

Research Article

Design and Synthesis of New Compounds Derived from Phenyl Hydrazine and Different Aldehydes as Anticancer Agents

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In this work we synthesized new derivatives from Phenyl Hydrazine and series of different Aldehydes (derivatives of benzylidenes). The synthesized compounds contain different aromatic Aldehydes which attached by Benzene ring via Hydrazine moiety. These derivatives were characterized by TLC, melting points, Infrared Red, Proton Nuclear Magnetic Resonance, Carbon Thirteen Nuclear Magnetic Resonance and Mass Spectroscopy. Finally, these synthesized derivatives were tested for antiproliferative activity against multiple normal and cancerous cell lines, HepG2 (Liver Cancer) and MCF-7 (Breast Cancer) cell lines were used for cytotoxic assay.

Keywords: Phenyl hydrazine; Aromatic aldehydes; Benzylidene synthesis; Cytotoxic assay; Anticancer; HepG2; MCF-7**Introduction**

Cancer is a public health menace. The disease is of a great concern to both developed and developing countries due to its high morbidity and mortality. In many countries, it has become second largest killer after cardiovascular disease [1]. In 2012, there were 14 million new cases and 8.2 million deaths [1]. Among men, lung cancer was the most predominant, while among women, it was breast cancer. It was reported that there were 24 million cancer cases annually and 14.6 million annual deaths by the end of 2015 [2]. These troubling figures compel policy makers and the researchers to combat this disease. Cancer is a collection of different life-threatening diseases characterized by uncontrolled growth of cells leading to invasion of surrounding tissue and often spreading to other parts of the body [3]. When it comes to understanding and controlling cancer scientists are now working from a position of strength because a foundation of knowledge about cancer has been built over the past 50 years. There is an urgent need for novel effective drug regimens for the treatment of cancer because the current chemotherapy suffers from a slim therapeutic index, with significant toxicity from effective drug doses or tumor recurrence at low drug doses. The new anticancer chemotherapeutic agents search continues to be an active area of research at many companies and research centers [3,4]. Searching for new anticancer agents having heterocyclic nucleus continues worldwide at various laboratories [5-7]. So these organic compounds synthesized and tested as anticancer drugs. Synthesized compounds have benzene ring attached to five or six membered rings (Benzimidazole) or (Phthalazine, Quinazoline, Quinoxalines). In this work we aimed the synthesis of organic compounds formed of benzene ring attached by Hydrazine moiety which is two nitrogen atoms but not fused in the ring as Phthalazines, Quinazolines, Quinoxalines or Benzimidazoles. These new compounds have two nitrogen atoms in side chain as a bridge between benzene ring and aromatic aldehydes [8-44].

Materials**Reagents**

All solvents and reagents were obtained from commercial sources and were used without further purification except Glacial Acetic acid and Petroleum Ether (PE). Phenyl Hydrazine was purchased from Sigma Aldrich (Cairo, Egypt). Series of Aromatic Aldehydes were acquired from Sigma Aldrich (Cairo, Egypt). Absolute Ethanol, Ethanol 95%, Glacial Acetic Acid, Ethyl Acetate, Petroleum Ether and Chloroform were purchased from Picochem (Cairo, Egypt). Distilled water was used for the experiments.

Instruments

Progress of chemical reactions was observed using TLC (Merck, silica gel plates 60 F254) and visualized using a UV-Vis spectrometer at 254nm. Melting points were determined by Mel-Temp apparatus. NMR spectra were performed in Chloroform(1) (7.26ppm), with trimethyl silane as an internal standard, using Bruker Avance 500 spectrometer at ambient temperature, at drug discovery unit, Faculty of Pharmacy, Ain Shams University (ASU, Cairo, Egypt). All chemical shifts were expressed in parts per million (δ), and coupling constants (J) in Hz. FTIR spectra were recorded using KBr pellets on a model 883 double beam infrared spectrophotometer Bruker in 200-4000 cm^{-1} , at drug discovery unit, Faculty of Pharmacy, Ain Shams University (ASU, Cairo, Egypt). MS spectra were recorded using a Bruker Esquire 2000 by APC or ES ionization, at drug discovery unit, Faculty of Pharmacy, Ain Shams University (ASU, Cairo, Egypt).

Cell culture: HepG2, MCF-7

Cell line was obtained from Nawah Scientific Inc., (Mokatam, Cairo, Egypt). Cells were maintained in DMEM media supplemented with 100mg/mL of streptomycin, 100units/mL of penicillin and 10% of heat-inactivated fetal bovine serum in humidified, 5% (v/v) CO₂ atmosphere at 37°C [45,46].

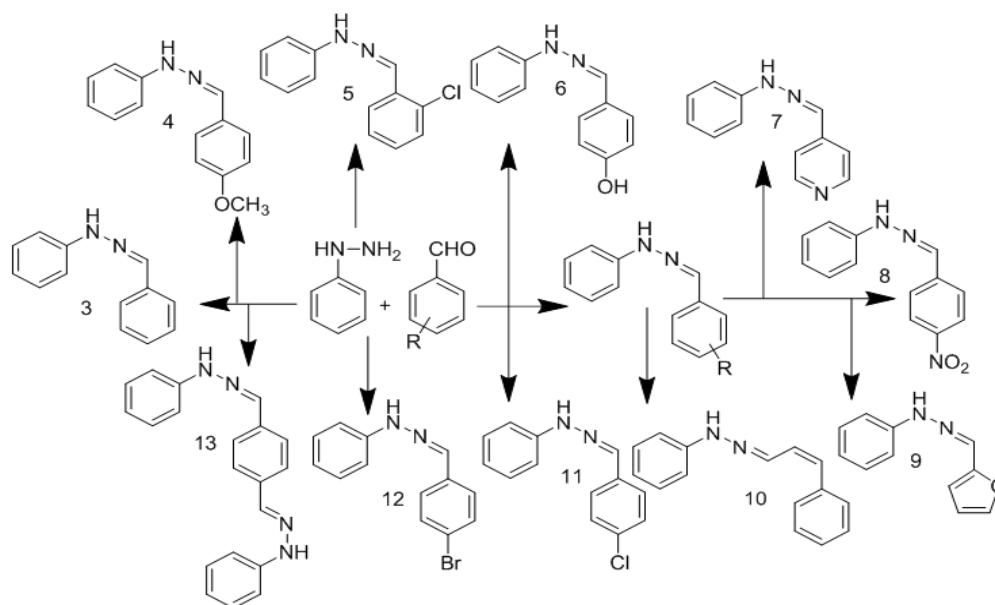


Figure 1: Reagents and conditions: (i) Series of Aromatic Aldehydes, Refluxing Glacial Acetic Acid, 135°C, 1-16h.

Cytotoxicity assay: Hep G2, MCF-7

Cell viability was assessed by SRB assay. Aliquots of 100 μ L cell suspension (5×10^3 cells) were in 96-well plates and incubated in complete media for 24h. Cells were treated with another aliquot of 100 μ L media containing drugs at various concentrations. After 72h of drug exposure, cells were fixed by replacing media with 150 μ L of 10% TCA and incubated at 4°C for 1h. The TCA solution was removed, and the cells were washed 5 times with distilled water. Aliquots of 70 μ L SRB solution (0.4%w/v) were added and incubated in a dark place at room temperature for 10min. Plates were washed 3 times with 1% acetic acid and allowed to air-dry overnight. Then, 150 μ L of TRIS (10mM) was added to dissolve protein-bound SRB stain; the absorbance was measured at 540nm using a BMG LABTECH-FLUOstar Omega microplate reader (Ortenberg, Germany) [45,46].

Chemistry and Scheme

Scheme

See Figure 1.

Procedure and synthesis of Compounds 3-13

Compound 3: (E)-1-benzylidene-2-phenylhydrazine: Equimolar mixture of Phenyl Hydrazine (20ml, 22gm, 0.202mole) and Benzaldehyde (20.5ml, 21.53gm, 0.202mole) are stirred together in refluxing Glacial Acetic acid for 1hour, TLC was made to ensure the completion of the reaction by system 2:1 Petroleum Ether: Ethyl Acetate. Precipitate was obtained from organic layer then water was added and more precipitate was retrieved. Product was purified by crystallization in Absolute Ethanol. The desired product was obtained in good yield 70%. m.p = 154-156 °C.

IR: 688.75, 747.51 cm^{-1} (aromatic, bending), 880.40 cm^{-1} (N-H, overtone), 1064.45 cm^{-1} (C-N), 1518 cm^{-1} (N-H, bending), 1590 cm^{-1} (C=C, aromatic), 2450 cm^{-1} (aromatic, overtone), 3090 cm^{-1} (C-H, aromatic) and 3300 cm^{-1} (N-H, stretching).

^1H NMR (400MHz, CDCl_3): δ 6.90-7.50ppm (m, aromatic protons), 7.65ppm (s, -CH-) and 10.3ppm (s,-NH-).

^{13}C NMR (100MHz, CDCl_3): δ C1 (144.5ppm), C2 (117ppm), C3 (114ppm), C4 (137ppm), C5 (114ppm), C6 (117ppm), C7 (146ppm), C1 (147.5ppm), C2 (115ppm), C3 (130ppm), C4 (125ppm), C5 (130ppm) and C6(115 ppm).

Compound 4: (E)-1-(4-methoxybenzylidene)-2-phenylhydrazine: Equimolar mixture of Phenyl Hydrazine (4ml, 4.47gm, 0.042mole) and 4-Methoxybenzaldehyde (5ml, 5.6gm, 0.042mole) are stirred together in refluxing Glacial Acetic acid for 1 and half hours, TLC was made to ensure the completion of the reaction by system 2:1 Petroleum Ether: Ethyl Acetate. Precipitate was obtained from organic layer then water was added and more precipitate was retrieved. Product was purified by crystallization in Absolute Ethanol. The desired product was obtained in good yield 82.5%. m.p = 128-130 °C.

^1H NMR (400MHz, CDCl_3): δ 3.86ppm (s,-CH₃-), 6.85-7.35ppm (m, aromatic protons), 7.65ppm (s,-CH-) and 9.9ppm (s,-NH-).

^{13}C NMR(100MHz, CDCl_3): δ C1 (54.3ppm), C2 (158.9ppm), C3 (113.6ppm), C4 (129.8ppm), C5 (124.8ppm), C6 (129.8), C7 (113.6ppm), C8 (143.8ppm), C1 (145.2ppm), C2 (112.2ppm), C3 (129.5ppm), C4 (128.8ppm), C5 (129.5ppm) and C6 (112.2ppm).

Compound 5: (E)-1-(2-chlorobenzylidene)-2-phenylhydrazine: Equimolar mixture of Phenyl Hydrazine (4.4ml, 4.8gm, 0.044mole) and 2-Chlorobenzaldehyde (5ml, 6.24gm, 0.044mole) are stirred together in refluxing Glacial Acetic acid for 15 hours, TLC was made to ensure the completion of the reaction by system 2:1 Petroleum Ether: Ethyl Acetate. Precipitate was obtained from organic layer then water was added and more precipitate was retrieved. Product was purified by crystallization in Absolute Ethanol. The desired product was obtained in good yield 73%. m.p = 129-131 °C.

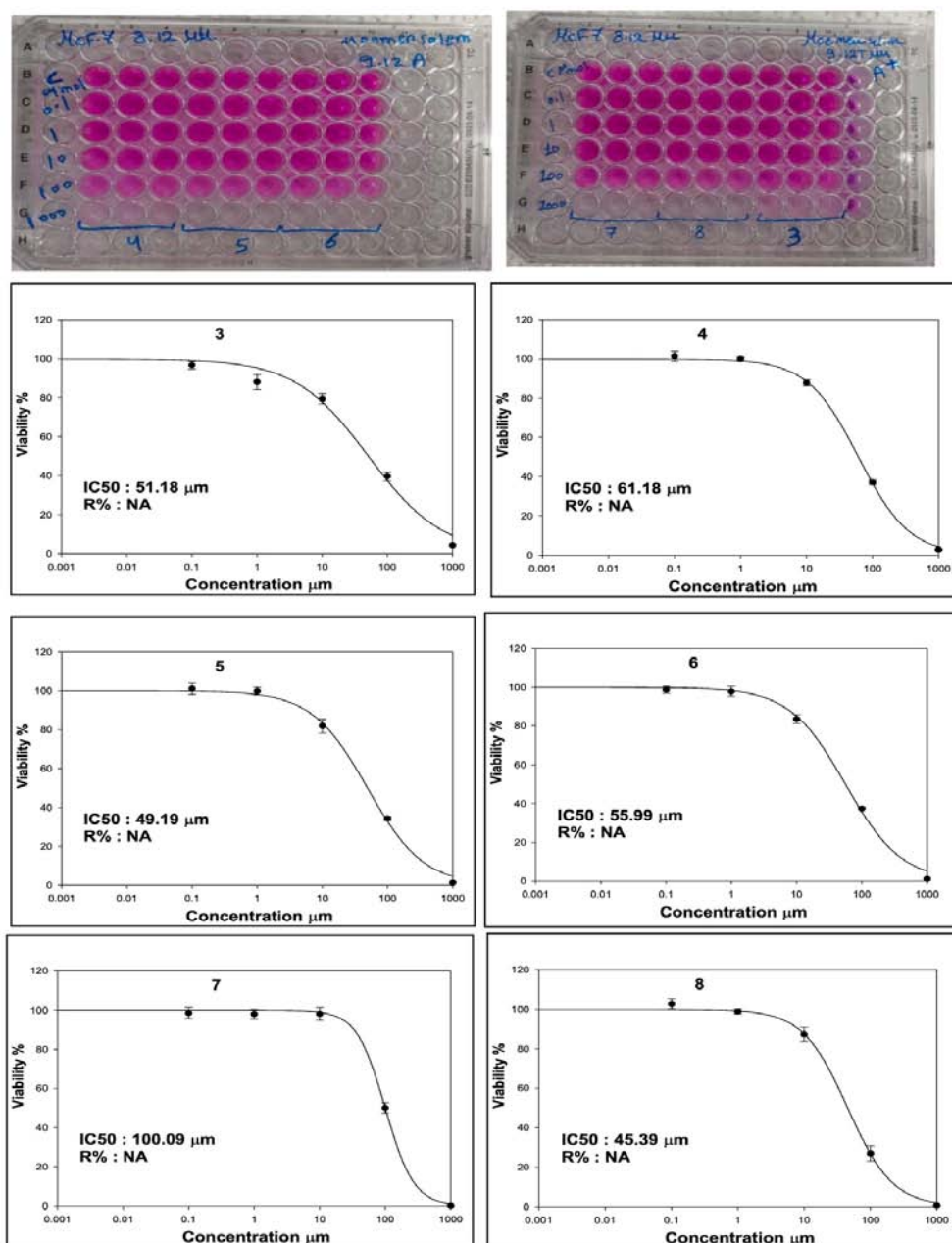


Figure 2: IC₅₀ Value results of compounds 3-8 against MCF-7 Cell line.

¹HNMR (400MHz, CDCl₃): δ6.75-7.75ppm (m, aromatic protons), 7.85ppm (s, -CH-) and 10.5ppm (s, -NH-).

MS Spectroscopy: m/z: 230.06 (100.0%), (M+1) 231.05 (87.9%), (M+2) 229.05 (12.1%).

Compound 6: 4-((2-phenylhydrazono) methyl)phenol: Equimolar mixture of Phenyl Hydrazine (4ml, 4.43gm, 0.041mole) and 4-Hydroxybenzaldehyde (5gm, 0.041mole) are stirred together in refluxing Glacial Acetic acid for 1 hour, TLC was made to ensure the completion of the reaction by system 2:1 Petroleum Ether: Ethyl Acetate. Precipitate was obtained from organic layer then water was added and more precipitate was retrieved. Product was purified by

crystallization in Absolute Ethanol. The desired product was obtained in good yield 86 %. m.p = 178-181 °C.

IR: 690.59, 743.83cm⁻¹ (aromatic, bending), 884.73cm⁻¹ (N-H, overtone), 1098.33cm⁻¹ (C-N), 1504cm⁻¹ (N-H, bending), 1596.49cm⁻¹ (C=C, aromatic), 1700cm⁻¹ (C=N), 3045cm⁻¹ (C-H, aromatic), 3290cm⁻¹ (N-H, stretching) and 2900-3625cm⁻¹ (OH).

¹HNMR(400MHz, CDCl₃): δ6.85-7.55ppm (m, aromatic protons), 7.7ppm (s, -CH-), 7.85ppm (s, -OH) and 9.88ppm (s, -NH-).

¹³CNMR(100 MHz, CDCl₃): δC1 (158.82ppm), C2 (117.56ppm), C3 (130.8ppm), C4 (125.4ppm), C5 (130.8ppm), C6 (117.56), C7 (140.7ppm), C1 (146.22ppm), C2 (113.9ppm), C3 (129.5ppm), C4

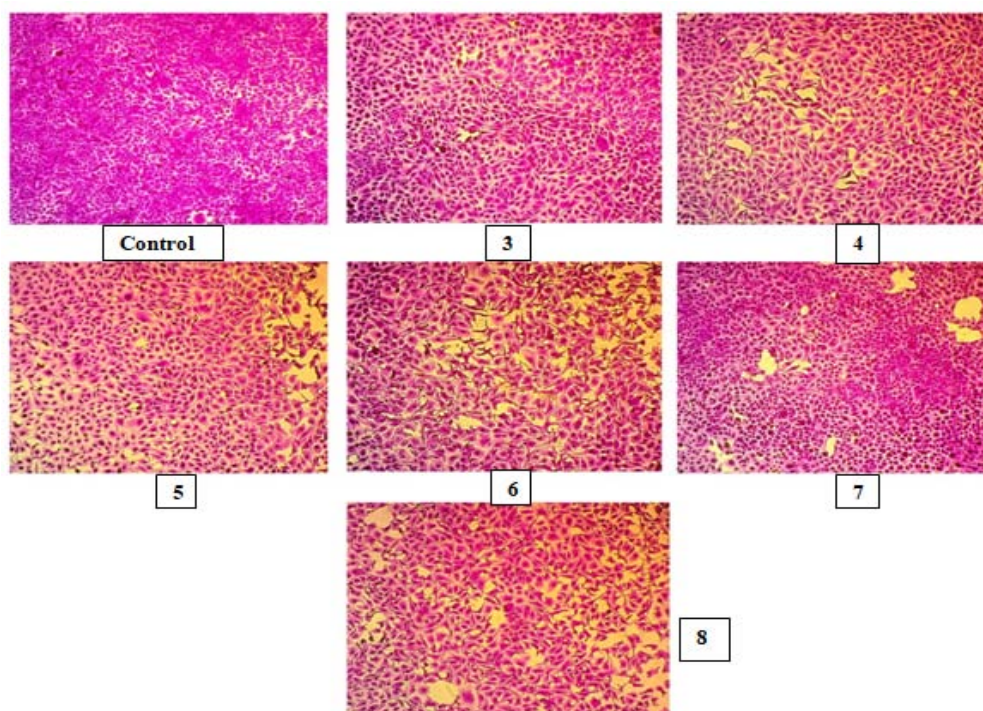


Figure 3: MCF-7 cell lines under microscopic examination of control and compounds (3-8) at 100 μ m concentration.

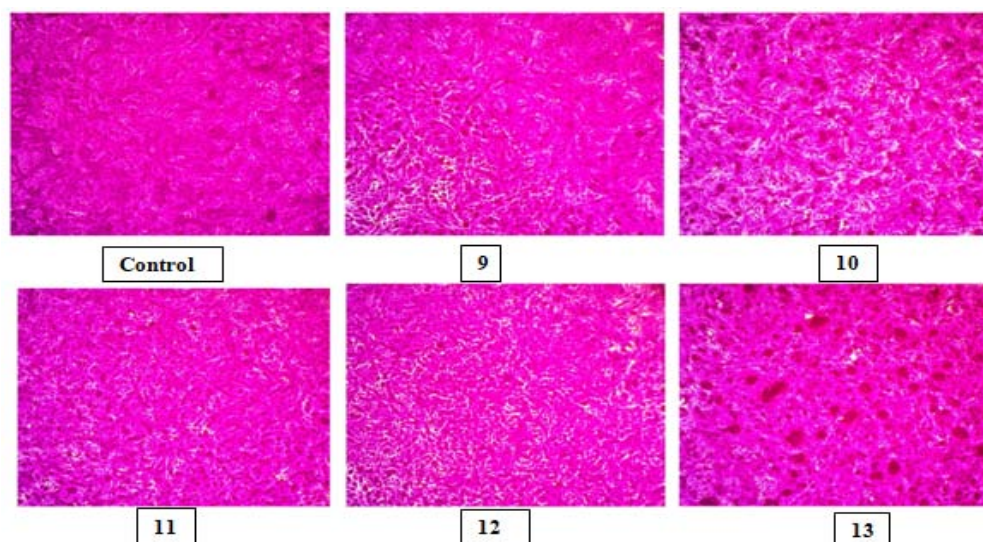


Figure 4: HepG2 cell lines under microscopic examination of control and compounds (9-13) at 100 μ m concentration.

(122.8ppm), C5 (129.5ppm) and C6 (113.9ppm).

Compound 7: 4-((2-phenylhydrazono) methyl)pyridine:

Equimolar mixture of Phenyl Hydrazine (10ml, 11gm, 0.102mole) and 4-Pyridinecarldehyde (9.6ml, 10.88gm, 0.102mole) are stirred together in refluxing Ethanol for 1hour, TLC was made to ensure the completion of the reaction by system 2:1 Petroleum Ether: Ethyl Acetate. Precipitate was obtained from organic layer then water was added and more precipitate was retrieved.

Product was purified by crystallization in Absolute Ethanol. The desired product was obtained in good yield 73%. m.p = 179-181 °C.

^1H NMR (400MHz, CDCl_3): δ 6.90-8.55ppm (m, aromatic protons), 7.60ppm (s, -CH-) and 8.15 (s, -NH-).

^{13}C NMR (100MHz, CDCl_3): δ C2 (149.98ppm), C3 (120.13ppm), C4 (143.47ppm), C5 (120.13ppm), C6 (149.98ppm), C7 (142.84ppm), C1 (133.55ppm), C2 (113.09ppm), C3 (129.42ppm), C4 (121.13ppm), C5 (129.42ppm) and C6 (113.09ppm).

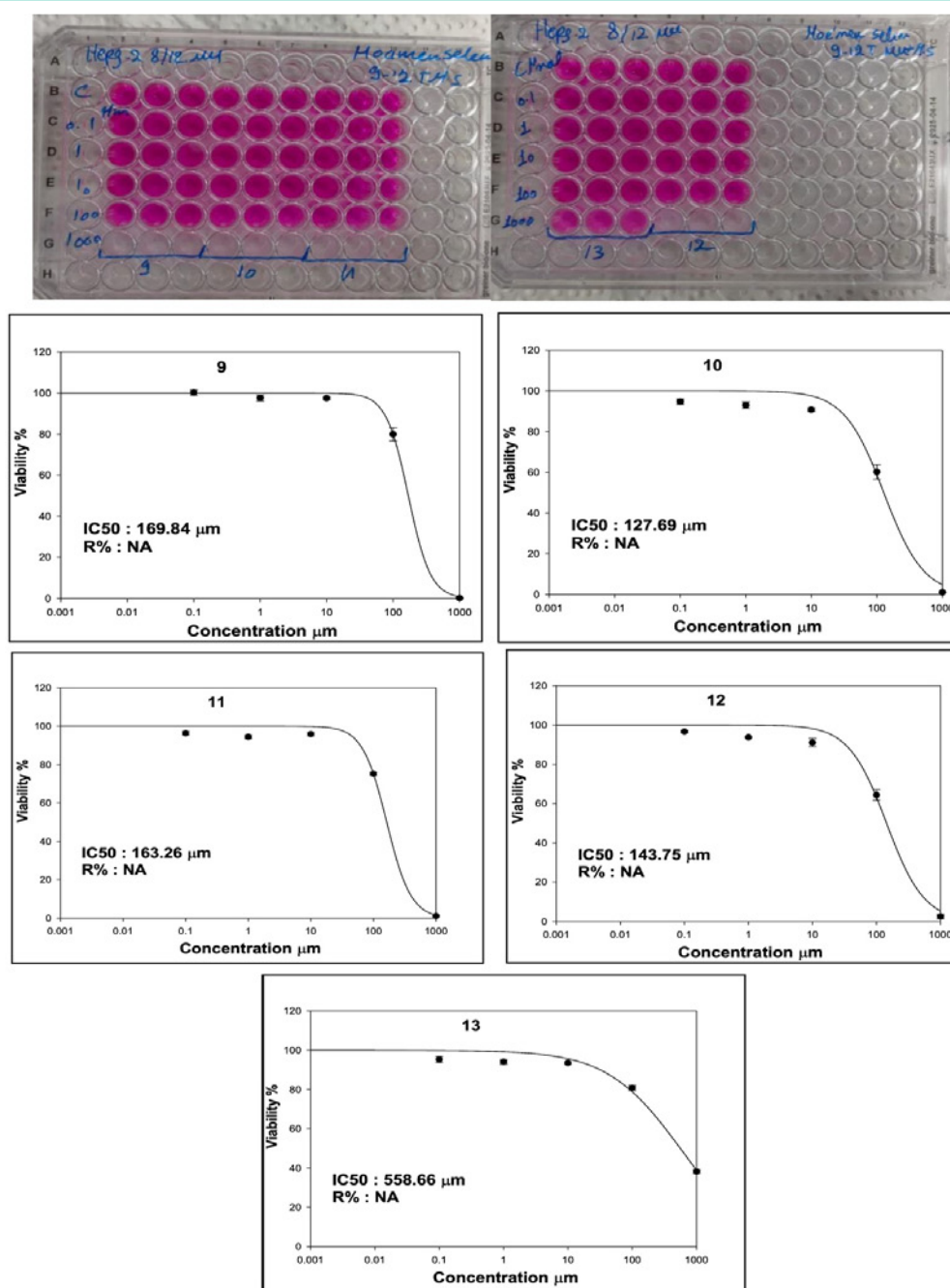


Figure 5: IC₅₀ Value results of compounds 9-13 against HepG2 Cell line.

Compound 8: (E)-1-(4-nitrobenzylidene)-2-phenylhydrazine: Equal mixture of Phenyl Hydrazine (1ml, 1.016gm, 0.009mole) and 4-Nitrobenzaldehyde (1.42gm, 0.009mole) are stirred together in refluxing Glacial Acetic acid for 6hours, TLC was made to ensure the completion of the reaction by system 2:1 Petroleum Ether: Ethyl Acetate. No precipitate was obtained from acetic acid layer then equal amounts of water and ethanol 95% were added to obtain the product. Product was purified by crystallization in Absolute Ethanol. The desired product was obtained in relatively low yield 32.2%. m.p = 110-112 °C.

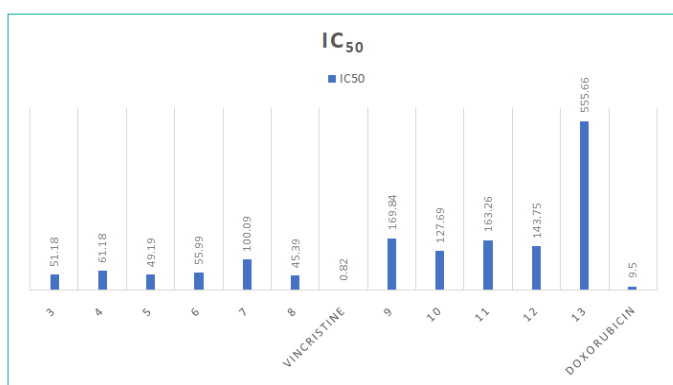
¹HNMR (400MHz, CDCl₃): δ6.80-7.40ppm (m, aromatic protons), 7.55ppm (s, -CH-) and 9.88ppm (s, -NH-).

¹³CNMR (100 MHz, CDCl₃): δC1 (147.18ppm), C2 (119.06ppm), C3 (119.84ppm), C4 (144.93ppm), C5 (119.84ppm), C6 (119.06ppm), C7 (137.29ppm), C1 (145.85ppm), C2 (111.66ppm), C3 (129.28ppm), C4 (112.71ppm), C5 (129.28ppm) and C6 (111.66ppm).

Compound 9: (E)-1-(furan-2-ylmethylene)-2-phenylhydrazine: Equal amounts of Phenyl Hydrazine (5ml, 5.5gm, 0.05mole) and Furan-2-carbaldehyde (4.2ml, 4.88gm, 0.05mole) are stirred together in refluxing Glacial Acetic acid for 10hours, TLC was made to

Table 1: Summary of the cytotoxic assay results of all compounds in two different cell lines.

Compound	IC ₅₀	Cell Line Type	Standard Drug	IC ₅₀
3	51.18	MCF-7	Vincristine	0.82
4	61.18	MCF-7	Vincristine	0.82
5	49.19	MCF-7	Vincristine	0.82
6	55.99	MCF-7	Vincristine	0.82
7	100.09	MCF-7	Vincristine	0.82
8	45.39	MCF-7	Vincristine	0.82
9	169.84	HepG2	Doxorubicin	9.5
10	127.69	HepG2	Doxorubicin	9.5
11	163.26	HepG2	Doxorubicin	9.5
12	143.75	HepG2	Doxorubicin	9.5
13	555.66	HepG2	Doxorubicin	9.5



monitor the reaction by system 2:1 Petroleum Ether: Ethyl Acetate. No precipitate was obtained from acetic acid layer. Water was added to obtain the product. Product was purified by crystallization in Absolute Ethanol. The desired product was obtained in average yield 65%. m.p = 113-115 °C.

IR: 692.95, 743.06cm⁻¹ (aromatic, bending), 818.48cm⁻¹ (N-H, overtone), 1153.57cm⁻¹ (C-N), 1342.30cm⁻¹(C-O), 1602.35 cm⁻¹(C=C, aromatic), 1604cm⁻¹(N-H, bending), 1655cm⁻¹ (C=N), 2025cm⁻¹ (C-H, aromatic overtone), 3090cm⁻¹ (C-H, aromatic) and 3317.56cm⁻¹ (N-H, stretching).

¹HNMR (400MHz, CDCl₃): δ6.85-7.55ppm (m, aromatic protons), 7.60ppm (s, -CH-) and 9.75ppm (s, -NH-).

¹³CNMR (100MHz, CDCl₃): δC2 (144.36ppm), C3 (112.89ppm), C4 (120.46ppm), C5 (150.55ppm), C6 (142.72ppm), C1 (143ppm), C2 (112.96ppm), C3 (129.31ppm), C4 (127.83ppm), C5 (129.31ppm) and C6 (112.96ppm).

Compound 10: (E)-1-phenyl-2-((E)-3-phenylallylidene)hydrazine: Equimolar mixture of Phenyl Hydrazine (5ml, 5.5gm, 0.05mole) and Cinnamaldehyde (6.4ml, 6.72gm, 0.05mole) are stirred together in refluxing Glacial Acetic acid for 1 hours, TLC was made to ensure the completion of the reaction by system 2:1 Petroleum Ether: Ethyl Acetate. Precipitate was obtained from acetic acid layer; water was added to obtain more of the product. Product was purified by crystallization in Absolute Ethanol. The desired product was obtained in high yield 80.5%. m.p = 150-152 °C.

¹HNMR (400 MHz, CDCl₃): δ6.75ppm (t, -CH-), 7.05ppm (d, -CH-), 6.85-7.50ppm (m, aromatic protons), 7.55ppm (s, -CH-) and 9.75ppm (s, -NH-).

¹³CNMR (100 MHz, CDCl₃): δC1 (132.5ppm), C2 (130ppm), C3 (127ppm), C4 (125ppm), C5 (127ppm), C6 (130ppm), C7 (134ppm), C8 (123ppm), C9 (140ppm), C1 (145ppm), C2 (118ppm), C3 (129ppm), C4 (122ppm), C5 (129ppm) and C6 (118ppm).

Compound 11: (E)-1-(4-chlorobenzylidene)-2-phenylhydrazine: Equimolar mixture of Phenyl Hydrazine (0.85ml, 0.92gm, 0.0085mole) and 4-Chlorobenzaldehyde (1.2gm, 0.0085mole) are stirred together in refluxing Glacial Acetic acid for 5 hours, TLC was made to ensure the completion of the reaction by system 2:1 Petroleum Ether: Ethyl Acetate. Precipitate was obtained from acetic acid layer; water was added to obtain more of the product. Product was purified by crystallization in Absolute Ethanol. The desired product was obtained in high yield 80.1%. m.p = 119-121 °C.

IR: 691.09, 746.28cm⁻¹ (mono-sub.), 819.32 cm⁻¹ (para-di-sub.) (aromatic, bending), 882.19 cm⁻¹ (N-H, overtone), 1133.08 cm⁻¹ (C-N), 1518.02 cm⁻¹ (N-H, bending), 1598.38 cm⁻¹ (C=C, aromatic), 1620.02cm⁻¹ (C=N), 2000 cm⁻¹ (C=C, aromatic), 3000 cm⁻¹ (C-H, aromatic) and 3310.61cm⁻¹ (N-H, stretching).

¹HNMR (400 MHz, CDCl₃): δ6.95-7.50ppm (m, aromatic protons), 7.90ppm (s, -CH-) and 10.10ppm (s, -NH-).

¹³CNMR (100MHz, CDCl₃): δC1 (134.5ppm), C2 (130.2ppm), C3 (132.3ppm), C4 (136.9ppm), C5 (132.3 ppm), C6 (130.2ppm), C7 (140.5ppm), C1 (144.8ppm), C2 (112ppm), C3 (129.7ppm), C4 (122.9ppm), C5 (129ppm) and C6 (112ppm).

Mass Spectroscopy: m/z: 230.06 (100.0%), (M+1) 231.10 (63.7%), (M+2) 229.05 (36.3%).

Compound 12: (E)-1-(4-bromobenzylidene)-2-phenylhydrazine: Equimolar mixture of Phenyl Hydrazine (0.28ml, 0.3gm, 0.0028mole) and 4-Bromobenzaldehyde (0.52gm, 0.0028mole) are stirred together in refluxing Glacial Acetic acid for 7 hours, TLC was made to ensure the completion of the reaction by system 2:1 Petroleum Ether: Ethyl Acetate. No precipitate was obtained from the organic layer, water was added to quench the reaction and from which the product was obtained. Product was purified by crystallization in Absolute Ethanol. The desired product was obtained in relatively high yield 71%. m.p = 115-117 °C.

¹HNMR (400 MHz, CDCl₃): δ7.0-7.60ppm (m, aromatic protons), 7.98ppm (s, -CH-) and 9.85ppm (s, -NH-).

¹³CNMR (100 MHz, CDCl₃): δC1 (129.3ppm), C2 (133.3ppm), C3 (131.5ppm), C4 (136.7ppm), C5 (131.5 ppm), C6 (133.3ppm), C7 (142.8ppm), C1 (145.6ppm), C2 (113.8ppm), C3 (128ppm), C4 (121.4ppm), C5 (128ppm) and C6 (113.8ppm).

Mass Spectroscopy: m/z: 276 (100.0%), (M+1) 278.95 (70 %), (M+2) 280.95 (30%).

Compound 13: 1,4-bis((2-phenylhydrazono) methyl)benzene: Equimolar mixture of Phenyl Hydrazine (2.93ml, 3.22gm, 0.029mole) and Terephthalaldehyde (4gm, 0.029mole) are stirred together in refluxing Glacial Acetic acid for 1hour, TLC was made to ensure the completion of the reaction by system 2:1 Petroleum Ether: Ethyl

Acetate. Precipitate was obtained from the organic layer, later on, water was added to quench the reaction and from which the product was obtained. Product was purified by crystallization in Absolute Ethanol. The desired product was obtained in average yield 62%. m.p = 220-22°C.

IR: 690.53, 743.71cm⁻¹ (aromatic, bending), 885.30cm⁻¹ (N-H, overtone), 1130.68cm⁻¹ (C-N), 1522.08cm⁻¹ (N-H, bending), 1588.48cm⁻¹(C=C, aromatic), 1600.36cm⁻¹ (C=N), 1925.25cm⁻¹ (C-H, aromatic overtone), 3075.25cm⁻¹ (C-H, aromatic) and 3299.42cm⁻¹ (N-H, stretching).

¹HNMR (400MHz, CDCl₃): δ6.95-7.90ppm (m, aromatic protons), 7.75ppm (s, -CH-), 10.03ppm (s,-NH-).

¹³CNMR (100MHz, CDCl₃): δC1 (145ppm), C2 (115ppm), C3 (130ppm), C4 (122ppm), C5 (130ppm), C6 (115ppm), C7 (140ppm), C8 (136ppm), C9 (129ppm), C10 (129ppm), C11 (136ppm), C12 (129ppm), C11 (136ppm), C12 (129ppm), C13 (129ppm), C14 (140ppm), C15 (145ppm), C16 (115ppm), C17 (130ppm), C18 (122ppm), C19 (130ppm) and C20 (115ppm).

Results

Cytotoxicity results of MCF-7

See Figure 2, 3 and 4.

Cytotoxicity results of HepG2

See Figure 5 and Table 1.

Conclusion

From the above findings, we concluded that all tested compounds have potential antiproliferative activity on both cell lines which were tested. For MCF-7 cell line, compound 8 was found to be the most potent compound in the group scoring 45.39µm IC₅₀, compound 7 was the lowest in potency scoring 100.09µm IC₅₀. For HepG2 cell line, compound 10 was found to be the most potent compound among the other compounds scoring 127.69µm IC₅₀ and compound 13 was the lowest in potency in this group.

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