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Synthesis, Spectral Studies and Biological Evaluation of Hydrazone Derivative as Antimicrobial and Anticancer Agents

Diksha Saini, Monika Gupta

Department of Pharmaceutical Chemistry, Amar Shaheed Baba Ajit Singh Jujhar Singh Memorial College of Pharmacy, Bela, Ropar, Punjab 140111, India

Abstract: ***Hydrazone is a class of organic compounds formed by the fusion of carbonyl compounds with hydrazine. In the last few decades, the hydrazones have emerged as biologically important pharmacophores. The present work deals with the synthesis and evaluation of a new series of (2,4-Dinitrophenyl)hydrazone derivatives as the hydrazones were synthesized by the reaction of various ketones with substituted hydrazine. The antimicrobial activity of the synthesized derivatives was carried out against Gram-negative bacteria Escherichia coli (MTCC 40) and Gram-positive bacteria Bacillus subtilis (MTCC 441) and Staphylococcus aureus (MTCC 87) by using Cup-plate agar diffusion method. Ciprofloxacin was used as standard drug for antibacterial activity. Six compounds 3a, 3d, 3e, 3f, 3g and 3j possessed good to moderate anti microbial activity. Compound 3f and 3g exhibited good antimicrobial activity against all the strains. The derivatives 3a-3j were further screened for anticancer activity against MCF-7 cell line using Adriamycin as standard. Seven compound 3a, 3d, 3e, 3f, 3g, 3i, 3j possessed good to moderate anticancer activity. Compound 3f exhibited best results in both assay. The structures of the synthesized compounds were established by IR and NMR spectral studies.***

Keywords: Hydrazones, (2,4-Dinitrophenyl)hydrazones, cytotoxicity, Anti proliferative, Antimicrobial activity

1. Introduction

The discovery and identification of compounds with desired potency, selective cytotoxicity and antimicrobial potential against cancer cells and micro-organisms that may lead to the development of new anti-cancer and antimicrobial drugs remains an important objective of medicinal chemists.

Hydrazones act as precursor for the synthesis of different heterocyclic scaffolds [1], like 1,3,4-oxadiazolines [2], azetidin-2-ones [3], coumarins [4], 1,3-thiazolidin-4-ones [5.6], and 1,3-ben- zothiazin-4-ones [7].

The hydrazones exhibit geometrical isomerism (syn and anti) due to the presence of the double bond between C and N. This geometrical isomerism have been reported to play important role in the bioactivity of the acyl hydrazones hence their studies are very crucial to develop synthetic methods for selective synthesis of a particular isomer [8]. The hydrazones have been reported to possess diverse biological activities such as anti-inflammatory, analgesic, anticonvulsant, antituberculosis, antitumor, anti-HIV and antimicrobial activity [9].

Recent years has witnessed some of the marketed hydrazone derivatives such as Iproniazide [10] as antidepressant; Nifuroxazide and Furazolidone [11] an oral antibiotic; used in colitis and enteritis treatment respectively, Nitrofurazone [12] in the treatment of skin infections due to skin grafts and Nitrofurantoin [13] in urinary tract infections.

The (2,4-dinitrophenyl)hydrazones of diaryl ketones have been reported to possess antiestrogenic and antineoplastic activities. One of the compound A-007, (4,4'-dihydrobenzophenone-2,4-dinitrophenyl-hydrazone **(1)** is in phase II clinical trials [14]. These hydrazones are reported to have pseudo ring structure that stereo-chemically occupies the estrogenic region of the receptor thereby inhibiting it from undergoing allosteric change to prevent transcription of estrogenic information to the cell nucleus [15].

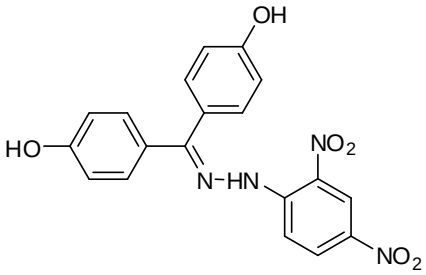


Figure 1: A-007

Small molecules like guanylhydrazone (2) have been reported to block estrogen receptor (ER) activity through a nontraditional mechanism, by directly interfering with coactivator binding to agonist-liganded ER [16].

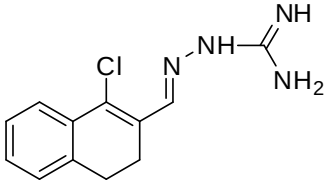


Figure 2: Guanylhydrazone

From a study of the estrogen-ligand pharmacophore model [17], subtle changes in the spatial conformation of the triarylethylene core lead to some major pharmacological changes. This hypothesis has led to the development of many different ER agonists/antagonists, the most promising being the triaryl pyrazole-based ER antagonists [18, 19, 20]. These observations prompted us to design and investigate the hydrazone pharmacophore as the acyclic prototype of monoaryl and diaryl substituted hydrazone for ER affinity and activity. Here, our motivating factor was to see the

flexibility effect of the benzylic group, and also the effect of distance between A and B rings as in tamoxifen (3) at 2-position and in pyrazole (4) at 3-position on the biological activity against ER+ve and ER−ve breast cancer cell lines. The flexible alignment of an acyclic or pseudo ring (5) may help in the adoption of a favorable (antagonist) conformation by the molecules within the receptor cavity resulting in the enhanced affinity of the ligand to the receptor amino acids thereby potentiating the biological efficacy of hydrazones.

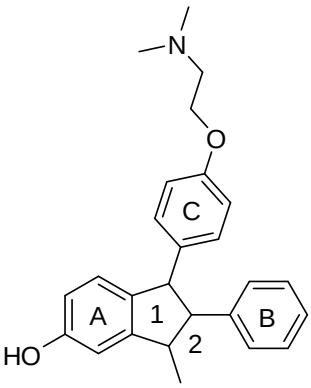


Figure 3: Tamoxifen

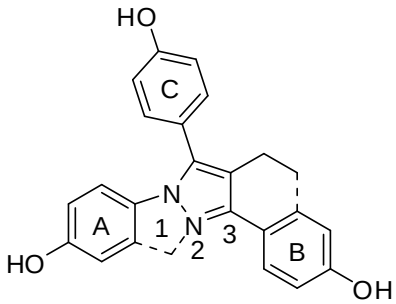


Figure 4: Triphenolic Pyrazole

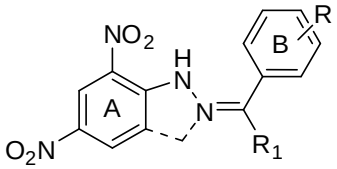


Figure 5: Proposed Hydrazone

Anticancer activity of Hydrazones

Anticancer agents act on various tumour cells by different mechanisms such as they act by inhibiting protein kinases, cause microtubule dysfunctioning, inhibiting topoisomerase I or II, down regulating oncogenes expression, induce apoptosis and interrupt cell division. Researchers synthesized various substituted hydrazones and screened for their anticancer activity on various cell lines *i.e.* human breast cancer, human colon carcinoma, leukemia, lung cancer, human gastric cancer, human esophageal cancer and pancreatic cancer during last five years. They have been reported to act by inhibiting RNA and DNA synthesis [21], inhibiting mitosis [21] induced caspase-dependent apoptosis [22], inhibiting tubulin polymerization and cause cell cycle arrests in G2/M phase [22], cancer cycle arrest at sub G1/G0 phase [23], tumor cell apoptosis [24].

Antibacterial activity of Hydrazones

The emerging bacterial resistance causes a widespread problem for the treatment of various infections. Therefore, the search for better antibacterial agent is a never-ending task. Now-a-days a number of hydrazone derivatives have been developed and evaluated for their antibacterial activity.

2. Experimental

The commercial chemicals employed for the present work were purchased from Sigma-Aldrich, and Loba Chem. All the solvents used were LR grade and were utilized in the reaction. The melting point was determined by open capillaries on Buchi-apparatus and is uncorrected. The reaction were monitored with the help of TLC using pre-coated aluminium sheets coated with $60F_{254}$ silica gel, 0.2 mm thickness from Merck. Various solvents systems used for developing chromatograms were (a) chloroform: ethanol (7:3), (b) chloroform: methanol (9.6:0.4) and U.V light chamber were used for the visualization of the TLC spots. The IR Spectra were recorded on an Bruker alpha-E FTIR-ATR. The 1H NMR spectra was recorded on Bruker Avance II (400MHz) spectrometer using $CDCl_3$ as solvent, where TMS was used as internal standard and chemical shifts are expressed as δ *ppm.*

Synthesis of 7-Hydroxy-4-methyl-2H-chromene-2-one

Resorcinol (3.7g) was add in ethyl acetoacetate (4.5g) and stirred the mixture until a complete solution is obtained. This solution was slowly added to the sulphuric acid at temp. 0°C, and continue the stirring for 30 minutes. The mixture was poured on to crushed ice; the solid 7-hydroxy-4-methyl-coumarin got separate. Filtered of the coumarin at the pump. Purified, the coumarin by dissolving it in cold 10% aqueous sodium hydroxide solution and reprecipitate it by adding dilute hydrochloric acid and then recrystallised it from ethanol**.**

7-Hydroxy-4-methyl-2H-chromene-2-one (1): Yield: 76.2%; State: cream color powder; Melting point: 180-185ºC; R_f 0.66 (Chloroform: Ethanol 7:3).

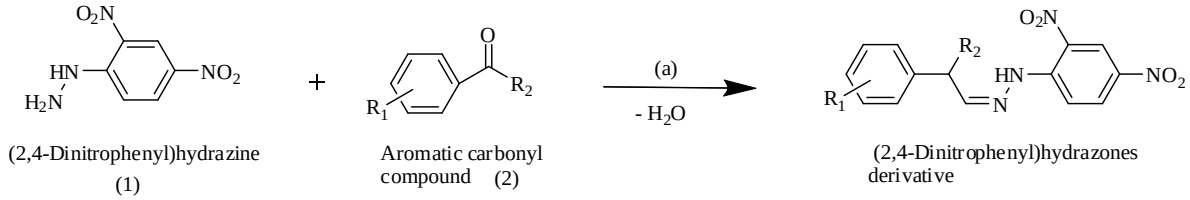
Synthesis of 3-acetyl-7-hydroxy-4-methyl-2H-chromen-2-one

7-hydroxy-4-methyl-2H-chromen-2-one (3g) in acetic acid (16ml) and phosphorus oxychloride (5.6ml) was added. The mixture was heated at reflux for 30 min. After cooling, the precipitate was collected and recrystallized from ethanol.

3-acetyl-7-hydroxy-4-methyl-2H-chromen-2-one (2): Yield: 38.33%; State: light brown powder; Melting point: 100-105ºC; R_f 0.70 (Chloroform: Ethanol 7:3).

Synthesis of Substituted (2,4-Dinitrophenyl)hydrazone derivative (3a-3j):

1g (0.005 mole) (2,4-dinitrophenyl)hydrazine was suspended in 20 ml of methanol and 2 ml concentrated Sulphuric acid was added in it. Filtered the warm solution and added a solution of 0.8g (0.003-0.006 mole) of the aromatic carbonyl compound in a small volume of methanol. The precipitated solid was collected by suction filteration and washed it with a little aqueous methanol. Recrystallised from ethanol (Figure 6 & 7)



(2,4-Dinitrophenyl)hydrazine (1)

Aromatic carbonyl compound (2)

(2,4-Dinitrophenyl)hydrazones derivative

Compounds	R_1	R_2
3a	2-Br, 4-Cl	CH_3
3b	3-Br	CH_3
3c	H	CH_3
3d	4-Cl	CH_3
3e	2,4-dichloro	HC=C(H)–C$_6$H$_5$
3g	H	C$_6$H$_5$
3h	4-CH_3	CH_3
3i	4-OCH_3	NH_2
3j	4-NO_2	CH_2Br

Figure 6: Scheme 1 for the synthesis of (2,4-Dinitrophenyl)hydrazone derivative

Reagents and Conditions: (1) Methanol, Sulphuric acid, stirring, 10 min (2) Methanol, stirring, 10 min **(a)** stir, 20 min.

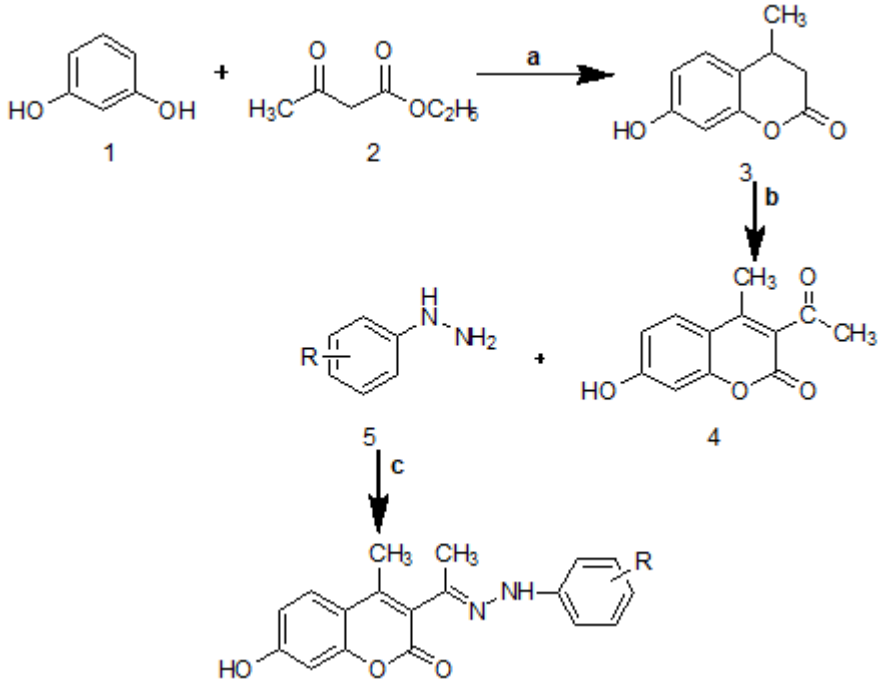


Figure 7: Scheme 2 for the synthesis of 3-(1-(2-(2,4-Dinitrophenyl)hydrazono)ethyl)-7-hydroxy-4-methyl-2H-chromen-2-one

Reagents and Conditions for scheme 2 : (a) Sulphuric acid, ethanol, stirring, 0.5h **(b)** Glacial acetic acid, Phosphorus Oxychloride, ethanol, reflux, 0.5h **(c)** Sulphuric acid, Methanol, stirring. Rt, 30 min.

1-(2-Bromo-4-chlorophenyl)ethylidene-2-(2,4-dinitrophenyl)hydrazine (3a): Yield: 78.5%; State: orange powder; Melting point: 192-194°C; R_f 0.72 (Chloroform: Methanol 9.6:0.4); IR (KBr, cm^{-1}): 3085.57 (Ar-C-H stretch), 730.84 (Ar-C-Cl stretch), 639.64 (Ar-C-Br stretch), 3281.30 cm^{-1} (N-H stretch), 1601.61 cm^{-1} (C=N stretch), 1324.28 (NO_2 stretch), 1500.51 cm^{-1} (NO_2 stretch), 1424.57 cm^{-1} (Ar-C=C stretch); ^{1}HNMR ($CDCl_3$, 400MHz, δ ppm): 0.91 (s, 1H, CH_3), 4.39 (s, 2H, CH_3), 11.56 (s, 1H, N-H), 7.44-8.11 (m. 6H, Ar-H).

1-(3-Bromophenyl)ethylidene-2-(2,4-dinitrophenyl)hydrazine (3b): Yield: 76.2%; State: orange powder; Melting point: 210-214°C; R_f 0.71 (Chloroform: Methanol 9.6:0.4); IR (KBr, cm^{-1}): 3094.46 cm^{-1} (Ar-C-H stretch), 678.60 cm^{-1} (Ar-C-Br stretch), 3302.19 cm^{-1} (N-H stretch), 1685.46 cm^{-1} (C=N stretch), 1332.02 cm^{-1} (C-NO_2 stretch), 1583.37 (C-NO_2 stretch), 1500.24 cm^{-1} (Ar-C=C stretch); ^{1}HNMR ($CDCl_3$, 400MHz, δ ppm): 2.43 (s, 3H, CH_3), 11.35 (s, 1H, N-H), 7.30-9.16 (m. 7H, Ar-H).

1-(2,4-dinitrophenyl)-2-(1,3-diphenylallylidene)hydrazine (3c) Yield: 65.5%; State: orange powder; Melting point: 170-174ºC; R_f 0.62 (Chloroform: Methanol 9.6:0.4); IR (KBr, cm^{-1}): 3100.34 cm^{-1} (Ar-C-H stretch), 3250.22 cm^{-1} (N-H stretch), 1763.26 cm^{-1} (C=N stretch), 1322.73 cm^{-1} (C-NO_2 stretch), 1419.21 (C-NO_2 stretch), 1604.74 cm^{-1} (ethylene-C=C stretch),

1498.49 cm^{-1} (Ar-C=C stretch); ^{1}HNMR ($CDCl_3$, 400MHz, δ ppm): 4.96-5.01 (d, 1H, CH-ethylene), 5.28-5.30 (d, 1H, CH-ethylene), 11.11 (s, 1H, N-H), 6.52-9.16 (m, 13H, Ar-H).

1-((4-Chlorophenyl)(phenyl)methylene)-2-(2,4-dinitrophenyl)hydrazine (3d) Yield: 76%; State: orange powder; Melting point: 203-205ºC; R_f 0.70 (Chloroform: Methanol 9.6:0.4); IR (KBr, cm^{-1}): 3100.38 cm^{-1} (Ar-C-H stretch), 3269.67 cm^{-1} (N-H stretch), 1581.01 cm^{-1} (C=N stretch), 1304.59 cm^{-1} (C-NO_2 stretch), 1494.88 (C-NO_2 stretch), 1412.01 cm^{-1} (Ar-C=C stretch); ^{1}HNMR ($CDCl_3$, 400MHz, δ ppm): 11.23 (s, 1H, N-H), 7.33-9.08 (m. 12H, Ar-H).

1-(2,4-Dichlorophenyl)ethylidene-2-(2,4-dinitrophenyl)hydrazine (3e) Yield: 66.3%; State: yellow crystalline powder; Melting point: 120-124ºC; R_f 0.71 (Chloroform: Methanol 9.6:0.4); IR (KBr, cm^{-1}): 3074.93 (Ar-C-H stretch), 695.80 (Ar-C-Cl stretch), 740.91 (Ar-C-Cl stretch), 3290.92 cm^{-1} (N-H stretch), 1686.35 cm^{-1} (C=N stretch), 1507.73 (NO_2 stretch), 1584.01 cm^{-1} (NO_2 stretch), 1610.46 cm^{-1} (Ar-C=C stretch); ^{1}HNMR ($CDCl_3$, 400MHz, δ ppm): 2.46 (s, 3H, CH_3), 11.29 (s, 1H, N-H), 7.39-9.16 (m. 6H, Ar-H).

3-(1-(2-(2,4-Dinitrophenyl)hydrazono)ethyl)-7-hydroxy-4-methyl-2H-chromen-2-one (3f) Yield: 60.1%; State: orange powder; Melting point: 166-170°C; R_f 0.63 (Chloroform: Methanol 9.6:0.4); IR (KBr, cm^{-1}): 3311.33 cm^{-1} (Ar-C-H stretch), 1745.45 cm^{-1} (C=O stretch), 1326.57 cm^{-1} (C-O stretch), 2956.62 cm^{-1} (C-CH_3 stretch), 3068.45 cm^{-1} (C-CH_3 stretch), 3510.91 cm^{-1} (N-H stretch), 3693.42 cm^{-1} (O-H stretch), 1513.72 cm^{-1} (NO_2 stretch), 1579.47 cm^{-1} (NO_2 stretch), 1421.05 cm^{-1} (Ar-C=C stretch), 1674.27 cm^{-1} (C=N stretch); ^{1}HNMR ($CDCl_3$, 400MHz, δ ppm): 1.52 (s, 3H, CH_3), 3.87 (s, 3H, CH_3), 11.52 (s, 1H, N-H), 9.17 (s, 1H, O-H), 6.98-8.10 (m, 6H, Ar-H).

N'-(2,4-dinitrophenyl)benzohydrazonamide (3g): Yield: 50.6%; State: orange powder; Melting point: 174-176ºC; R_f 0.54 (Chloroform: Methanol 9.6:0.4); IR (KBr, cm^{-1}): 3094.89 cm^{-1} (Ar-C-H stretch), 3356.46 cm^{-1} (C-NH_2 stretch), 3249.65 cm^{-1} (N-H stretch), 1506.53 cm^{-1} (NO_2 stretch), 1527.81 cm^{-1} (NO_2), 1431.60 cm^{-1} (Ar-C=C stretch), 1591.79 cm^{-1} (C=N stretch).

1-(2-bromo-1-p-tolylethylidene)-2-(2,4-dinitrophenyl) hydrazine (3h): Yield: 65.4%; State: orange powder; Melting point: 182-184ºC; R_f 0.71 (Chloroform: Methanol 9.6:0.4); IR (KBr, cm^{-1}): 3107.22 cm^{-1} (Ar-C-H stretch), 2918.14 cm^{-1} (C-CH_3 stretch), 3283.96 cm^{-1} (C-CH_2 stretch), 651.03cm^{-1} (C-Br stretch), 3391.60 cm^{-1} (N-H stretch), 1326.58 cm^{-1} (NO_2 stretch), 1368.90 cm^{-1} (NO_2 stretch), 1504.16cm^{-1} (Ar-C=C stretch), 1655.59 cm^{-1} (C=N stretch).

1-(2-Bromo-1-(4-methoxyphenyl)-ethylidene)-2-(2,4-dinitrophenyl)hydrazine (3i): Yield: 68.%; State: red crystalline powder; Melting point: 194-196°C; R_f 0.70 (Chloroform: Methanol 9.6:0.4); IR (KBr, cm^{-1}): 3104.03 cm^{-1} (Ar-C-H stretch), 1170.47 cm^{-1} (C-O-CH_3 stretch), 3257.81 cm^{-1} (C-CH_2 stretch), 651.46 cm^{-1} (C-Br stretch), 3334.26 cm^{-1} (N-H stretch), 1583.97 cm^{-1} (C=N stretch), 1321.89 cm^{-1} (NO_2 stretch), 1361.73 cm^{-1} (NO_2 stretch), 1494.01 cm^{-1} (Ar-C=C stretch); ^{1}HNMR ($CDCl_3$, 400MHz, δ ppm): 2.38 (s, 3H, CH_3), 3.99 (s, 2H, CH_2), 9.41 (s, 1H, N-H), 6.13-9.09 (m, 7H, Ar-H).

1-(2-Bromo-1-(4-nitrophenyl)ethylidene)-2-(2,4-dinitrophenyl)hydrazine (3j): Yield: 71.4%; State: yellow powder; Melting point: 211-213ºC; R_f 0.70 (Chloroform: Methanol 9.6:0.4); IR (KBr, cm^{-1}): 3087.86 cm^{-1} (Ar-C-H stretch), 3275.50 (C-CH_2 stretch), 640.16 cm^{-1} (C-Br stretch), 3421.93 cm^{-1} (N-H stretch), 1693.73 cm^{-1} (C=N stretch), 1331.71 cm^{-1} (NO_2 stretch), 1545.62 cm^{-1} (NO_2 stretch), 1592.62 cm^{-1} (NO_2 stretch), 1493.46 cm^{-1} (Ar-C=C stretch).

Biological screening of the synthesized compounds

Antimicrobial screening by Cup-plate agar diffusion method

The antibacterial screening of the synthesized compounds was carried out by cup plate agar diffusion method. All the synthesized compounds were subjected to antibacterial activity against Gram negative *Escherichia coli* (MTCC 40) and Gram positive *Staphylococcus aureus* (MTCC 87), *Bacillus subtilis* (MTCC 441). The standard Ciprofloxacin was dissolved in DMSO to get a concentration 1000 µg/ml, 100 µg/ml and 50 µg/ml for testing antibacterial activity. Each test compounds was dissolved in DMSO to get a concentration of 1000 µg/ml, 100 µg/ml and 50 µg/ml for testing antibacterial activity. The zone of inhibition were observed and measured in cm.

***In-vitro* Anticancer screening**

All the synthesized hydrazone derivatives were screened against MCF-7 cell line to determine the growth inhibitory effect of compounds. *In vitro* testing was done using SRB assay protocol, each derivative was tested at 4 dose levels (10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml).

3. Results and Discussion

All the synthesized derivatives of (2,4-Dinitrophenyl) hydrazones were synthesized in good yields by reacting different aromatic ketones with (2,4-Dinitrophenyl) hydrazine in the presence of methanol and under mild acidic conditions by using H_2SO_4. All the reactions were monitored through TLC observation till the completion using suitable mobile phase. After completion of the reaction, the products were purified by using suitable solvents *e.g* ethanol. The outcome of the present work has been summarized in Table 1.

Table 1: Physical characteristic of synthesized compounds

Sr. No	*C. No.*	*Mol.Formula*	*Color*	*Mol. Weight (g)*	*Mel. Point (ºC)*	R_f^*	*Yield (%)*
1.	3a	$C_{14}H_{10}BrClN_4O_4$	Light Orange	413.61	192-194°C	0.72	78.5%
2.	3b	$C_{14}H_{11}BrN_4O_4$	Royal Orange	379.17	210-214°C	0.71	76.2%
3.	3c	$C_{21}H_{16}N_4O_4$	Dark Orange	388.38	170-174ºC	0.62	65.5%
4.	3d	$C_{19}H_{13}ClN_4O_4$	Reddish Orange	396.78	203-205°C	0.70	76%
5.	3e	$C_{14}H_{10}Cl_2N_4O_4$	Lemon	369.16	120-124ºC	0.71	66.3%
6.	3f	$C_{18}H_{14}N_4O_7$	Mustard	398.33	166-170°C	0.63	60.1%
7.	3g	$C_{13}H_{11}N_5O_4$	Brownish Orange	301.26	174-176ºC	0.54	50.6%
8.	3h	$C_{15}H_{13}BrN_4O_4$	Dark Orange	393.19	182-184ºC	0.71	65.4%
9.	3i	$C_{15}H_{13}BrN_4O_5$	Blood Red	409.19	194-196°C	0.70	68.4%
10.	3j	$C_{14}H_{10}BrN_5O_6$	Pale Yellow	424.16	211-213ºC	0.70	71.4%

The purity of the compound was confirmed by TLC using precoated silica gel as a stationary phase, using appropriate solvent system as mobile phase and visualized under UV-light as well as analyzed. Structures of the title compounds were confirmed by FT-IR and 1H NMR spectral studies. The R_f for the title compounds was observed between 0.54-0.72 using Solvent system; Chloroform: Methanol (9.6:0.4). The melting point of all the compounds are in range between 120-214ºC and uncorrected. The yield of all the synthesized derivatives was found in between 50.6-78.5%.

Antimicrobial activity

The synthesized derivatives 3a, 3d, 3e, 3f, 3g and 3j exhibited antimicrobial potential against *Escherichia coli* (MTCC 40), *Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus* (MTCC 87) during the primary screening of the compounds at a concentration 1000 µg/ml (Table 3). The active compounds were further screened at a concentration of 100 µg/ml and 50 µg/ml for test and standard against the same strains. The compound 3f had marked antimicrobial activity at all concentrations against all the strains. The compound 3g had exhibited moderate activity and compounds 3a, 3d, 3e and 3j compounds exhibited less activity. The higher active compound 3f can be attributed the presence of coumarin nucleus. The other less active compounds had electron withdrawing substituent *e.g.* chloro, bromo and nitro at various positions on phenyl rings. The detail with regard to zone of inhibition (in cm) has been summarized in Table 3.

Table 2: In vitro antimicrobial activity of synthesized compounds

C. NO.	*Bacterial strains*								
	Gram -ve bacteria E.coli (MTCC 40)			*Gram +ve bacteria S. aureus (MTCC 87)*			*Gram +ve bacteria B.subtites (MTCC)*		
Conc. (µg/ml)	*1000*	*100*	*50*	*1000*	*100*	*50*	*1000*	*100*	*50*
3a	+	-	-	-	-	-	-	-	-
3b	-	-	-	-	-	-	-	-	-
3c	-	-	-	-	-	-	-	-	-
3d	+	-	-	+	-	-	+	-	-
3e	-	-	-	+	-	-	-	-	-
3f	+	+	+	+	+	+	+	+	+
3g	+	-	-	+	-	-	+	+	+
3h	-	-	-	-	-	-	-	-	-
3i	-	-	-	-	-	-	-	-	-
3j	-	-	-	-	-	-	+	-	-

(-) not active **(+)** active

Table 3: Measured zone of inhibition of bacterial strains at different concentration of synthesized compounds

C. No.	*Zone of inhibition (ZOI) of Bacterial strains (in cm)*								
	Gram -ve bacteria E.coli (MTCC 40)			*Gram +ve bacteria S. aureus (MTCC 87)*			*Gram +ve bacteria B.subtites (MTCC)*		
Conc. (µg/ml)	*1000*	*100*	*50*	*1000*	*100*	*50*	*1000*	*100*	*50*
3a	0.5	-	-	-	-	-	-	-	-
3d	0.4	-	-	0.3	-	-	0.4	-	-
3e	-	-	-	0.6	-	-	-	-	-
3f	2.1	1.6	1.5	1.9	1.9	1.6	1.7	1.5	1.8
3g	1.5	-	-	1.9	-	-	1.5	0.6	1
3j	-	-	-	-	-	-	0.9	-	-
St.*	2.5	2.3	2.2	2.6	2.5	2.4	2.8	2.5	2.9

* Ciprofloxacin

Anticancer screening using *SRB Assay*

Anticancer activities of (2,4-Dinitrophenyl)hydrazone derivative was carried out on MCF-7 cancer cell-line at Advanced Center for Treatment Research and Education in Cancer (ACTREC), Tata Memorial Centre Kharghar, Navi Mumbai. Seven of the synthesized compound *i.e.* 3a, 3d, 3e, 3f, 3g, 3i, 3j possessed good to moderate anticancer activity. The compound 3f at concentration 10 µg/ml possessed % control growth inhibition comparable to standard drug Andriamycin. The order for the % control growth inhibition of MCF-7 was found to be 3j> 3d> 3g> 3e> 3a> 3f> 3i>3h>3b>3c as shown in Table 4-8. All the seven compounds inhibited 50% of the cell growth at the conc. <10 µg/ml (Table 8, Figure 8 & 9). TGI (Concentration of drug causing total inhibition of cell growth) of compound 3f was <10 (8)

Table 4: In vitro percentage control growth of MCF-7 cell line at different concentrations of compounds (Experiment 1)

C. No.	*% Control Growth*			
	Drug Concentrations (µg/ml)			
	10	20	40	80
3a	-8.6	-7.6	-6.5	-5.8
3b	12.2	11.3	8.9	7.1
3c	32.0	26.8	22.4	18.5
3d	-15.6	-8.2	-6.1	-4.7
3e	-19.1	-15.3	-12.2	-11.3
3f	-22.4	-18.8	-16.6	-14.2
3g	-7.9	-8.7	-10.7	-17.6
3h	25.2	19.2	8.2	6.5
3i	-18.6	-25.3	-25.3	-20.6
3j	-5.4	-3.9	-2.9	-1.1
ADR	-27.2	-38.6	-49.8	-52.4

Table 5: In vitro percentage control growth of MCF-7 cell line at different concentrations of compounds (Experiment 2)

C. No.	*% Control Growth*			
	Drug Concentrations (µg/ml)			
	10	20	40	80
3a	-20.7	-16.7	-13.2	-10.2
3b	28.3	24.8	20.9	15.2
3c	29.9	25.1	22.7	17.9
3d	-18.9	-16.1	-15.9	-12.5
3e	-16.1	-14.7	-11.8	-9.2
3f	-28.3	-24.6	-20.2	-14.9
3g	-5.8	-8.0	-10.0	-11.8
3h	26.1	15.6	10.9	7.1
3i	-14.7	-24.2	-34.8	-42.9
3j	-4.1	-3.6	-2.2	-1.4
ADR	-29.9	-37.2	-45.6	-50.3

Table 6: In vitro percentage control growth of MCF-7 cell line at different concentrations of compounds (Experiment 3)

C. No.	*% Control Growth*			
	Drug Concentrations (µg/ml)			
	10	**20**	**40**	**80**
3a	-34.5	-31.6	-28.9	-19.7
3b	36.3	28.3	24.5	22.2
3c	29.3	27.1	23.6	22.1
3d	-13.3	-8.3	-7.1	6.8
3e	-29.4	-22.8	-16.4	-11.9
3f	-27.3	-26.3	-23.7	-20.2
3g	-7.7	-11.2	-12.0	-19.1
3h	19.9	18.2	9.6	6.4
3i	-17.5	-28.9	-35.9	-39.8
3j	-5.2	-4.2	-3.8	-1.9
ADR	-26.2	-36.7	-40.3	-48.3

Table 7: Average values of percentage control growth of MCF-7 cell line at different drugs concentrations

C. No.	*% Control Growth*			
	Drug Concentrations (µg/ml)			
	10	**20**	**40**	**80**
3a	-21.3	-18.6	-16.2	-11.9
3b	25.6	21.5	18.1	14.8
3c	30.4	26.3	22.9	19.5
3d	-15.9	-10.9	-9.7	-3.5
3e	-21.5	-17.6	-13.5	-10.8
3f	-26.0	-23.2	-20.1	-16.4
3g	-7.2	-5.6	-10.9	-16.2
3h	23.8	11.6	9.6	6.6
3i	-16.9	-16.5	-32.0	-34.4
3j	-4.9	-2.5	-3.0	-1.4
ADR	-27.8	-37.5	-45.2	-50.3

Table 8: Synthesized compound 3a- 3j concentrations (µg/ml) as GI_{50} calculated for MCF-7 cell line

Drug concentrations (µg/ml) calculated from graph	
MCF-7	***GI_{50}****
3a	<10
3b	>80
3c	>80
3d	<10
3e	<10
3f	<10
3g	<10
3h	>80
3i	<10
3j	<10
ADR	<10

ADR = Adriamycin, Positive control compound; GI_{50} = Concentration of drug causing 50% inhibition of cell growth. GI_{50} value of ≤ 10µg/ml is considered to demonstrate activity in case of pure compounds.

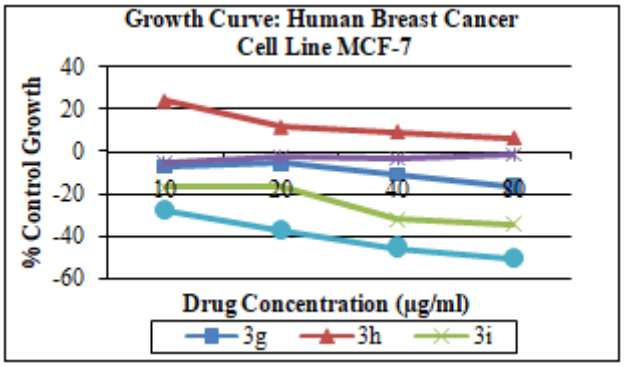


Figure 8: A graph plot of % control growth versus drug conc. of compound 3a-3f

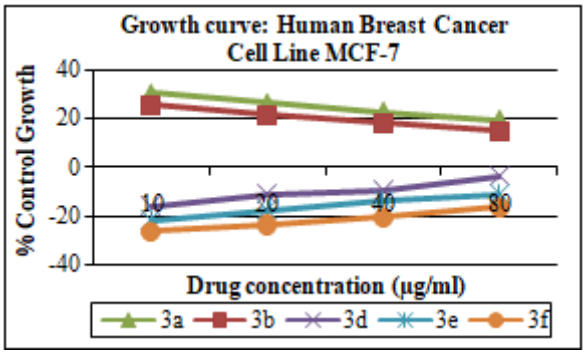


Figure 9: A graph plot of % control growth versus drug conc. of compound 3g-3j

The better activity of 3a and 3e can be attributed to the presence of electron withdrawing substituent at position 2 and 4 of the aromatic phenyl ring. Similarly the compound 3d had 4-Chloro substitution at phenyl ring. Compound 3f possess better activity due to presence of coumarin nucleus. The activity of Compounds 3i and 3j can be attributed due to presence of substitutions like 4-methoxyphenyl and 4-nitrophenyl respectively. Therefore the substituent and its position affected the activity in the order 4-methoxy>coumarin>2-bromo,4-chloro>2,4-dichloro>4-Chloro>4-Nitro>4-methyl>3-bromo.

4. Conclusion

In the present study a total of 10 (2,4-Dinitrophenyl) hydrazone derivatives were synthesized. The compounds 3f and 3g exhibited good antimicrobial activity, the compounds 3a, 3d, 3e, 3f, 3g, 3j have exhibited marked activity in both the antimicrobial as well as anticancer assays but compound 3f exhibited best results in both assay. The synthesized derivatives can be further explored for better antimicrobial and anticancer activity by studying their structure activity relationship. The compound 3f can be utilized further to develop potential antimicrobial and anticancer agents.

5. Conflict of Interest

The authors confirm that this article content has no conflicts of interest.

6. Acknowledgement

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References

[1] S. Rollas, S.G. Kucukguzel, Biological activities of hydrazone derivatives, Molecules, 12 (2007) 1910-1939.
[2] H.N. Dogan, A. Duran, S. Rollas, G. Sener, Y. Armutak, M.U. Keyer, Synthesis and structure elucidation of some new hydrazones and oxadiazolines anticonvulsant activities of 2-(3-acetyloxy-2-naphtyl)-4-acetyl-5-substituted-1,3,4-oxadiazolines, Med. Sci. Res., 26 (1998) 755-758.
[3] R. Kalsi, M. Shrimali, T.N. Bhalla, J.P. Barthwal, Synthesis and anti-inflammatory activity of indolyl azetidinones, Indian J. Pharm. Sci., 41 (2006) 353-359.
[4] R.M. Mohareb, D.H. Fleita, O.K. Sakka, Novel synthesis of hydrazide–hydrazone derivatives and their utilizaiton in the synthesis of coumarin, pyridine, thiazole and thiopehene derivativeswith antitumor activity, Molecules, 16 (2011) 16-27.
[5] L. Popiolek, A. Biernasiuk, A. Malm, Synthesis and antimicrobial activity of new 1,3-thiazolidin-4-one derivatives obtained from carboxylic acid hydrazides. Phosphorus, Sulfur, and Silicon and the Related Elements, 190 (2015) 251-260.
[6] L. Popiolek, J. Stefanska, M. Kielczykowska, I. Musik, A. Biernasiuk, A. Malm, M. Wujec, Synthesis, dissociation constants, and antimicrobial activity of novel 2,3-disubstituted-1,3-Thiazolidin-4-one derivatives, J. Heterocycl. Chem., 53 (2016) 393-402.
[7] L. Popiolek, A. Biernasiuk, A. Malm, Design, synthesis, and in vitro antimicrobial activity of new Furan/Thiophene-1,3-Benzothiazin-4-one hybrids. J. Heterocycl. Chem., 53 (2016) 479-486.
[8] D. Saini, M. Gupta, Hydrazones as potential anticancer agents: An update AJPP, 4 (2018) 116-122
[9] P. Kumar, B. Narasimhan, Hydrazides/hydrazones as antimicrobial and anticancer agents in the new millennium, Mini Rev. Med. Chem., 13 (2013) 971–987
[10] V. Singh, V.K. Srivastava, G. Palit, K. Shankar, Coumarin congeners as antidepressants, Drug Res.**,** 42 (1992) 993- 996**.**
[11] S.N. Chatterjee, S. Ghosh, Mechanism of action of furazolidone: inter-strand cross-linking in DNA & liquid holding recovery of Vibrio cholerae cells, Indian J. Biochem. Biophys., 16 (1979) 125-130.
[12] D.R. Mccalla, A. Reuvers, C. Kaiser, Mode of Action of Nitrofurazone, J. Bacteriol., 104 (1970) 1126-1134.
[13] M.J. Munoz-Davila, Role of Old Antibiotics in the Era of Antibiotic Resistance. Highlighted Nitrofurantoin for the Treatment of Lower Urinary Tract Infections, Antibiot., 3 (2014) 39-48.
[14] Morgan LR, Thangaraj K, LeBlanc B, Rodgers A, Wolford LT, Hooper CL, Fan D, Jursic BS (2003) Design, synthesis, and anticancer properties of 4,4'-dihydroxybenzophenone-2,4-dinitrophenylhydrazone and analogues. J Med Chem 46:4552–4563
[15] R. Shankar, R.K. Rawal, U.S Singh, P. Chaudhary, R. Konwar, K. Hajela, Design, synthesis and biological evaluation of hydrazone derivatives as anti-proliferative agents, Med. Chem. Res., 26 (2017) 1459-1468.
[16] A.L. Lafrate, J.R. Gunther, K.E. Carlson, J.A. Katzenellenbogen, Synthesis and biological evaluation of guanylhydrazone coactivator binding inhibitors for the estrogen receptor, Bioorg. Med. Chem., 16 (2008) 10075–10084.
[17] S. Agatonovic-Kustrin, J.V. Turner, Molecular structural characteristics of estrogen receptor modulators as determinants of estrogen receptor selectivity, Mini Rev. Med. Chem., 8 (2008) 943–951.
[18] S.R. Stauffer, C.J. Coletta, R. Tedesco, J. Sun, J.A. Katzenellenbogen, Pyrazole ligands: structure-affinity/activity relationships and estrogen receptor-alpha-selective agonists, J. Med. Chem., 43 (2000) 4934–4947.
[19] Stauffer SR, Huang YR, Aron ZD, Coletta CJ, Sun J, Katzenellenbogen BS, Katzenellenbogen JA (2001) Triarylpyrazoles with basic side chains: development of pyrazole-based estrogen receptor antagonists. Bioorg Med Chem 9:151–161.
[20] J. Sun, M.J. Meyers, B.E. Fink, R. Rajendran, J.A. Katzenellenbogen, B.S. Katzenellenbogen, Novel ligands that function as selective estrogens or antiestrogens for estrogen receptor-alpha or estrogen receptor-beta, Endocrinology, 140 (1999) 800–804.
[21] R. Kaplanek, M. Jakubek, J. Rak, Z. Kejik, M. Havlik, B. Dolensky, I. Frydrych, M. Hajduch, M. Kolar, K. Bogdanova, J. Kralova, P. Dzubak, V. Kral, Caffeine-hydrazones as anticancer agents with pronounced selectivity toward T-lymphoblastic leukaemia cells, Bioorg. Chem., 60 (2015) 19-29.
[22] M.P. Tantak, L. Klingler, L. Arun, A. Kumar, R. Sadana, D. Kumar, Design and synthesis of bis(indolyl)ketohydrazide-hydrazones: Identification of potent and selective novel tubulin inhibitors, Eur. J. Med. Chem., 136 (2017) 184-194.
[23] S Senkardes, N.K. Basu, I. Durmaz, D. Manvar, A. Basu, R. Atalay, S.G. Kucukguzel, Synthesis of Novel Diflunisal Hydrazide-Hydrazones as Anti-Hepatitis C Virus Agents and Hepatocellular Carcinoma Inhibitors, Eur. J. Med. Chem., 108 (2016) 301-308.
[24] S. Shen, H. Chen, T. Zhu, X. Ma, J. Xu, W. Zhu, R. Chen, J. Xie, T. Ma, L. Jia, Y. Wang, C. Peng, Synthesis, characterization and anticancer activities of transition metal complexes with a nicotinohydrazone ligand, Oncol. Lett., 13 (2017) 3169-3176.