# Synthesis, Characterization and Cytotoxic Effect of Some New Thiazolyl Hydrazone Derivatives of 1-Indanone

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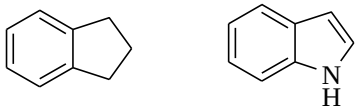
**Summary:** In the present study, a series of twelve thiazolyl hydrazone derivatives of 1-indanone was synthesized and characterized by various spectroscopic techniques such as UV-Visible, NMR, IR and Mass Spectrometry. All the synthesized target compounds were subjected to MTT assay for cytotoxicity screening and evaluation of their anti-cancer activity on various cell lines of human cancer including glioblastoma (SNB-19), prostate cancer (PC-3), Lung cancer (NCI-H460), human ovarian carcinoma (SK-OV-3 and IGROV-1), human leukemia (K-562) and human colon cancer(HCT116).Three synthesized compounds showed promising anti-cancer activity against the colon cancer cell HCT 116 cells with $IC_{50}$ ranging from 1.25±0.02 to 5.04±0.2 µM. On the other hand all the compounds didn't show cytotoxic activity against other forms of human cancer cells.

Keywords: Thiazolyl hydrazone, Derivatives, 1- indanone, Cytotoxic activity, Spectroscopy.

## Introduction

Cancer is one of the most threatening disease in the world. Despite of enormous research in the biomedical sciences, which have resulted in higher cure rates for various forms of tumors, cancer remains the second leading health threat after heart disorders in developing as well as developed countries. Although many advances have been made in this area of research, new and more efficient anti-cancer agents are needed to cope with various malignancies[2].

As a part of our efforts to find new chemotherapeutic as potential anti-cancer agent, we have synthesized a series of indanone derivatives. Indanone derivatives represent many important classes of medicinal agents in medicinal chemistry with various bioactivities[3, 4] including anti-cancer activity[5-7]. Moreover, indane nucleus is an isostere of indole ring[7] (Fig. 1), which along with its various derivatives have already been established as an important nucleus for various purposes including cytotoxic[1, 8], anti-inflammatory, analgesic and antiplatelet activities[7, 9-13].




Indane and indole ring systems

Fig. 1: Isosteres structures of Indane and Indole ring

Derivatives comprising the thiazole heterocycle exhibited a broad range of biological activities among the wide range of compounds tested as potential anti-cancer agents and also found in several potent biologically active molecules, such as sulfathiazole (antimicrobial drug), ritonavir

(Antiretroviral drug), Abafungin (antifungal drug) and Tiazofurin (antineoplastic drug). So far, introducing the thiazole ring have proven highly effective with improved potency inmany synthetic derivatives with lesser toxicity[14][14, 15]. It has also been reported that some thiazolyl hydrazones have remarkable antiproliferative effect on a variety of cells[16].

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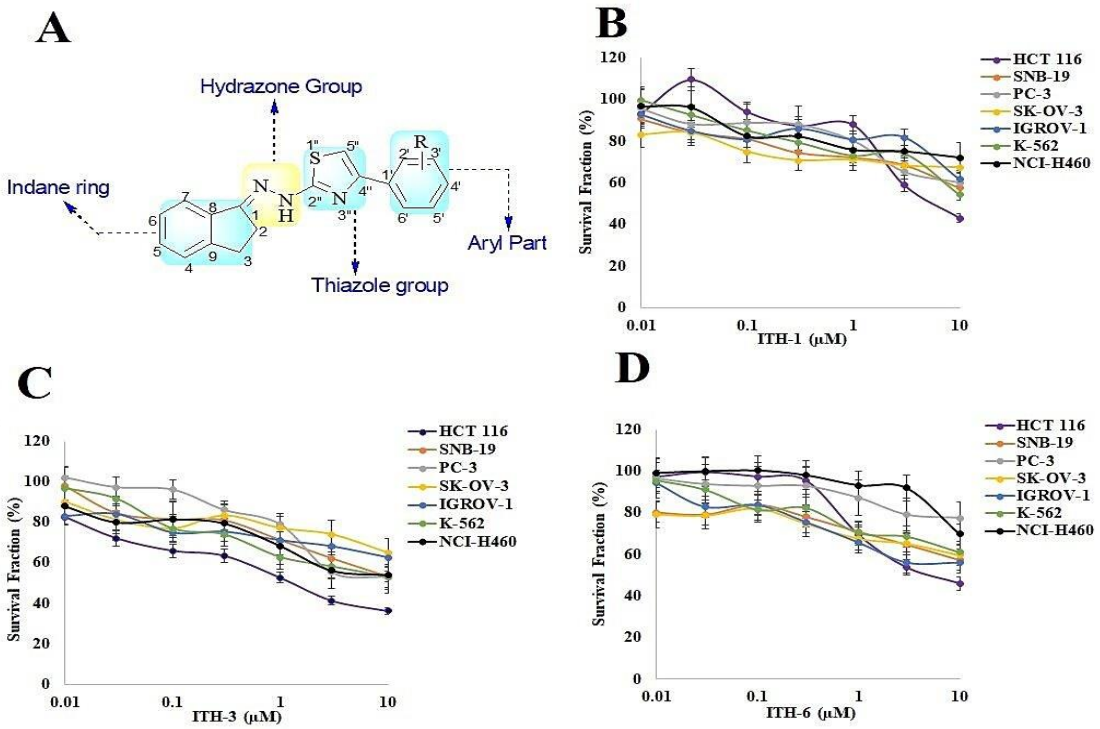



Fig. 2: (A). General structure of synthetic thiazolyl hydrazone derivatives of 1-indanone.
Cytotoxicity of ITH-1(B), ITH-3(C) and ITH-6(D) on HCT 116, SNB-19, PC-3, SK-OV-3, IGROV-1, K-562 and NCI-H460 cell lines. Survival fraction (%) was measured after treatment with ITH-1, ITH-3 and ITH-6 (μM) for 72 h on HCT 116 (violet), SNB-19 (red), PC-3 (grey), SK-OV-3 (yellow), IGROV-1 (blue), K-562 (green) and NCI-H460 (black) cell lines. Points with error bars represent the mean ± SD for independent determinations in triplicate. The figures are representative of three independent experiments.

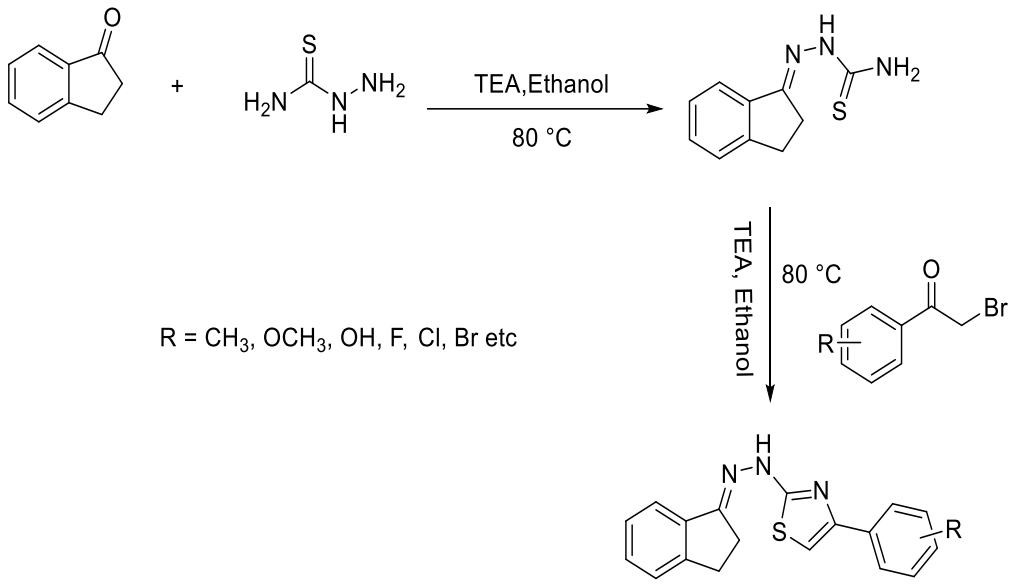
In view of these facts, it was rationalized to synthesize and investigate the cytotoxic activity of some new derivatives comprising the indane and the derived aryl substituted thiazolyl hydrazone pharmacophores (Fig. 2A). Aryl substitution on the thiazole moiety with varying substituents was considered as an interesting structure variation in order to study the influence of such modification on the anticipated cytotoxic activity. The substitution pattern of aryl part was selected so as to confer different electronic environment that would affect the lipophilicity, and hence the activity of the target molecules. The objective of forming these derivatives is to obtain some new active antitumor agents with promising activity and selectivity toward cancerous cell.

*Materials and methods*

All reagents and solvents were used as supplied by the supplier or recrystallized / redistilled as necessary. Melting points were taken on a Fisher-Johns melting point apparatus and are uncorrected. Infrared spectra (KBr discs) were run on a Shimadzu 8400 or a Shimadzu Prestige-21 FT-IR spectrometer. The $^1$H-NMR spectra were recorded in DMSO-$d_6$on Bruker (Rhenis-tetten-Forchheim, Germany) AM 300 spectrometers operating at 300 and 400 MHz, using TMS as an internal standard. $^1$H chemical shifts are reported in (ppm) and coupling constants in Hz. The electron impact mass spectra (EI MS) were determined with a Finnigan MAT-312 and a JEOL MSRoute mass spectrometer. The progress of the reaction and purity of the products were checked on TLC plates coated with Merck silica gel 60 GF254and the spots were visualized under ultraviolet light at 254 and 366 nm and / or spraying with iodine vapors.

*Synthesis*

Thiazolyl hydrazones were synthesized by taking 1 mmol of 1-indanone with thiosemicarbazide 1 mmol in the presence of catalytic amounts of acetic acid in 10 mL of ethanol as solvent to obtain intermediate thiosemicarbazone. The thiosemicarbazone (1 mmol) was reacted with differently substituted phenacyl bromide (1 mmol) in the same solvent (Scheme-1)[20]. All synthetic products were purified by washing with petroleum ether, hexane, and diethyl ether.




Scheme-1: Synthetic route for the synthesis of thiosemicarbazone and thiazolyl hydrazone of 1-indanone.

To the best of our knowledge only compounds ITH- **4, 7, 12**[21]**, 1** (CAS reg. no: 887352-02-5), **6** (CAS#887351-99-7) were previously reported while remaining analogs are new.

The synthesized compounds were characterized as under:

*N-Indan-1-ylidene-N'-[4-(4-methoxy-phenyl)-thiazol-2-yl]-hydrazine (ITH-1)*

Yield: 93%, mp 153-154 °C; IR (KBr) $cm^{-1}$:3337 (NH), 1558 (C=N), 1122 (C-S); $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.02 (s, 1H, NH), 7.80 (d, 2H, $J$ = 8.4 Hz ,H-2′, H-6′), 7.61 (d, 1H, $J$ = 7.2 Hz, H-7), 7.36 (overlapping multiplet, 2H, H-4, H-5), 7.32 (ovp, 1H, H-6), 7.12 (s, 1H, H-5″), 6.96 (d, 2H, $J$ = 8.8 Hz, H-3′, H-5′), 3.77 (s, 3H,-$OCH_3$), 3.10 (t, 2H, $J$ = 6.8 Hz, 3-$CH_2$), 2.89 (t, 2H, $J$ = 6.4 Hz, 2-$CH_2$); $^{13}$C-NMR (100 MHz, DMSO-$d_6$): $\delta$ 169.3, 158.6,147.7, 137.7, 129.8, 126.9, 126.7, 125.6, 120.5, 113.9, 101.5, 55.0, 28.1, 27.4; EI-Mass *m/z*: 335.1 $[M]^+$; HREI-MS: *m/z* Calcd for $C_{19}H_{17}N_3OS$: 335.1092; found 335.1098; λmax = 272 nm.

*N-Indan-1-ylidene-N'-[4-(3, 5-Dichloro-phenyl)-thiazol-2-yl]-hydrazine (ITH-2)*

Yield: 91%, mp 176-177 °C; IR (KBr) $cm^{-1}$:3329 (NH), 1558 (C=N), 1118 (C-S); $^1$H-NMR (300 MHz, DMSO-$d_6$): $\delta$ 11.12 (s, 1H, NH), 8.10 (d, 1H, $J$ = 1.8 Hz, H-7), 7.86 (dd, 1H, $J$ = 2.1 Hz, H-4′), 7.67 (overlapping multiplet, 2H, H-2′, H-6′), 7.52 (s, 1H, H-5″), 7.37 (overlapping multiplet, 3H, H-4, H-5, H-6), 3.10 (t, 2H, $J$ = 6.6 Hz, 3-$CH_2$), 2.89 (t, 2H, $J$ = 6.6 Hz, 2-$CH_2$); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): $\delta$ 169.6, 156.3, 147.9, 147.8, 137.6, 135.4, 130.7, 129.9, 127.1, 126.9, 125.6, 125.4, 120.6, 105.9, 28.1, 27.4; EI-Mass *m/z*: 373.1$[M]^+$; HREI-MS: *m/z* Calcd for $C_{18}H_{13}Cl_2N_3S$: 373.0207; found 373.0179; λmax = 261 nm.

*N-Indan-1-ylidene-N'-[4-(4-Bromo-phenyl)-thiazol-2-yl]-hydrazine (ITH-3)*

Yield: 93%, mp 184-185 °C; IR (KBr) $cm^{-1}$: 3315 (NH), 1558 (C=N), 1128 (C-S); $^1$H-NMR (300 MHz, DMSO-$d_6$): $\delta$ 11.10 (s, 1H, NH), 7.83 (d, 2H, $J$ = 8.7 Hz, H-7, H-4), 7.62 (overlapping multiplet, 3H, H-6, H-5, H-5″), 7.38 (overlapping multiplet, 4H, H-2′, H-3′, H-5′, H-6′), 3.10 (t, 2H, $J$ = 6.6 Hz, 3-$CH_2$), 2.89 (t, $J$ = 6.6 Hz, 1H, 2-$CH_2$); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): $\delta$ 169.5, 156.1, 147.8, 131.4, 129.9, 127.5, 126.9, 125.6, 120.6, 120.3,104.6, 28.1, 27.4; EI-Mass *m/z*: 383.01 $[M]^+$; HREI-MS: *m/z* Calcd for $C_{18}H_{14}BrN_3S$: 383.0085; found 383.0086; λmax = 261 nm.

*N-Indan-1-ylidene-N'-[4-(4-Chloro-phenyl)-thiazol-2-yl]-hydrazine (ITH-4)*

Yield: 92%, mp 201-202 °C. IR (KBr) $cm^{-1}$ : 3321 (NH), 1558 (C=N), 1128 (C-S); $^1$H-NMR (300 MHz, DMSO-$d_6$): $\delta$ 11.10 (s, 1H, NH), 7.89 (d, 2H, $J$ = 8.7 Hz, H-2′, H-6′), 7.62 (d, 1H, $J$ = 7.2 Hz, H-7), 7.47 (d, 2H, $J$ = 8.7 Hz, H-3′, H-5′), 7.37 (overlapping multiplet, 4H, H-4, H-5, H-6, H-5″), 3.10 (t, 2H, $J$ = 6.6 Hz, 3-$CH_2$), 2.89 (t, 1H, $J$ = 6.9 Hz, 2-$CH_2$); $^{13}$C-NMR (100 MHz, DMSO-$d_6$): $\delta$ 156.1, 149.3, 147.8, 137.7, 133.7, 131.7, 129.9, 128.5, 127.1, 126.9, 125.6, 120.6, 104.5, 28.1, 27.4; EI-Mass *m/z*: 339.3 $[M]^+$; HREI-MS: *m/z* Calcd for

$C_{18}H_{14}ClN_3S$: 339.0597; found 339.0594; λmax = 261 nm.

*N-Indan-1-ylidene-N'-[4-(3-nitro-phenyl)-thiazol-2-yl]-hydrazine (ITH-5)*

Yield: 94%, mp 190-191 ˚C; IR (KBr) $cm^{-1}$: 3275 (NH), 1566 (C=N), 1105 (C-S), 1346 ($NO_2$); $^1$H-NMR (300 MHz, DMSO-$d_6$): $\delta$ 11.11 (s, 1H, NH), 7.83 (d, 2H, *J* = 8.4 Hz, H-4′, H-5′), 7.62 (m, 3H, H-2′, H-6′, H-7), 7.38 (overlapping multiplet, 3H, H-4, H-5, H-5″), 7.32 (overlapping multiplet, 1H, H-6), 3.10 (t, *J* = 6.6 Hz, 1H, 3-$CH_2$), 2.89 (t, *J* = 6.6 Hz 1H, 2-$CH_2$); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): $\delta$ 169.7, 156.4, 148.2, 147.9, 137.6, 131.4, 130.1, 129.9, 126.9, 125.7, 121.8, 120.6, 119.9, 106.4, 28.1, 27.5, 27.0; EI-Mass *m/z*: 350.2 $[M]^+$; HREI-MS: *m/z* Calcd for $C_{18}H_{14}N_4O_2S$: 350.0832; found, 350.037; λmax = 248nm.

*N-Indan-1-ylidene-N'-(4-Biphenyl-4-yl-thiazol-2-yl)-hydrazine (ITH-6)*

Yield: 90.5%, mp 200 ˚C (decomposed); IR (KBr) $cm^{-1}$: 3342 (NH), 1556 (C=N), 1113 (C-S); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 11.10 (s, 1H, NH), 7.97 (d, 2H, *J* = 8.1 Hz, H-4, H-7), 7.73 (d, 4H, H-2′, H-3′, H-5′, H-6′), 7.63 (d, 1H, *J* =7.2 Hz, H-4‴), 7.49 (t, 2H, *J* = 7.2 Hz H-3‴, H-5‴), 7.37 (ovp, 4H,H-5, H-6, H-2‴, H-6‴), 3.11 (t, *J* = 6.6 Hz, 2H, 3-$CH_2$), 2.91 (t, *J*= 6.6 Hz, 2H, 2-$CH_2$); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): $\delta$ 169.4, 147.7, 139.6, 129.8, 128.9, 127.3, 126.9, 126.7, 126.4, 126.0, 125.6, 120.5, 103.9, 28.1, 27.4; EI-Mass *m/z*: 381.5 $[M]^+$; HREI-MS: *m/z* Calcd for $C_{24}H_{19}N_3S$: 381.1300; found 381.1276; λmax = 276 nm.

*N-Indan-1-ylidene-N'-(4-phenyl-thiazol-2-yl)-hydrazine (ITH-7)*

Yield: 91%, mp 178-179 ˚C (decomposed). IR (KBr) $cm^{-1}$: 3380 (NH), 1558 (C=N), 1120 (C-S); $^1$H-NMR (300 MHz, DMSO-$d_6$): $\delta$ 11.07 (s, 1H, NH), 7.87 (d, 2H, *J* = 7.2 Hz, H-2′, H-6′), 7.62 (d, 1H, *J* = 7.2 Hz, H-7), 7.42 (overlapping multiplet, 4H, H-3′, H-4′, H-5′, H-5″), 7.37 (overlapping multiplet, H-4, H-5, H-6), 3.11 (t, *J* = 6.9 Hz, 3-$CH_2$), 2.90 (t, *J* = 6.6.Hz, 1H, 2-$CH_2$); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): $\delta$ 169.4, 155.9, 150.6, 147.7, 137.7, 134.8, 129.8, 128.5, 127.3, 126.9, 125.6, 125.4, 120.5, 103.7, 28.1, 27.4; EI-Mass *m/z*: 305.3 $[M]^+$; HREI-MS: *m/z* Calcd for$C_{18}H_{15}N_3S$:305.0987; found 305.0968; λmax = 260 nm.

*N-Indan-1-ylidene-N'-[4-(3-Bromo-phenyl)-thiazol-2-yl]-hydrazine (ITH-8)*

Yield: 89%, mp 169-170 ˚C (decomposed). IR (KBr) $cm^{-1}$: 3327 (NH), 1556 (C=N), 1115 (C-S); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 11.10 (s, 1H, NH), 8.07 (s, 1H, H-2′), 7.88 (d, 1H, *J* = 7.5 Hz, H-7), 7.62 (d, 1H, *J* = 7.2 Hz, H-4), 7.49 (overlapping multiplet, 2H, H-4′, H-5′), 7.38 (s, 1H, H-5″), 7.36 (overlapping multiplet, 3H, H-5, H-6, H-6′), 3.11 (t, *J* = 6.6 Hz, 2H, 3-$CH_2$), 2.89 (t, *J* = 6.6 Hz 1H, 2-$CH_2$); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): $\delta$ 169.5, 156.2, 148.8, 147.8, 137.6, 137.1, 130.7, 129.9, 128.1, 126.9, 125,6, 124.3, 122.0, 120.6, 105.3, 28.1, 27.4; EI-Mass *m/z*: 383.1 $[M]^+$; HREI-MS: *m/z* Calcd for $C_{18}H_{14}BrN_3S$: 383.0092; found 383.0065; λmax = 260 nm.

*N-Indan-1-ylidene-N'-[4-(4-nitro-phenyl)-thiazol-2-yl]-hydrazine (ITH-9)*

Yield: 95%, mp: (decomposed); IR(KBr) $cm^{-1}$: 3332 (NH), 1558 (C=N), 1118 (C-S), 1346 ($NO_2$); $^1$H-NMR (300 MHz, DMSO-$d_6$): $\delta$ 11.24 (s, 1H, NH), 8.29 (d, 2H, *J* = 9.0 Hz, H-3′, H-5′), 8.13 (d, 2H, *J* = 9.0, H-2′, H-6′), 7.71 (s, 1H, H-5″), 7.63 (d, 1H, *J* = 7.2 Hz, H-7), 7.37 (overlapping multiplet, 3H, H-4, H-5, H-6), 3.11 (t, *J* = 6.6 Hz, 2H, 3-$CH_2$), 2.90 (t, *J* = 6.6 Hz, 2H, 2-$CH_2$); $^{13}$C-NMR 100 MHz, DMSO-$d_6$): $\delta$ 130.0, 127.3, 126.9, 126.7, 126.2, 125.6, 124.0, 123.2, 120.9, 120.6, 108.6, 28.1, 27.9, 27.5; EI-Mass *m/z*: 350.2 $[M]^+$; HREI-MS: *m/z* Calcd for $C_{18}H_{14}N_4O_2S$: 350.0837; found 350.0837; λmax = 320 nm.

*N-Indan-1-ylidene-N'-[4-(2-hydroxy-phenyl)-thiazol-2-yl]-hydrazine (ITH-10)*

Yield: 94%, mp: 200 ˚C (decomposed). IR (KBr) $cm^{-1}$: 3232.5 (NH), 3400 (Ar-OH), 1560 (C=N), 1244 (C-O bond of Ar-OH), 1109 (C-S); $^1$H-NMR (300 MHz, DMSO-$d_6$): $\delta$ 11.44 (s, 1H, NH), 11.20 (s, 1H, OH), 7.81 (dd, 1H, *J* = 6.3 Hz, *J* = 1.2 Hz, H-3′), 7.64 (d, 1H, *J*= 7.2 Hz, H-6′), 7.38 (overlapping multiplet, 4H, H-4, H-5, H-7, H-5″), 7.17 (overlapping multiplet, 1H, H-6), 6.88 (m, 1H, H-4′, H-5′), 3.12 (t, *J* = 6.9 Hz, 2H, 3-$CH_2$), 2.91 (t, *J* = 6.9 Hz, 2H, 2-$CH_2$); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): $\delta$ 169.3, 157.2, 155.6, 148.4, 138.1, 130.5, 129.3, 127.5, 127.2, 126.1, 121.0, 119.3, 119.0, 117.2, 103.7, 28.6, 28.0; EI-Mass *m/z*: 321$[M]^+$; HREI-MS: *m/z* Calcd for $C_{18}H_{15}N_3OS$:321.0936; found 321.0924; λmax = 266 nm.

*N-Indan-1-ylidene-N'-[4-(2, 4-Dichloro-phenyl)-thiazol-2-yl]-hydrazine (ITH-11)*

Yield: 93%, mp: 175-176 ˚C. IR (KBr) $cm^{-1}$:3340 (NH), 1570 (C=N), 1115 (C-S);$^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 11.13 (s, 1H, NH), 7.94 (d, 1H, *J* = 8.4 Hz, H-7), 7.68 (d, 1H, *J* = 2.1 Hz, H-3′), 7.62 (d, 1H, *J* = 7.2 Hz, H-6), 7.52 (dd, 1H, *J* = 6.6 Hz, *J* = 2.1 Hz, H-5′), 7.39 (overlapping multiplet, 3H, H4, H5, H-6), 7.33 (ovp, 1H, H-6′), 3.10 (t, *J* = 6.9 Hz,2H, 3-$CH_2$), 2.89 (t, *J*

= 6.6 Hz, 2H, 2-$CH_2$); $^{13}$C-NMR (100 MHz, DMSO-$d_6$): $\delta$ 168.6, 156.2, 147.8, 137.6, 132.3, 132.1, 131.4, 129.9, 129.7, 127.4, 126.9, 125.6, 120.6, 109.2, 28.1, 27.4; EI-Mass *m/z*: 373.2 $[M]^+$; HREI-MS: *m/z* Calcd for $C_{18}H_{13}Cl_2N_3S$:373.0207; found 373.0182; λmax = 213nm.

*N-Indan-1-ylidene-N'-[4-(4-methyl-phenyl)-thiazol-2-yl]-hydrazine (ITH-12)*

Yield: 90%, mp: 171-172 ˚C. IR (KBr) $cm^{-1}$: 3346 (NH), 1556 (C=N), 1340 (Ar-$CH_3$), 1113 (C-S); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 11.04 (s, 1H, NH), 7.76 (d, 2H, *J* = 8.1 Hz, H-2′, H-6′), 7.61 (d, 1H, *J* = 6.9 Hz, H-7), 7.36 (overlapping multiplet, 3H, H-5″, H-5, H-6), 7.21 (d, 3H, *J* = 7.8 Hz, H-3′, H-5′, H-4), 3.10 (t, *J* = 6.9 Hz, 2H, 3-$CH_2$), 2.89 (t, *J* = 6.6Hz, 2H, 2-$CH_2$), 2.31 (s, 3H, $CH_3$); EI-Mass *m/z*: 319.2 $[M]^+$; HREI-MS: *m/z* Calcd for$C_{19}H_{17}N_3S$: 319.1143; found 319.1113; λmax = 261 nm.

*Cell lines and cell culture*

HEK293 (human embryonic kidney cell line), 3T3 (mouse fibroblast), SNB-19 (human astrocytoma cell line), PC-3 (human prostate cancer cell line), NCI-H460 (human lung cancer cell line), IGROV-1 (human ovarian cancer cell line), SK-OV-3 (human ovarian cancer cell line), K-562 (human chronic myeloid leukemia cell line) and HCT 116 (human colon cancer cell line) were used. All the cells were grown in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin in a humidified incubator at 37 ℃ with 5% $CO_2$.

*MTT cytotoxicity assay*

The cytotoxicity of the 12 compounds to cultured cancer cells was tested by MTT colorimetric assay. The assay assesses cell viability by detecting the formazan product formed from the reduction of 3-(4, 5-dimethylthiazole-2-yl)-2, 5-biphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase of metabolically active cells. Cells were seeded in 96-well plates at 5000 cells/well. After 24 h of incubation, various concentrations (ranging from 0–30 μM) of the 12 compounds were added respectively to the cells for a 72 h continuous drug incubation. At the end of the 68$^{th}$ hour of incubation, MTT reagent (4 mg/ml) was added and the plates were incubated at 37 °C for 4 h till the whole drug incubation ended. Subsequently, the supernatant was removed and 100 μl of DMSO were added to dissolve the formazan crystals. The plates were well shaken for 5 min, and the absorbance was determined at 570 nm by the accuSkan™ GO UV/Vis Microplate Spectrophotometer (Fisher Sci., Fair Lawn, NJ). The $IC_{50}$ (concentration that inhibited the survival of cells by 50%) values were calculated to represent the cytotoxicity of the compounds[22].

*Statistical analysis*

All experiments were repeated at least three times and the differences were determined using a one-way analysis of variance (ANOVA). The statistical significance was determined at $p < 0.05$, $p < 0.001$ and $p < 0.0001$. The post hoc analysis was performed using Tukey's test. The data were analyzed using GraphPad Prism, version

**Results and Discussion**

The present work describes the synthesis and evaluation of cytotoxic activity of twelve thiazolyl-hydrazone derivatives of 1-indanone.

*Chemistry*

Synthesis of thiazolyl hydrazones was accomplished by the reaction of 1-indanone with thiosemicarbazide in ethanol with catalytic amounts of acetic acid to obtain thiosemicarbazones. The resulted thiosemicarbazone were subsequently converted into thiazolyl hydrazones by reaction with differently substituted phenacyl bromide in the same solvent. The synthesized products were purified by washing with hexane and diethyl ethers and were collected with good yields. The synthetic pathways adopted for the preparation of the intermediate and target products are illustrated in scheme. 1 and proposed structures and their details are mentioned in Table-1. All the synthesized compounds were characterized by $^1$H &$^{13}$C NMR and HRMS and all the spectral data were in agreement with the proposed structures.

*Effect of Compounds on Normal cell lines*

To determine the cytotoxic effect of synthesized compounds on normal healthy cell lines, MTT assay was done against human embryonic kidney cell line, HEK293 and mouse fibroblast cell, 3T3. All the derivatives did not show any cytotoxicity on these normal healthy cell lines and $IC_{50}$ was more than 30 μM (Table-2**)** when compared with standard cytotoxic compound Cyclohexamide **(**$IC_{50}$ = 0.8 ± 0.2 μM**)** showing that the compounds are safe to normal (non-cancerous) cells.

Table-1: Structural information of Thiazolyl hydrazones derivatives.

| Compound | R | Mol. mass | formula | IUPAC Name | Color and physical statesstatestate |
|---|---|---|---|---|---|
| ITH-1 | 4′-$OCH_3$ | 335.42 | $C_{19}H_{17}N_3OS$ | *N*-Indan-1-ylidene-*N*'-[4-(4-methoxy-phenyl)-thiazol-2-yl]-hydrazine | Mustard powder |
| ITH-2 | 3′, 5′-Cl | 373.02 | $C_{18}H_{13}Cl_2N_3S$ | *N*-Indan-1-ylidene-*N*'-[4-(3, 5-Dichloro-phenyl)-thiazol-2-yl]-hydrazine | Dark orange powder |
| ITH-3 | 4′-Br | 383.01 | $C_{18}H_{14}BrN_3S$ | *N*-Indan-1-ylidene-*N*'-[4-(4-Bromo-phenyl)-thiazol-2-yl]-hydrazine | Brown powder |
| ITH-4 | 4′-Cl | 339.06 | $C_{18}H_{14}ClN_3S$ | *N*-Indan-1-ylidene-*N*'-[4-(4-Chloro-phenyl)-thiazol-2-yl]-hydrazine | Beige powder |
| ITH-5 | 3′-$NO_2$ | 350.08 | $C_{18}H_{14}N_4O_2S$ | *N*-Indan-1-ylidene-*N*'-[4-(3-nitro-phenyl)-thiazol-2-yl]-hydrazine | Light brown powder |
| ITH-6 | 4′-$C_6H_5$ | 381.13 | $C_{24}H_{19}N_3S$ | *N*-Indan-1-ylidene-*N*'-(4-Biphenyl-4-yl-thiazol-2-yl)-hydrazine | Yellow powder |
| ITH-7 | -H | 305.10 | $C_{18}H_{15}N_3S$ | *N*-Indan-1-ylidene-*N*'-(4-phenyl-thiazol-2-yl)-hydrazine | Orange powder |
| ITH-8 | 3′-Br | 383 | $C_{18}H_{14}BrN_3S$ | *N*-Indan-1-ylidene-*N*'-[4-(3-Bromo-phenyl)-thiazol- | Brown |
| ITH-9 | 4′-$NO_2$ | 350.08 | $C_{18}H_{14}N_4O_2S$ | *N*-Indan-1-ylidene-*N*'-[4-(4-nitro-phenyl)-thiazol-2-yl]-hydrazine | Orange powder |
| ITH-10 | 2′-OH | 321.09 | $C_{18}H_{15}N_3OS$ | *N*-Indan-1-ylidene-*N*'-[4-(2-hydroxy-phenyl)-thiazol-2-yl]-hydrazine | Peach powder |
| ITH-11 | 2′,4′-Cl | 374.02 | $C_{18}H_{13}Cl_2N_3S$ | *N*-Indan-1-ylidene-*N*'-[4-(2, 4-Dichloro-phenyl)-thiazol-2-yl]-hydrazine | Pale yellow powder |
| ITH-12 | 4′-$CH_3$ | 319.11 | $C_{19}H_{17}N_3S$ | *N*-Indan-1-ylidene-*N*'-[4-(4-methyl-phenyl)-thiazol-2-yl]-hydrazine | Yellow powder |

Table-2: The effect ofThiazolyl Hydrazone derivatives of 1-Indanone (ITH-1 to ITH-12) on normal cell lines.

| Compounds | $IC_{50} \pm SD^a(\mu M)$ | |
|---|---|---|
| | 3T3 (Mouse fibroblasts) | HEK293 (Human embryonic kidney cells) |
| ITH-1 | >30 | >30 |
| ITH-2 | >30 | >30 |
| ITH-3 | >30 | >30 |
| ITH-4 | >30 | >30 |
| ITH-5 | >30 | >30 |
| ITH-6 | >30 | >30 |
| ITH-7 | >30 | >30 |
| ITH-8 | >30 | >30 |
| ITH-9 | >30 | >30 |
| ITH-10 | >30 | >30 |
| ITH-11 | >30 | >30 |
| ITH-12 | >30 | >30 |
| Cyclohexamide (Standard) | 0.8 ± 0.2 | |

μM - Micromole.
The cytotoxic effects of the test compounds on HEK293 (human embryonic kidney cells) and 3T3 (mouse fibroblasts).
Values in tables are representative of at least three independent experiments performed in triplicates.
$IC_{50}$: concentration that inhibits cell survival by 50% (mean ± SD).

*Anti-cancer Activity*

All the synthesized thiazolyl hydrazone derivatives were tested for their cytotoxic effects. In order to determine the cytotoxicity of compounds on different cancer cells, MTT assay was performed and screened against 7 cell lines (as mentioned in cell lines and cell culture). As shown in Table-2, it has been noticed that three of the tested compounds exhibit remarkable anti-cancer activity against tested human colon cancer cells, HCT 116. This may attract the attention towards selective cytotoxic properties of the constructed ring system (Table-2).

Indanone and its derivatives are well known for their extensive bioactivities. Past studies have shown that the indanone derivatives as active agents for anti-cancer, anti-inflammatory, analgesic, antimicrobial, anticholinergic, and antimalarial activity. The 3-aryl substituted analogue of indanone was found to be significantly active against the cell lines HeLa and K562[23]. Among the present indanone derivatives**,** compounds ITH-1, ITH-3 and ITH-6 had good cytotoxicity on HCT 116 with $IC_{50}$ values of 5.04μM, 1.25 μM and 5μM respectively and no activity against other cancer cell lines (Fig.2B, Fig.2C and Fig.2D). Among this, ITH-6 showed potential cytotoxicity (>1 μM) against other colon cancer cell lines[24].

However, from the observed data as shown in Table-2, it has been noticed that all the other tested derivatives have no activity against most of the tested human tumor cells (HCT 116, SNB-19, PC-3, NCI-H460, IGROV-1, SK-OV-3 and K-562). (Fig. 3)

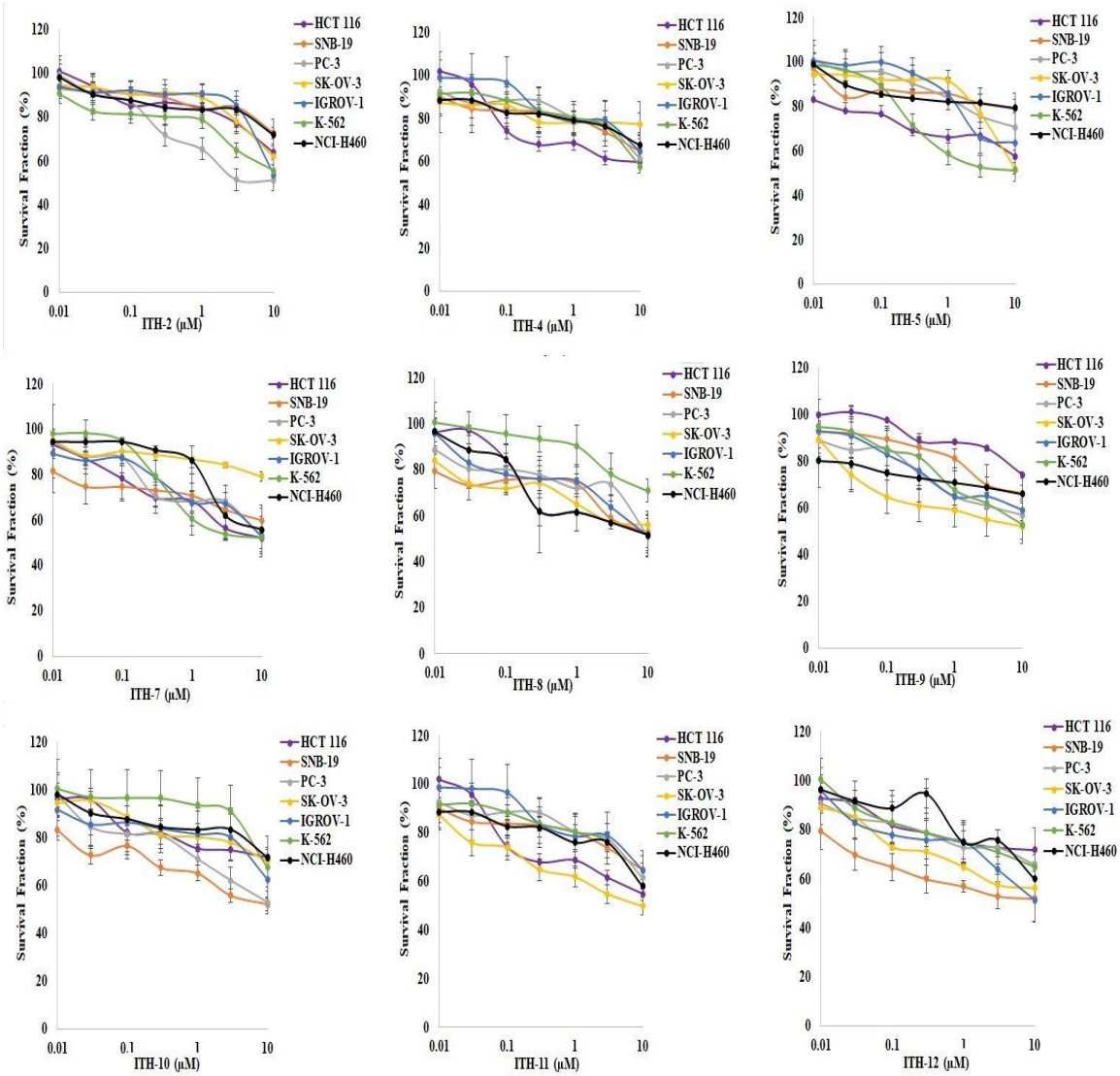



Fig. 3: Cytotoxicity of ITH-2, ITH-4, ITH-5, ITH-7, ITH-8, ITH-9, ITH-10, ITH-11 and ITH-12 on HCT 116, SNB-19, PC-3, SK-OV-3, IGROV-1, K-562 and NCI-H460 cell lines. Survival fraction (%) was measured after treatment with these compounds (µM) for 72 h on HCT 116 (violet), SNB-19 (red), PC-3 (grey), SK-OV-3 (yellow), IGROV-1 (blue), K-562 (green) and NCI-H460 (black) cell lines. Points with error bars represent the mean ± SD for independent determinations in triplicate. The figures are representative of three independent experiments.

*Structure-activity relationship*

In SAR studies of the synthesized compounds, we found that the electronic properties of substituents of the phenyl ring (specifically at 4-or *para*-position) at the thiazole ring strongly affected the anti-cancer activity amongst synthesized thiazolyl hydrazone derivatives. Unsubstituted phenyl derivative (ITH-7) was found to be totally inactive. Moreover, the aromatic ring substitution with electron-donating groups significantly contributed to high cytotoxicity as compared to electron withdrawing groups. Compound ITH-1 with methoxy (4-OMe) group and ITH-6 with another phenyl (4-Ph)group substitution showed best cytotoxic effect as the phenyl group is said to be activated, suggesting that all these substituents bonded to it increase the electron density to the phenyl ring for better receptor interaction supported by a previous finding in which 4-OMe group substitution on phenyl ring attached in molecular structure effectively increased the anti-cancer activity against Colon carcinoma (HCT-116)

and liver carcinoma (HEPG2) cell lines with IC50 close to Doxorubicin[25] and also a diphenyl thiazole derivative showed promising anti-cancer potential[26]. 4-phenyl substituted derivative ITH-6 being the most active member within the series seems to be most potent active anti-tumor agent than the other derivatives. This electron-donating effect of phenyl ring substituents might result in more DNA cleavage of tumorous cells possibly via enhanced superoxide anion and radical formation resulting in more pronounced cytotoxicity as shown by a previous study[27]. Introducing other electron donating groups such as -OH to 2- or *ortho*-position of phenyl ring (ITH-10) exhibited no anti-proliferative activity.

Moreover, ITH-3 also showed significant cytotoxic effect possessing 4-or *p*-bromine substitution. This is in close agreement with various reports in which 4-bromo phenyl substituted thiazole compounds were identified as promising cytotoxic agents[28, 29]. In addition, to compare the effects of *meta*- and *para*- substitutions, a bromo atom (-Br) was also introduced to 3- or *meta*-position of phenyl ring (ITH-8). This 3-or *m*-substituents did not exhibit equal activity. 4- or *p*- bromo substituted derivative (ITH-7) had the best activity for examined Colon carcinoma cells. The replacement of (2-)*ortho,(3-)meta* or (4-) *para*- positions with or halogen atoms such as 4′-Cl (ITH-4), 3′, 5′-*di* Cl(ITH-2) and 2, 4′-*di* Cl (ITH-11) did not exert any cytotoxic activity. Furthermore, the introduction of strong electron withdrawing group *i.e*. -NO2 on 3- or *m*- (ITH-5) and 4- or *p*-position (ITH-9) of phenyl ring resulted in total loss of activity.

## Conclusion

In conclusion, a distinctive pattern of sensitivity toward individual colon cancer cells was demonstrated by the three compounds. It has been shown that their anti-cancer ability depends on the electronic effect of various substituents on the aromatic ring of thiazolyl hydrazone derivatives on the indanone scaffold. This combination of electronic effects could be responsible for the cytotoxic effect of the active derivatives, the mechanism of which remains to be determined. This preliminary structure-activity relationship (SAR) study may serve as a basis for chemical modifications directed towards the development of potential anti-cancer compounds of medicinal interest.

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