-k)** the signal due to NH_2 proton was absent and the presence of a prominent singlet at δ 8.58- 8.46 due to the N=CH proton confirmed the formation of hydrazones**(4)**.

Table 1: Characterization data of 5-methyl-1-(*p*-nitrophenyl)-N'-[(aryl substituted)methylidene]-1H-1,2,3-triazole-4-carbohydrazone (4a-k)

Comp. No. *	Ar	M.P ^{0}C (Yield)	Molecular Formula (Mol. Wt)	Analysis % Found (Clculated)		
				C	H	N
4a	Nitrofuran	256-258 (88)	$C_{15}H_{11}N_7O_6$ (385.29)	46.74 (46.76)	2.85 (2.88)	25.44 (25.45)
4b	Nitrothiophene	258-260 (76)	$C_{15}H_{11}N_7O_5S$ (401.35)	44.86 (44.89)	2.76 (2.76)	24.42 (24.43)
4c	C_6H_5	230-232 (70)	$C_{17}H_{14}N_6O_3$ (350.33)	58.30 (58.28)	4.02 (4.03)	23.98 (23.99)
4d	*p*-$NO_2C_6H_4$	245-248 (82)	$C_{17}H_{13}N_7O_5$ (395.32)	51.64 (51.65)	3.30 (3.31)	24.82 (24.80)
4e	*p*-FC_6H_4	210-212 (90)	$C_{17}H_{13}FN_6O_3$ (368.32)	55.46 (55.44)	3.54 (3.56)	22.82 (22.82)
4f	*p*-BrC_6H_4	246-248 (82)	$C_{17}H_{13}BrN_6O_3$ (429.22)	47.59 (47.57)	3.02 (3.05)	19.54 (19.58)
4g	*m*-$NO_2C_6H_4$	235-238 (76)	$C_{17}H_{13}N_7O_5$ (395.32)	51.64 (51.65)	3.32 (3.31)	24.84 (24.80)
4h	*m*-BrC_6H_4	258-260 (78)	$C_{17}H_{13}BrN_6O_3$ (429.22)	47.56 (47.57)	3.08 (3.05)	19.60 (19.58)
4i	2,4-$Cl_2C_6H_3$	240-242 (70)	$C_{17}H_{12}Cl_2N_6O_3$ (419.22)	48.70 (48.71)	2.92 (2.89)	20.08 (20.05)
4j	3,4-$(OCH_3)_2C_6H_3$	222-226 (68)	$C_{19}H_{18}N_6O_5$ (410.38)	55.62 (55.61)	4.40 (4.42)	20.48 (20.48)
4k	3-OH-4-$OCH_3C_6H_3$	215-218 (84)	$C_{18}H_{16}N_6O_5$ (396.35)	54.56 (54.54)	4.06 (4.07)	21.24 (21.20)

**Solvent for re-crystalizationEtOH+DMF mixture*



ORTEP Diagram for the compound 4i

Pharmacological Evaluation

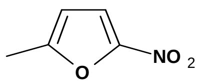
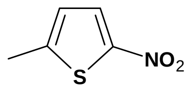
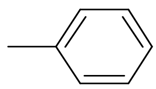
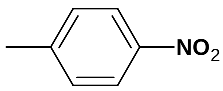
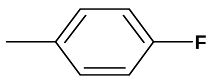
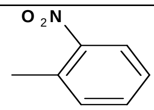
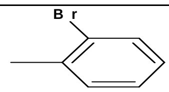
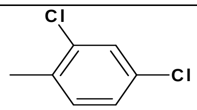
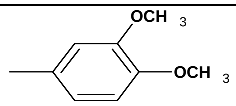
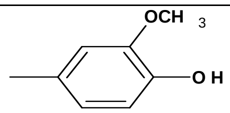
In vitro Cytotoxicactivity

In vitro cytotoxicity of some of the synthesized compounds was assessed by standard MTT bioassay in HeLa cells. The results indicated that the parent hydrazine derivative showed IC_{50} value 19.17. In HeLa cells, the IC_{50}values for nitrofuran derivative **(4a)** was 24.25 and for nitrothiophene derivative **(4b)** IC_{50} value was 33.81. In case of hydrazone possessing phenyl moiety**(4c)** showed excellent activity 12.49. **(Table 2)**

Antioxidant activity

The DPPH scavenging activity of hydrazones showed activity ranging from 69.29% to 45.1%, whereas standard drug BHT showed 90.42% inhibition **(Table 2)**. Compound **4a** and **4g** displayed significant radical scavenging activity, 69.29 and 67.61% respectively among the set of compounds tested in the present study. Whereas compounds **4d**, **4e** and **4c** showed moderate antioxidant activity comparable with BHT.

Table 2: Cytotoxic and DPPH radical scavenging activity data of compounds 3 and 4a-k

Compd. No.	Ar	Cytotoxicity (%) IC_{50}(µg/ml)	Antioxidant Activity
		HeLa cells	DPPH Assay in %
3	-	19.17	72.34
4a		24.25	69.29
4b		33.81	48.69
4c		12.49	50.60
4d		40.56	59.49
4e		59.47	52.54
4g		41.79	67.61
4h		95.21	45.10
4i		49.72	11.32
4j		76.69	-
4k		39.72	49.1
Standard			90.42(BHT)

CONCLUSION

In the present study, we have described the syntheses and in vitro cytotoxicity screening and antioxidant study of 5-methyl-1-(*p*-nitrophenyl)-N'-[(aryl substituted)methylidene]-1H-1,2,3-triazole-4-carbohydrazones**(4)**. Various heterocyclic and substituted arylsystem were incorporated to increase the biological activity of hydrazones.

An interesting observation made during cytotoxic study was that the intermediate possessing nitrofuran and phenyl were more active than the other substitution.

Most of the compounds showed significant free radical scavenging activity but the intermediates especially possessing nitrofuran and *p*-nitrophenyl substituents showed excellent activity compared to that of standard.

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