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New chalcones bearing isatin scaffold: synthesis, molecular modeling and biological evaluation as anticancer agents

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Abstract Derivatives of isatin have been reported to possess cytotoxic effects against different human carcinoma cell lines. A series of new isatin-linked chalcones was synthesized starting from isatin. Most of the newly synthesized compounds were screened for their in vitro anticancer activity against human breast (MCF-7), liver (HepG-2), and colon (HCT-116) cancer cell lines. All the tested compounds exhibited antitumor activity, with IC_{50} ranging from 2.88 to 62.88 μM in comparison to the reference drug used in this study, Imatinib. Compounds **2–5** were the most active, with IC_{50} ranging from 2.88 to 18.12 μM for the three cell lines, while compound **7b** also showed moderate activity against HepG-2, MCF-7 and HCT-116 with IC_{50} 13.95, 31.66

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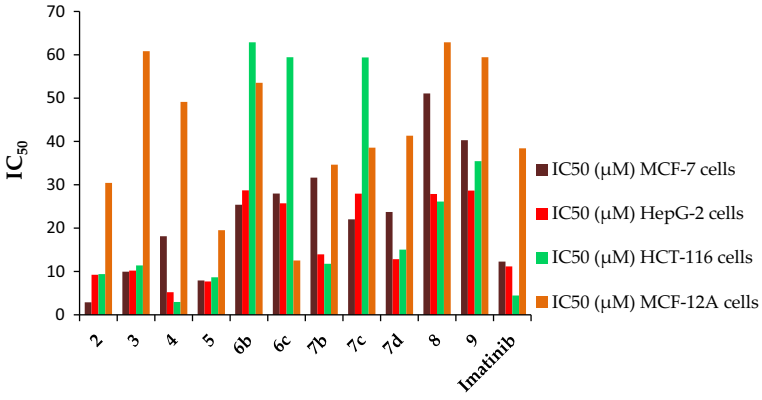
and 11.78 μM, respectively. Furthermore, compound **7d** showed high activity against HepG-2 cells with IC_{50} 12.84 μM. Compound **4** was shown to be the most potent against both HepG-2 and HCT-116 cell lines, while compound **2** is the most potent against MCF-7. The compounds were also screened for their cytotoxic activity against normal breast cell line MCF-12A, and were found to possess mild cytotoxicity. A docking study was performed for the most active compounds in this study, **2**–**5**, inside the active site of CDK2. All the docked compounds have shown favorable binding interactions and energy scores. Compound **4** has proved to be the best in binding interactions and energy score. These findings may explain the cytotoxic activity of the target compounds.

Graphical Abstract A novel series of isatin-linked chalcones was synthesized. The target compounds were evaluated for their cytotoxic activity towards human breast (MCF-7), liver (HepG-2), colon (HCT-116) cancer cell lines and (MCF-12A) normal breast cell line.



Keywords Isatin · Chalcones · Indolines · MCF-7 · HepG-2 · Anticancer

Introduction

Cancer remains a major medical challenge worldwide. It can be treated by surgery, radiation, chemotherapy, and hormonal and biological therapy [1–3]. However, the method of treatment depends on the location and the progression of the disease. Chemotherapeutic agents are mainly used in cases of metastasis and hypoxic tumors, but the major limitation to their use is toxicity [4, 5], which occurs due to low selectivity of the current chemotherapeutic drugs [6]. Also, one of the major side effects of chemotherapy is drug resistance, which may occur after a long period of using the chemotherapeutic agent [7], which has led to a continuous search for new anticancer agents offering both selectivity towards malignant cells and lowered resistance. Isatin was first isolated from the fruits of the Cannonball tree *Couroupita guianensis* [8]. This plant species has been used in folk medicine [9]. Derivatives of isatin have been reported to possess cytotoxic effects against various human carcinoma cell lines derived from breast, prostate [10], human acute lymphoblastic

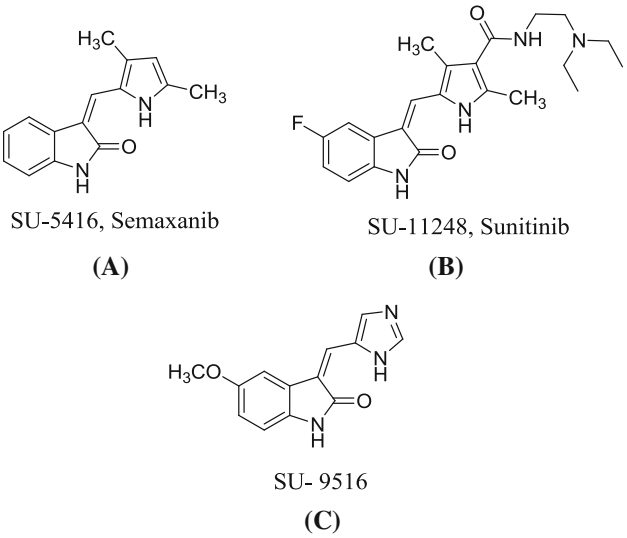


Fig. 1 Isatin-containing drugs

leukemia (MOLT-4) [11, 12], colon [13, 14] and lung [15, 16]. Significant interest in the 2-oxoindoles derivatives as kinase inhibitors came after the disclosure of the tyrosine kinase inhibitory properties and anti-angiogenesis properties of SU-5416 (Semaxanib, **A**) [17–20] and SU-11248 (Sunitinib, **B**) (Fig. 1); the latter molecule has recently completed phase III clinical trials with success. On the other hand, the structurally relevant SU-9516 (**C**) was reported as a potential CDK inhibitor which induces apoptosis in colon carcinoma cells [21, 22]. Moreover, indolinones with their dual receptor tyrosine kinase and cyclin/CDK complex inhibitory properties have been reported to treat tumor cell proliferation [23].

Chalcones are secondary metabolite precursors of flavonoids and isoflavonoids. The major factors contributing to the increased interest in exploring their pharmacological activities are their good safety profile, their possibility of oral administration [24, 25] and easy synthesis. Chalcones have been reported to possess several biological activities such as anti-inflammatory [26], antibacterial [27], antifungal [28] and antitumor [29–32]. The double bond of the enone system in chalcones was found to be essential for their anticancer activity [33, 34]. The advantage of chalcones is the low probability of interacting with DNA and the decrease in the risk of mutagenicity as a common side effect of current chemotherapeutic agents [35]. Literature surveys revealed that the structural modifications of chalcones mostly focused on the replacement of the phenyl rings with heterocyclic rings and polyaromatic groups [36, 37], the introduction of different substituents on the phenyl moieties [38] and cyclization of the chalcone to give rigid analogs [39].

In an attempt to improve the chalcone's anticancer activity, isatin was introduced, generating a class of isatin–chalcone hybrids. The isatin group is fixed, and diversity was created by introducing different substituents on the other ring. The target compounds were evaluated against MCF-7, HepG-2 and HCT-116 cancer cell

lines, and the most active compounds were docked inside the active site of CDK2/CyA [40] (cyclin-dependent kinase 2/cyclin A), to determine their possible mechanism of action as anticancer agents.

Experimental

Chemistry

All melting points were measured on an Electrothermal LA9000 series Digital melting point apparatus and were uncorrected. IR spectra were determined using the KBr disc technique on a NikoletIR 200 FT-IR Spectrophotometer at the Pharmaceutical Analytical Unit, Faculty of Pharmacy, Al-Azhar University, Egypt, and values are represented in cm^{-1}. The ^{1}HNMR and ^{13}CNMR spectra were recorded on Gemini 300 MHz, and Mercury 400 MHz NMR Spectrometers at the Main Chemical Warfare Laboratories, Chemical Warfare Department, Ministry of Defense, Cairo, Egypt. DMSO-d_6 was used as solvent, and chemical shifts were measured in δ ppm, relative to TMS as an internal standard. Mass spectrum was recorded at 70 eV on a DI-50 unit of a Shimadzu GC/MS-QP5050A Spectrometer at the Regional Center for Mycology and Biotechnology (RCMB), at Al-Azhar University, represented as *m/z* (relative abundance %) (formula). Element analysis (C, H, N) were also carried out at Regional Center for Mycology and Biotechnology, and the values were found to be within ±0.4% of the theoretical ones unless otherwise indicated. The progress of the reaction was monitored by TLC using TLC sheets pre-coated with UV fluorescent silica gel Merck 60 F254 plates and was visualized using a UV lamp.

*3-[(2-(4-Aminophenyl)-2-oxoethylidene] indolin-2-one (**4**)*

To a solution of **3** (2.82 g, 0.01 mol) in ethanol (30 mL), conc. HCl (2 dps) was added, the mixture was refluxed for 30 min, cooled and treated with ice-cold water, and the obtained product was filtered and crystallized from ethanol. Dark red powder; yield (86%); m.p.: 170–172 °C; IR: υ/cm^{-1} = 3455, 3328, 3213 (NH_2 & NH), 2940, 2833 (aliph.), 1706 (2CO).^{1}H NMR: δ/ppm = 6.38 [s, 2H, NH_2], 6.60 [d, 2H, J = 6 Hz, Ar–H], 6.84 [d,1H J = 6 Hz, indolin-7], 6.90 [t, 1H, indolin-6], 7.26 [t, 1H, indolin-5], 7.58 [s, 1H, CH=], 7.76 [d, 2H, J = 6 Hz, indolin-4], 7.85 [d, 2H, J = 6 Hz, Ar–H], 10.69 [s, 1H, NH]. ^{13}C NMR; 113.38, 120.70, 121.99, 126.83, 128.63, 131.84, 132.35, 134.54, 144.63, 155.29 (Ar–C + C=C), 166.81, 188.39 (2CO). Mass spectrum exhibited a molecular ion peak at *m/z* 264 (M^{+}, 23.21%) with 265 (M^{+1}, 4.05%) with a base peak at *m/z* 236; Anal. Calcd. for $C_{16}H_{12}N_2O_2$ (264.28): C, 72.72; H, 4.58; N, 10.6; Found: C, 72.94; H, 4.63; N, 10.75.

*4-(2-(2-Oxoindolin-3-ylidene)acetyl)phenyl)acetamide (**5**)*

To a solution of **3** (2.82 g, 0.01 mol) in acetic acid (30 mL), conc. HCl (2 mL) was added, the mixture was refluxed for 30 min. cooled and treated with ice-cold water, and the obtained product filtered and crystallized from ethanol. Orange powder;

yield (78%); m.p.: 250–252 °C; $R_f = 0.60$; IR: υ/cm^{-1} = 3423, 3230 (2NH), 3080 (arom.), 2890, 2817 (aliph.), 1703 (CO), 1603 (C=C).^{1}H NMR: δ/ppm = 2.09 [s, 3H, CH_3], 6.88 [d, 1H, $J = 9$ Hz, indolin-7], 6.95 [t, 1H, indolin-6], 7.32 [t, 1H, indolin-5], 7.68 [s, 1H,CH=], 7.79 [d, 2H, $J = 9$ Hz, Ar–H], 7.96 [d, 1H, $J = 9$ Hz, indolin-4], 8.05 [d, 2H, $J = 9$ Hz, Ar–H], 10.75, 10.36 [s, 2H, 2NH]. ^{13}C NMR: 23.5 (CH_3), 119.9 (2), 127.4 (2), 129.0 (2), 129.90 (2), 134.02 (2), 138.31 (2), 138.42, 154.04 (C=C), 159.02 (CO), 170.50 (CO), 179.73 (CO). Mass spectrum exhibited a molecular ion peak at *m/z* 306 (M^+, 0.91%) with a base peak at *m/z*: 43; Anal. Calcd. for $C_{18}H_{14}N_2O_3$ (306.32): C, 70.58; H, 4.61; N, 9.15; Found: C, 70.81; H, 4.69; N, 9.27.

General procedure for the synthesis of compounds 6a–d

A mixture of isatin (1.47 g, 0.01 mol), the corresponding Schiff bases (0.01 mol) and diethyl-amine (3 mL) in ethanol (30 mL) was stirred for 5 h., left overnight, cooled and treated with ice-cold water, and the obtained product was filtered and crystallized from benzene.

*3-[2-(4-Benzylideneamino) phenyl)-2-oxoethyl]-3-hydroxyindolin-2-one (**6a**)*

Pale yellow crystal; yield (83%); m.p.: 125–127 °C; $R_f = 0.52$; IR: υ/cm^{-1} = 3362–3500 (br. OH & NH), 3050 (arom.), 1715 (2CO), 1600 (CN); ^{1}H NMR δ/ppm = 3.40, 4.03 [2d, $J = 12$ Hz, 2H, CH_2], 6.10 [s, 1H, OH], 6.60–7.92 [m, 14H, Ar–H + N=CH], 10.20 [s, 1H, NH]. Mass spectrum exhibited a molecular ion peak at *m/z* 370 (M^+, 1.54%) with a base peak at *m/z*: 103; Anal. Calcd. for $C_{23}H_{18}N_2O_3$ (370.40): C, 74.58; H, 4.90; N, 7.56; Found: C, 79.81; H, 4.98; N, 7.72.

*3-Hydroxy-3-[2-(4-(2-hydroxybenzylideneamino)phenyl)-2-oxoethyl]indolin-2-one (**6b**)*

Orange powder; yield (83%); m.p.: 218–220 °C; $R_f = 0.45$; IR: υ/cm^{-1} = 3380, 3204 (NH& 2OH), 3059 (arom.), 2900, 2808 (aliph.), 1695 (2 CO), 1611 (CN); ^{1}H NMR: δ/ppm = 3.60, 4.09 [2d, 2H $J = 18$ Hz, CH_2], 6.03 [s, 1H, OH], 6.79–7.79 [m, 12H, Ar–H], 8.97 [s, 1H, CH=N], 10.23, 12.62 [2 s, 2H, NH &OH]. ^{13}C NMR; 46.18 (CH_2), 73.49 (C_3-isatin), 109.84, 117.15, 119.78, 121.56, 129.37, 129.98, 132.19, 133.05, 134.68, 134.66, 143.38, 152.99 (Ar–C), 160.75 (CN), 165.35 (C–OH), 178.77, 195.98 (2 CO). Mass spectrum exhibited a molecular ion peak at *m/z* 386 (M^+, 1.21%) with a base peak at *m/z*: 224; Anal. Calcd. for $C_{23}H_{18}N_2O_4$ (386.40): C, 71.49; H, 4.70; N, 7.25; Found: C, 71.66; H, 4.81; N, 7.38.

*3-Hydroxy-3-[2-(4-(4-methoxybenzylideneamino)phenyl)-2oxoethyl)indolin-2-one (**6c**)*

Yellowish brown powder; yield (65%); m.p.: 185–187 °C; $R_f = 0.49$; IR: υ/cm^{-1} = 3364 (br. NH & OH), 3065 (arom,), 2952, 2891 (aliph.), 1725, 1672 (2

CO), 1599 (CN).^{1}H NMR: δ/ppm = 3.86 [s, 3H, OCH_3], 3.58, 4.07 [2d, 2H, J = 18 Hz, CH_2], 6.02 [s, 1H, OH], 6.78–7.91 [m, 12H, Ar–H], 8.53 [s, 1H, CH=N], 10.22 [s, 1H, NH]. ^{13}C NMR: 46.12 (OCH_3), 55.94 (CH_2), 73.52 (C_3-indoline), 109.86, 114.83, 121.55, 121.58, 124.06, 129.87, 131.39, 132.27, 133.66, 143.43, 156.63, 162.14 (Ar–C), 162.81 (CN), 178.85, 195.90 (2CO). Mass spectrum exhibited a molecular ion peak at m/z: 400 (M^+, 0.85%) with a base peak at m/z 239; Anal. Calcd. for $C_{24}H_{20}N_2O_4$ (400.43): C, 71.99; H, 5.03; N, 7.00; Found: C, 72.18; H, 5.09; N, 7.13.

*3-Hydroxy-3-[2-(4-nitrobenzylideneaminophenyl)-2-oxoethyl] indolin-2-one (**6d**)*

Orange powder; yield (53%); m.p.: 220–222 °C; R_f = 0.73; IR: υ/cm^{-1} = 3377, 3191 (NH & OH), 3067 (arom.), 2889 (aliph.), 1695 (2 CO), 1600 (CN).^{1}H NMR: δ/ppm = 3.60, 4.09 [2d, 2H, J = 18 Hz, CH_2], 6.04 [s, 1H, OH], 6.77 [d, 1H, J = 9 Hz, indolin-7], 6.87 [t, 1H, indolin-6], 7.15 [t, 1H, indolin-5], 7.28 [d, 2H, J = 6 Hz, Ar–H], 7.39 [d, 1H, J = 9 Hz, indolin-4], 7.97 [d, 2H, J = 6 Hz, Ar–H], 8.17 [d, 2H, J = 6 Hz, Ar–H], 8.42 [d, 2H, J = 6 Hz, Ar–H], 8.81 [s, 1H, CH=N], 10.24 [s, 1H, NH].^{13}C NMR: 45.28 (CH_2), 73.53 (C_3-indoline), 109.74, 112.90, 121.41, 123.87, 124.53, 129.15, 130.75, 132.61, 140.53, 143.48, 151.09, 154.24, 155.36, 162.14 (Ar–C), 161.47 (CN), 179.03, 192.81 (2CO). Mass spectrum exhibited a molecular ion peak at m/z: 415 (M^+, 0.40%) with a base peak at m/z 150; Anal. Calcd. for $C_{23}H_{17}N_3O_3$ (415.40): C, 66.50; H, 4.12; N, 10.12. Found: C, 66.73; H, 4.18; N, 10.31.

General procedure for the synthesis of compounds 7a–d

1st method: a mixture of **3** (2.82 g, 0.01 mol), and aldehyde derivatives (0.01 mol) in ethanol (30 mL) and acetic acid (3 mL) was refluxed for 2 h. The precipitated solid was filtered and crystallized from ethanol.

2nd method: a mixture of **4** (2.64 g, 0.01 mol), and aldehyde derivatives (0.01 mol) in ethanol (30 mL) and acetic acid (3 mL) was refluxed for 2 h. The precipitated formed was filtered and crystallized from ethanol.

3rd method: compound **6** (0.01 mol) in ethanol (30 mL), to which conc. HCl (2 mL) was added, the mixture was refluxed for 30 min, cooled and the obtained product was filtered and crystallized from ethanol.

*3-[2-(4-(2-Hydroxybenzylindeneamino) phenyl)-2-oxoethylidene] indolin-2-one (**7a**)*

Dark orange powder; yield (78%); m.p.: 170–172 °C; R_f = 0.68; IR: υ/cm^{-1} = 3379, 3196 (OH & NH), 3050 (arom.), 2950 (aliph.), 1711 (2 CO), 1601 (CN); ^{1}H NMR: δ/ppm = 6.60–7.91 [m, 13H, Ar–H + C=CH], 8.51 [s, 1H, CH=N], 10.67, 10.24 [2 s, 2H, NH, OH].^{13}C NMR: 110.56, 117.67, 119.83, 119.92, 120.70, 122.56, 122.73, 125.13, 126.83, 128.89, 129.59, 131.84, 132.35, 133.03, 136.85, 144.64, 155.22 (Ar–C + C=C), 161.17 (CN), 168.81 (C–OH), 188.39, 192.11 (2CO). Mass spectrum exhibited a molecular ion peak at m/z 368 (M^+,

32.27%) with a base peak at *m/z*: 116; Anal. Calcd. for $C_{23}H_{16}N_2O_3$ (368.38): C, 74.99; H, 4.38; N, 7.60; Found: C, 75.12; H, 4.42; N, 7.69.

*3-[2-(4-(4-Methoxybenzylideneamino) phenyl)-2-oxoethylidene] indoline-2-one (**7b**)*

Yellow powder; yield (69%); m.p.: 110–112 °C; $R_f = 0.63$; IR: υ/cm^{-1} = 3362 (NH), 3063 (arom.), 2955, 2847 (aliph.), 1725, 1675 (2 CO), 1595 (CN). ^{1}H NMR: δ/ppm = 3.84 [s, 3H, OCH_3], 7.07–8.00 [m, 13H, Ar–H + CH=], 8.55 [s, 1H, N=CH], 10.20 [s, 1H, NH]. ^{13}C NMR: 55.80 (OCH_3), 112.70, 118.71, 119.81(2), 119.90 (3), 129.01 (2), 129.92 (3), 130.02 (2), 132.75 (2), 138.31, 138.51, 142.32, 162.71 (2), 170.14 (CO), 196.05 (CO). Mass spectrum exhibited a molecular ion peak at *m/z* 382 (M^+, 10.42%) with a base peak at *m/z*: 236; Anal. Calcd. for $C_{24}H_{18}N_2O_3$ (382.13): C, 75.38; H, 4.74; N, 7.33. Found: C, 75.46; H, 4.81; N, 7.45.

*3-[2-(4-(4-Chlorobenzylideneamino) phenyl)-2-oxoethylidine] indolin-2-one (**7c**)*

Yellow powder; yield (64%); m.p.: 200–202 °C; $R_f = 0.78$; IR: υ/cm^{-1} = 3372 (NH), 3062 (arom.), 2879 (aliph.), 1670, 1647 (2 CO), 1591 (CN), 1490 (C=C). ^{1}H NMR: δ/ppm = 7.32–8.24 [m, 13H, Ar–H + CH=], 8.68 [s, 1H, N=CH], 10.02 [s, 1H, NH]. ^{13}C NMR: 118.31 (2), 119.74, 128.02 (2), 129.04 (2), 129.05 (2), 130.01, 130.06 (2), 130.10 (2), 130.2, 134.65, 138.39, 138.71, 139.23, 157.13, 165.01 (CN), 170.62 (CO), 196.96 (CO). Mass spectrum exhibited a molecular ion peak at *m/z* 388 (M + 2, 2.53%), 386 (M^+, 3.84%) with a base peak at *m/z* 97; Anal. Calcd. for $C_{23}H_{15}ClN_2O_2$ (386.83): C, 68.23; H, 4.23; N, 6.92. Found: C, 68.74; H, 4.31; N, 7.08.

*3-[2-(4-(4-Nitrobenzylideneamino) phenyl)-2-oxoethylidene] indoline-2-one (**7d**)*

Yellowish powder; yield (64%); m.p.: 120–122 °C; $R_f = 0.81$; IR: υ/cm^{-1} = 3385 (NH), 3050 (arom.), 2850 (aliph.), 1724 (2CO), 1597 (CN). ^{1}H NMR δ/ppm = 7.41–8.40 [m, 13H, Ar–H + CH=], 8.83 [s, 1H, N=CH], 10.20 [s, 1H, NH]. ^{13}C NMR: 111.01, 119.32, 120.00 (2), 121.98, 122.76, 128.89, 130.02 (2), 130.31 (2), 131.75 (2), 133.73, 136.41, 139.62 (2), 142.43, 151.41, 156.68, 160.31 (CN), 170.50 (CO), 189.73 (CO). Mass spectrum exhibited a molecular ion peak at *m/z* 397 (M^+, 2.56%) with 399 (M + 2, 1.27%), 398 (M + 1, 1.36%), with a base peak at *m/z*: 337; Anal. Calcd. for $C_{23}H_{15}N_3O_4$ (397.38): C, 66.73; H, 3.80; N, 10.57; Found: C, 66.76; H, 4.15; N, 10.36.

*2-[4-(2-(2-Oxoindolin-3-ylidine) acetyl) phenyl] isoindolin-1, 3-dione (**8**)*

A mixture of 4 (2.64 g, 0.01 mol) and phthalic anhydride (0.01 mol) in acetic acid (20 mL) was refluxed for 3 h., cooled and treated with ice-cold water, and the obtained product was filtered and crystallized from ethanol. Dark orange powder; yield (71%); m.p.: 215–217 °C; $R_f = 0.42$; IR: υ/cm^{-1} = 3193 (NH), 3050 (arom.), 2827 (aliph.), 1718 (CO). ^{1}H NMR: δ/ppm = 6.87–8.24 [m, 13H, Ar–H + CH=],

10.80 [s, 1H, NH]. ^{13}C NMR: 113.56, 121.76, 121.94, 125.16, 125.75, 126.25, 127.56, 128.12, 128.33, 129.806, 130.48, 131.17, 132.81, 134.87, 138.85, 141.32, 146.00, 158.15 (Ar–C), 161.13, 188.20 (CO). Mass spectrum exhibited a molecular ion peak at *m/z* 394 (M^+, 21.92%) with a base peak at *m/z*: 236; Anal. Calcd. for $C_{24}H_{14}N_2O_4$ (394.38): C, 73.09; H, 3.58; N, 7.10; Found: C, 73.35; H, 3.54; N, 7.21.

*5′-(4-Aminophenyl)-2′, 4′-dihydro spiro (indoline-3, 3′-pyrazol)-2-one (**9**)*

A mixture of 4 (2.64 g, 0.01 mol) and hydrazine hydrate (0.05 mol) in ethanol (30 mL) and acetic acid (3 mL) was refluxed for 3 h., cooled and treated with ice-cold water, and the obtained product was filtered and crystallized from ethanol. Yellow crystal; yield (60%); m.p.: 230–232 °C; $R_f = 0.39$; IR: $\upsilon/cm^{-1} = 3418$, 3261, 3166 (NH_2, 2NH), 3026 (arom.), 2819 (aliph.), 1650 (CO), 1596 (CN). ^{1}H NMR: δ/ppm = 3.38 [s, 2H, CH_2], 4.72 [s, 1H, NH], 5.59 [s, 2H, NH_2], 6.70 [d, 1H, $J = 9$ Hz, indolin-7], 7.53 [t, 1H, indolin-6], 7.74 [t, 1H, indolin-5], 8.02–8.11 [m, 4H, Ar–H], 8.14 [d, 1H, $J = 9$ Hz, indolin-4], 9.92 [s, 1H, NH]. ^{13}C NMR: 44.11 (CH_2), 83.71, 118.75, 119.40, 120.32, 129.14, 129.31, 130.01(2), 130.12 (2), 134.04, 138.35, 138.73, 157.54 (CN), 165.85 (CO). Mass spectrum exhibited a molecular ion peak at *m/z* 278 (M^+, 55.83%) with a base peak at *m/z* 219; Anal. Calcd. for $C_{16}H_{14}N_4O$ (278.31): C, 69.05; H, 5.07; N, 20.13; Found: C, 68.99; H, 4.95; N, 19.94.

*3-[2-(4-Aminophenyl)-2-(phenylhydrazanoethylidene] indolin-2-one (**10**)*

A mixture of 4 (2.64 g, 0.01 mol) and phenyl hydrazine (0.01 mol) in ethanol (30 mL) and acetic acid(3 mL) was refluxed for 3 h., cooled and treated with ice-cold water, and the obtained product was filtered and crystallized from ethanol. Orange red powder; yield (66%); m.p.: 185–187 °C; $R_f = 0.36$; IR: $\upsilon/cm^{-1} = 3157$ (br. 2NH & NH_2), 3050 (arom.), 2822 (aliph.), 1680 (CO), 1598 (CN). ^{1}HNMR: δ/ppm = 6.90–7.55 [m, 14H, Ar–H + CH=], 7.36 [s, 2H, NH_2], 10.99, 12.73 [2 s, 2H, 2NH]. ^{13}CNMR: 116.19, 116.70, 117.25, 120.04, 122.52, 125.07, 125.16, 125.67, 126.41, 126.72, 127.64, 127.94, 130.51, 135.55, 136.25, 140.39, 142.92, 161.67 (Ar–C), 161.69 (CN), 162.02 (CO). Mass spectrum exhibited a molecular ion peak at *m/z* 354 (M^+, 19.96%) with a base peak at *m/z*: 98; Anal. Calcd. for $C_{22}H_{18}N_4O$ (354.40): C, 74.56; H, 5.12; N, 15.81; Found: C, 74.71; H, 5.19; N, 15.97.

Biological activity

Cell lines

Human breast (MCF-7), hepatocellular (Hep-G2), colon (HCT-116) carcinoma cells and breast (MCF-12A) normal cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were grown in RPMI-1640 medium, supplemented with 10% inactivated fetal calf serum and 50 μg/ml gentamycin. The cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2.

Evaluation of the antitumor activity by MTT assay

The viability of control and treated cells was evaluated using the MTT assay in triplicate. The MTT assay is a laboratory test and a standard colorimetric assay (an assay which measures changes in color) for measuring cellular growth, Yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide, a tetrazole) is reduced to purple formazan in the mitochondria of living cells, and a solubilization solution (dimethyl sulfoxide) is added to dissolve the insoluble purple formazan product into a colored solution. Briefly, three tumor cell lines were seeded in 96-well plates containing 100 µL of the growth medium at a density of 1×10^4 cells/well. Cells were permitted to adhere for 24 h until confluence, washed with PBS, and then treated with different concentrations of compounds in fresh maintenance medium from 50 to 1.56 µg/mL and incubated at 37 °C for 24 h. A control of untreated cells was used in the absence of a test compound. Untreated cells used as negative control. Serial two-fold dilutions of the tested compounds were added into a 96-well tissue culture plate using a multichannel pipette (Eppendorf, Germany). After treatment (24 h), the culture supernatant was replaced by fresh medium. Then, the cells in each well were incubated at 37 °C with 100 µl of MTT solution (5 mg/mL) for 4 h. After incubation, the MTT solution was removed, then 100 µL of DMSO was added to each well. The absorbance was detected at 570 nm using a microplate reader (Sun Rise TECAN, USA). The absorbance of untreated cells was considered as 100%. The results were determined by three independent experiments [41].

Data analysis

The percentage cell viability was calculated as follows $= [1 - (ODt/ODc)] \times 100\%$, where ODt is the mean optical density of wells treated with the tested compound and ODc is the mean optical density of untreated cells. The tested compounds were compared using the IC_{50} value, i.e., the concentration of an individual compound leading to 50% cell death that was estimated from graphical plots of surviving cells versus compound concentrations.

Molecular modeling and docking

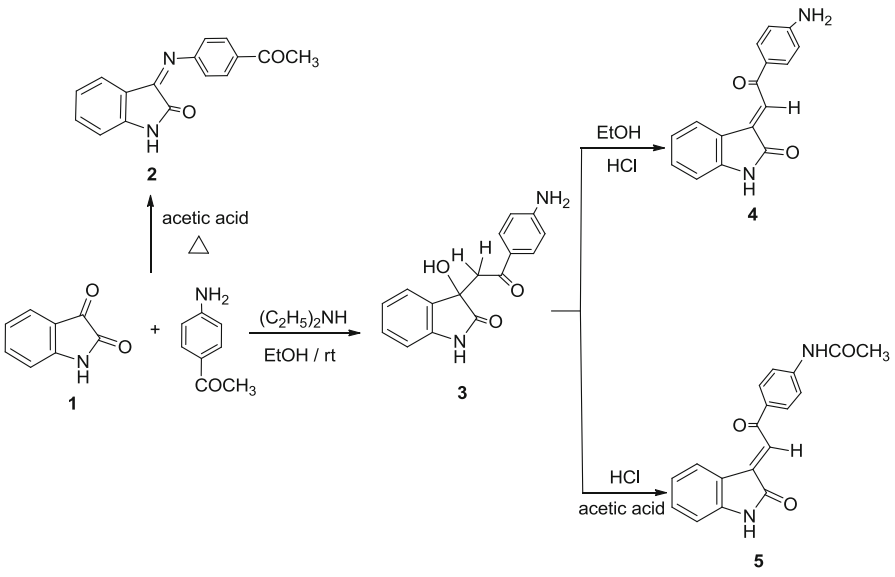
The molecular model of the new isatin derivatives was built using standard bond angles and lengths, with the Molecular Operating Environment (MOE) software suite 10.2008. Following geometry optimization, a systematic conformational search was carried out to RMS gradient of 0.05 Å with energy minimization of the resultant conformations employing the ConfSearch module implemented in MOE. All molecular mechanics computations were performed with the Merck Force Field (MMFF94s). The crystallographic structure of CDK2/CyA complex was obtained from the Protein Data Bank (PDB ID: 4BCQ). Hydrogen atoms were added to the enzyme and partial charges were calculated. Validation followed by docking of the compounds into the active site was carried out after removing the co-crystallized ligand. The target protein was kept rigid, while the ligands were left free to explore

the conformational space inside the enzyme cavity; 50 separate docking simulations were run using default parameters, and the conformations were chosen based on the combination of E conformation, S score data, and appropriate fitting with the relevant amino acids in the binding pocket.

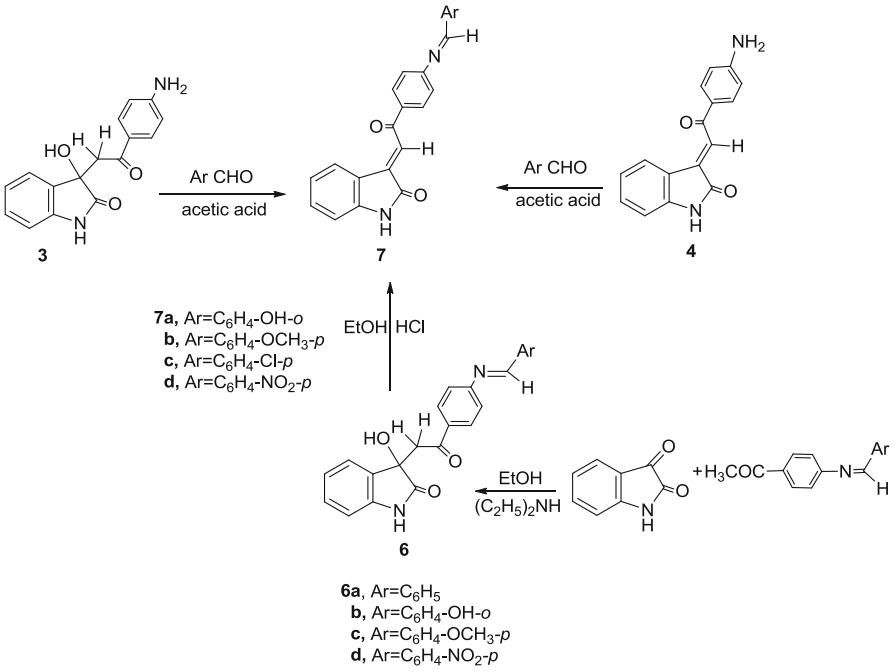
Results and discussion

Chemistry

The synthetic routes of the proposed compounds are outlined in Schemes 1, 2 and 3. *p*-amino-acetophenone has two nucleophilic centers, so its reaction with isatin depends on the reaction conditions. Condensation of *p*-aminoacetophenone with isatin in acetic acid afforded 3-[4-acetylphenyl) imino] indolin-2-one **2** [42]. On the other hand, crossed aldol condensation of *p*-aminoacetophenone with isatin in absolute ethanol, in the presence of 3 mL *N*,*N*-diethylamine (DEA) as a catalyst, gave 3-[2-(4-aminophenyl)-2-oxoethyl]-3-hydroxyindolin-2-one **3** [43]. This reaction undergoes aldol condensation through the reaction of the acetyl group of *p*-aminoacetophenone with the carbonyl function of isatin. Dehydration of compound **3** using dilute alcoholic hydrochloric acid yielded the expected α,β-unsaturated carbonyl compound, 3-[(2-(4-aminophenyl)-2-oxoethyl-idene]indolin-2-one **4**. The IR of **4** displayed absorption bands at 3455, 3328, and 3213 cm^{-1} due to the NH_2 and NH groups, and another at 1706 cm^{-1} due to the CO group. ^{1}HNMR exhibited the lack of the CH_2 and OH groups and the presence of signals due to NH_2 and aromatic protons. On repeating this reaction using a mixture of hydrochloric and



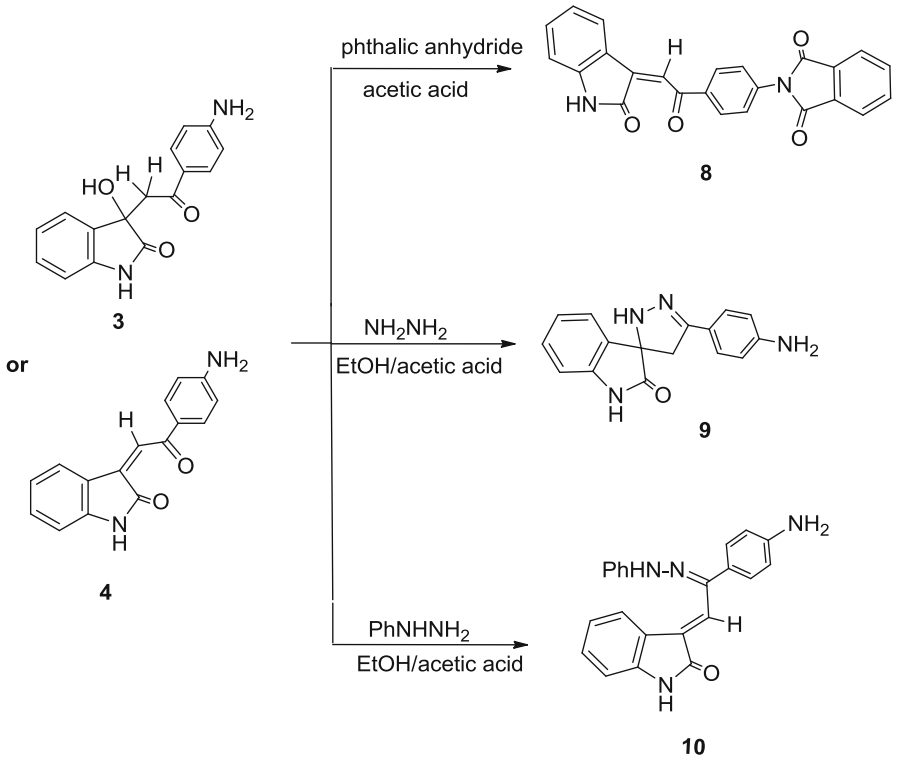
Scheme 1 Synthesis of the isatin derivatives **2–5**



Scheme 2 Synthesis of isatin-chalcone derivatives **6**, **7**

acetic acids (1:3), dehydration and acetylation of the amino group furnished the *N*-(4-(2-(oxoindolino-3-ylidene) acetamide **5** (Scheme 1). The IR of **5** revealed the lack of bands corresponding to the OH and NH_2 groups and showed broad bands around 3423 and 3230 cm^{-1} corresponding to the two NH groups and a band at 1703 cm^{-1} due to three CO groups. ^1H NMR showed the lack of the CH_2, OH and NH_2 groups and the presence of a singlet at 2.09 ppm for the CH_3, besides two singlet signals at 10.36 and 10.75 ppm corresponding to the two NH protons.

In the present work, a novel series of various substituted Schiff bases bearing the indolinone scaffold in their molecules were synthesized using three pathways; (1) condensation of compound **4** with some aromatic aldehydes afforded the corresponding Schiff bases **7a**–**d**; (2) condensation of the hydroxyl ethyl derivative **3** with aromatic aldehydes in acetic acid; and (3) the reaction of Schiff bases derived of *p*-aminoacetophenone with isatin furnished the hydroxyl ethyl derivatives **6a**–**d** which were subjected to dehydration with ethanolic hydrochloric acid to yield the same Schiff bases **7a**–**d** (Scheme 2). The IR of **6c** revealed the presence of OH and NH as a broad band around 3364 $cm^{-1}$and two carbonyl groups at 1725 and 1672 cm^{-1}. ^1H NMR exhibited two doublets at 3.58 and 4.07 ppm assigned to CH_2 protons, two singlets at 3.86 and 8.53 ppm specific for OCH_3 and azomethine protons, respectively, an other two singlets at 6.02 and 10.22 ppm due to OH and NH protons. ^{13}C NMR exhibited three signals at 46.12, 55.94 and 73.52 ppm due to OCH_3, CH_2 and C_3-indoline, besides two CO groups at 178.85 and 195.90 ppm. The mass spectrum showed a molecular ion peak at $m/z = 400$, corresponding to a



Scheme 3 Synthesis of the isatin derivatives **8–10**

molecular formula $C_{24}H_{20}N_2O_4$. The IR of **7b** showed the disappearance of absorption band corresponding to the NH_2 group and the presence of another at 3362 cm^{-1} due to the NH group, and 1725 and 1675 cm^{-1} due to the two CO groups. ^1H NMR exhibited two singlets at 3.84 and 8.55 ppm specific for the methoxy and azomethine protons, in addition to a singlet at 10.20 ppm exchangeable with D_2O due to the NH proton. The mass spectrum showed a molecular ion peak at $m/z = 382$ corresponding to the molecular formula $C_{24}H_{18}N_2O_3$.

In an attempt to collect indolinone and isoindolinone in one structure, condensation of the hydroxyethyl derivative **3** with phthalic anhydride in acetic acid furnished 2-[4-(2-(2-oxoindolin-3-ylidene)acetyl)phenyl]isoindoline-1,3-dione **8**. The IR of **8** is characterized by the absence of the amino and hydroxyl groups found in the starting material and the presence of absorption bands at 3193 and 1718 cm^{-1} corresponding to the NH and CO groups. ^{13}C NMR revealed aromatic carbons in the region 113.56–158.15 and four carbonyl carbons at 161.13 and 188.20 ppm. The mass spectrum showed a molecular ion peak at $m/z = 394$, corresponding to the molecular formula $C_{24}H_{14}N_2O_4$.

Finally, cyclo-condensation of α, β-unsaturated ketone **3** with hydrazine in ethanol containing acetic acid gave the spiro-pyrazoline derivative **9**. The IR of **9** showed bands at 3418, 3261, and 3166 cm^{-1} due to NH_2 and two NH groups, and a

Table 1 % Viability and IC_{50} of MCF-7 cells

Compound	1.56 μM	3.125 μM	6.25 μM	12.5 μM	25 μM	50 μM	IC_{50} (μM)
2	36.84 ± 0.026	28.91 ± 0.003	19.68 ± 0.001	13.82 ± 0.003	9.21 ± 0.014	5.43 ± 0.013	2.88
3	76.41 ± 0.012	43.52 ± 0.013	30.17 ± 0.002	21.48 ± 0.001	15.36 ± 0.007	8.51 ± 0.015	9.95
4	76.14 ± 0.014	58.21 ± 0.020	42.76 ± 0.012	34.07 ± 0.011	20.91 ± 0.015	14.86 ± 0.014	18.12
5	64.97 ± 0.042	38.05 ± 0.031	26.31 ± 0.017	19.79 ± 0.013	11.42 ± 0.012	7.53 ± 0.015	7.93
6b	89.95 ± 0.014	81.47 ± 0.001	64.73 ± 0.019	38.94 ± 0.016	26.18 ± 0.007	15.86 ± 0.031	25.41
6c	96.49 ± 0.012	91.83 ± 0.007	76.46 ± 0.002	43.23 ± 0.014	22.76 ± 0.014	13.58 ± 0.012	27.97
7b	93.52 ± 0.017	85.74 ± 0.019	74.65 ± 0.007	48.24 ± 0.006	32.69 ± 0.010‘	24.16 ± 0.013	31.66
7c	89.51 ± 0.001	74.86 ± 0.021	59.45 ± 0.023	37.21 ± 0.003	21.57 ± 0.005	12.46 ± 0.005	22.04
7d	91.96 ± 0.002	82.25 ± 0.031	68.94 ± 0.002	31.76 ± 0.012	17.62 ± 0.001	10.84 ± 0.003	23.73
8	97.81 ± 0.013	91.64 ± 0.005	83.97 ± 0.011	68.72 ± 0.008	38.53 ± 0.003	24.95 ± 0.012	51.08
9	94.08 ± 0.004	87.56 ± 0.009	72.68 ± 0.008	43.91 ± 0.009	29.14 ± 0.026	15.69 ± 0.017	40.28
Imatinib	79.46 ± 0.018	67.52 ± 0.012	48.89 ± 0.011	37.18 ± 0.014	26.72 ± 0.013	17.65 ± 0.014	12.29

band around 1650 cm^{-1} attributed to CO. ^1H NMR showed a doublet at 3.38 ppm due to the CH_2 and a singlet at 4.72 ppm attributed to the NH. On the other hand, the phenyl hydrazono derivative **10** was obtained by the treatment of hydroxyl ethyl derivative **3** or the unsaturated ketone **4** with hydrazine hydrate in ethanol containing acetic acid. The IR of **10** showed stretching frequencies around 3157 cm^{-1} due to NH_2 and two NH groups. ^1H NMR showed the absence of CH_2 protons and the presence of three singlet signals at 7.37, 10.99 and 12.73 ppm for the NH_2 and NH protons. ^{13}C NMR exhibited two signals at 161.69 and 162.67 ppm due to spiro pyrazoline derivative CN and CO groups.

Biological activity

Most of the newly synthesized compounds were screened for their in vitro anticancer activity against human breast (MCF-7), liver (HepG-2), colon (HCT-116) cancer cell lines and breast (MCF-12A) normal cell line. The tested compounds showed favorable activity against MCF-7 cells with IC_{50} ranging from 2.88 to 51.08 μM. Compounds **2**, **3** and **5** revealed higher antitumor activity (IC_{50} 2.88, 9.95 and 7.93 μM) against the MCF-7 cell line when compared with the standard drug Imatinib (IC_{50} 12.29 μM), as shown in Table 1 and Fig. 2. For the HepG-2 cell line, compounds **2–5** also showed higher activity (IC_{50} 9.23, 10.20, 5.22 and 7.70 μM), in comparison to Imatinib (IC_{50} 11.16 μM) (Table 2; Fig. 2). The same compounds, **2–5**, showed potent activity against the HCT-116 cell line in which the IC_{50} were 9.38, 11.4, 2.95 and 8.65 μM, respectively (Table 3; Fig. 2). In addition, compounds **7b** and **7d** showed anticancer activity against the HepG-2 cell line with IC_{50} of 13.95 and 12.84 μM, respectively. However, all the compounds show mild cytotoxicity against the normal breast cell line (MCF-12A) (Table 4). Furthermore, the most active compound against the HCT-116 cell line was compound **4** with IC_{50} of 2.95 μM followed by **5**, **2** and **3** as shown in Table 3 and Fig. 2. Also, compounds **7b** and **7d** showed moderate activity against the same cell line with IC_{50} of 11.78 and 15.04 μM, respectively.

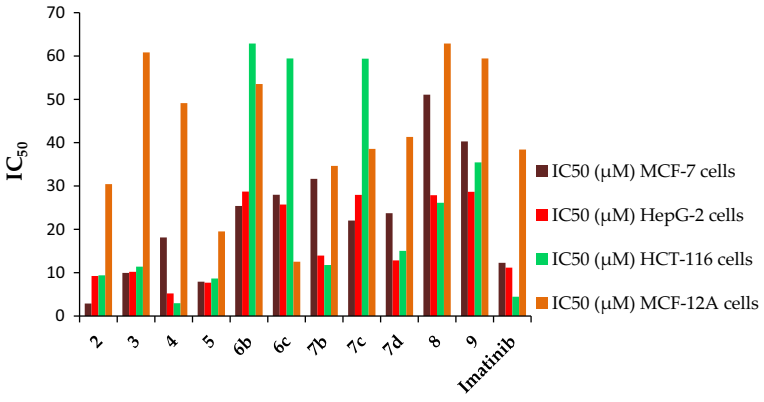


Fig. 2 IC_{50} of the tested compounds against human MCF-7, HepG-2, HCT-116 and MCF-12A cell lines. (Color figure online)

Table 2 % Viability and IC_{50} of HepG-2 cells

Compound	1.56 μM	3.125 μM	6.25 μM	12.5 μM	25 μM	50 μM	IC_{50} (μM)
2	56.49 ± 0.011	45.02 ± 0.003	30.84 ± 0.013	18.75 ± 0.003	11.69 ± 0.021	7.28 ± 0.006	9.23
3	67.38 ± 0.009	46.85 ± 0.016	34.67 ± 0.021	24.82 ± 0.021	12.96 ± 0.012	6.73 ± 0.011	10.20
4	46.89 ± 0.004	40.75 ± 0.018	32.80 ± 0.022	26.41 ± 0.012	17.93 ± 0.018	9.86 ± 0.021	5.22
5	58.46 ± 0.014	41.95 ± 0.020	29.43 ± 0.007	21.84 ± 0.010	12.37 ± 0.020	5.98 ± 0.026	7.70
6b	83.27 ± 0.021	76.54 ± 0.005	61.49 ± 0.002	46.78 ± 0.016	32.41 ± 0.006	21.35 ± 0.015	28.72
6c	86.48 ± 0.006	78.15 ± 0.033	65.32 ± 0.019	41.59 ± 0.028	30.98 ± 0.004	19.74 ± 0.002	25.72
7b	69.71 ± 0.005	61.48 ± 0.010	45.23 ± 0.031	36.47 ± 0.010	26.85 ± 0.016	17.29 ± 0.006	13.95
7c	87.61 ± 0.010‘	80.32 ± 0.019	62.47 ± 0.022	47.18 ± 0.006	34.85 ± 0.014	23.89 ± 0.003	27.96
7d	85.43 ± 0.017	68.19 ± 0.004	39.47 ± 0.019	26.85 ± 0.009	15.78 ± 0.011	8.92 ± 0.016	12.84
8	93.27 ± 0.018	84.65 ± 0.009	69.41 ± 0.010	43.87 ± 0.013	29.54 ± 0.007	16.36 ± 0.019	27.89
9	88.74 ± 0.011	76.21 ± 0.011	54.65 ± 0.021	37.86 ± 0.028	24.72 ± 0.005	12.81 ± 0.018	28.67
Imatinib	72.38 ± 0.007	61.94 ± 0.017	46.21 ± 0.006	34.67 ± 0.021	25.13 ± 0.013	14.98 ± 0.011	11.16

Table 3 % Viability and IC_{50} of HCT-116 cell

Compound	1.56 μM	3.125 μM	6.25 μM	12.5 μM	12.5 μM	25 μM	50 μM	IC_{50} (μM)
2	68.63 ± 0.021	36.95 ± 0.031	27.68 ± 0.014	20.47 ± 0.003	20.47 ± 0.003	13.94 ± 0.005	8.09 ± 0.021	9.38
3	78.17 ± 0.032	36.92 ± 0.013	18.74 ± 0.023	13.45 ± 0.018	13.45 ± 0.018	7.14 ± 0.021	50.43 ± 0.016	11.40
4	38.96 ± 0.013	31.84 ± 0.008	23.75 ± 0.027	17.08 ± 0.016	17.08 ± 0.016	12.63 ± 0.012	6.29 ± 0.009	2.95
5	71.48 ± 0.016	40.83 ± 0.012	25.07 ± 0.007	17.68 ± 0.023	17.68 ± 0.023	11.96 ± 0.019	6.43 ± 0.014	8.65
6b	98.78 ± 0.007	96.31 ± 0.019	86.24 ± 0.015	73.83 ± 0.009	73.83 ± 0.009	8.68 ± 0.006	32.57 ± 0.012	62.88
6c	98.61 ± 0.003	95.04 ± 0.021	87.43 ± 0.027	78.91 ± 0.003	78.91 ± 0.003	46.89 ± 0.003	34.62 ± 0.017	59.44
7b	73.47 ± 0.001	56.29 ± 0.020	41.95 ± 0.028	30.62 ± 0.012	30.62 ± 0.012	21.93 ± 0.012	14.87 ± 0.008	11.78
7c	98.43 ± 0.023	95.56 ± 0.014	86.39 ± 0.006	68.95 ± 0.017	68.95 ± 0.017	49.87 ± 0.011	36.94 ± 0.004	59.39
7d	87.69 ± 0.021	75.46 ± 0.006	47.64 ± 0.002	28.52 ± 0.016	28.52 ± 0.016	14.38 ± 0.030	7.06 ± 0.012	15.04
8	94.13 ± 0.017	86.57 ± 0.002	72.02 ± 0.016	38.14 ± 0.022	38.14 ± 0.022	24.61 ± 0.021	12.44 ± 0.025	26.14
9	95.92 ± 0.019	89.48 ± 0.028	70.89 ± 0.028	34.72 ± 0.021	34.72 ± 0.021	26.56 ± 0.015	13.47 ± 0.015	35.46
Imatinib	56.43 ± 0.022	6.92 ± 0.032	11.76 ± 0.021	20.45 ± 0.001	20.45 ± 0.001	29.18 ± 0.008	40.89 ± 0.018	4.46

Table 4 % Viability and IC_{50} on MCF-12A normal breast cell line

No.	1.56 μM	3.125 μM	6.25 μM	12.5 μM	25 μM	50 μM	IC_{50} (μM)
2	93.16 ± 0.013	86.69 ± 0.010	75.24 ± 0.006	48.65 ± 0.007	33.74 ± 0.019	21.64 ± 0.012	30.45
3	98.63 ± 0.004	94.32 ± 0.011	67.12 ± 0.017	61.32 ± 0.006	46.32 ± 0.014	36.12 ± 0.023	60.82
4	97.82 ± 0.011	91.12 ± 0.005	88.14 ± 0.013	67.92 ± 0.012	39.12 ± 0.003	24.72 ± 0.008	49.13
5	89.45 ± 0.023	75.16 ± 0.021	59.51 ± 0.001	38.46 ± 0.005	21.05 ± 0.005	13.21 ± 0.003	19.52
6b	97.97 ± 0.011	91.34 ± 0.005	84.81 ± 0.013	69.92 ± 0.012	41.12 ± 0.003	35.12 ± 0.023	53.54
6c	85.92 ± 0.016	65.78 ± 0.011	41.85 ± 0.009	30.47 ± 0.019	15.19 ± 0.004	9.43 ± 0.017	12.54
7b	95.92 ± 0.016	85.78 ± 0.011	70.85 ± 0.009	36.47 ± 0.019	27.19 ± 0.004	15.43 ± 0.017	34.64
7c	96.34 ± 0.015	90.12 ± 0.015	69.18 ± 0.021	37.21 ± 0.028	28.54 ± 0.028	18.89 ± 0.019	38.56
7d	95.11 ± 0.015	87.32 ± 0.015	72.34 ± 0.021	42.12 ± 0.028	28.21 ± 0.028	16.42 ± 0.019	41.33
8	98.24 ± 0.017	96.14 ± 0.026	87.91 ± 0.009	74.68 ± 0.008	31.56 ± 0.009	20.08 ± 0.004	62.88
9	98.31 ± 0.012	95.61 ± 0.006	82.51 ± 0.009	69.03 ± 0.015	50.04 ± 0.019	32.21 ± 0.007	59.44
Imatinib	96.14 ± 0.004	89.95 ± 0.011	68.98 ± 0.017	38.31 ± 0.006	29.14 ± 0.014	18.62 ± 0.023	38.41

Molecular docking

CDKs (cyclin-dependent kinases) are a group of serine-threonine protein kinases which play a significant role in cell cycle progression [44–46]. CDKs together with cyclins (Cy) manage cell division, cycle progression, neuronal function, differentiation, and apoptosis. These CDKs control cell division and survival through phosphorylation of proteins. Malignant tissues suffer from either overexpression of cyclins or CDK inhibitory proteins (CDKIs) suppression, resulting in tumor progression. From the literature review, it was found that indolinones posses cyclin/CDK complex inhibitory properties [23], which make them useful targets for chemotherapy [40].

In our present study to determine the possible mechanism of action of the target compounds, molecular docking of compounds **2**–**5** was performed in the active site of CDK2/CyA to explore their binding interactions with the amino acids inside the active site. The protein data bank file (PDB: 4BCQ) was selected for this purpose. The file contains CDK2/CyA enzyme co-crystallized with *N*,1,4,4-tetramethyl-8-((4-(4-methylpiperazin-1-yl)phenyl)amino)-4,5-dihydro-1H-pyrazolo (4,3-H) quinazoline-3-carboxamide (Pha-848125). All docking procedures were carried out using MOE software 10.2008. The docking protocol was verified by re-docking of the co-crystallized ligand in the vicinity of the active site of the enzyme with the energy score (S) = −10.82 kcal mol^{-1}and root mean standard deviation = 0.90. The co-crystallized ligand interacts with the active site of CDK2 by two interactions: Leu 83 with a hydrogen bond of 2.90 A° and Lys 33 with a hydrogen bond of 2.79 A°. All the docked compounds were fitted in the active site of the enzyme and the results of energy scores (S), as well as amino acids interactions, are listed in Figs. 3, 4, 5, 6 and Table 5. It is obvious that compound **4** shows the best

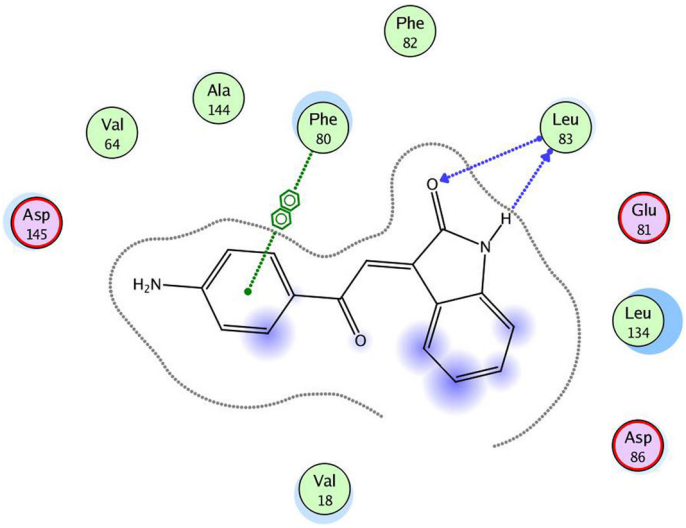


Fig. 3 2D ligand interaction of compound **4** with the active site amino acids of 4BCQ

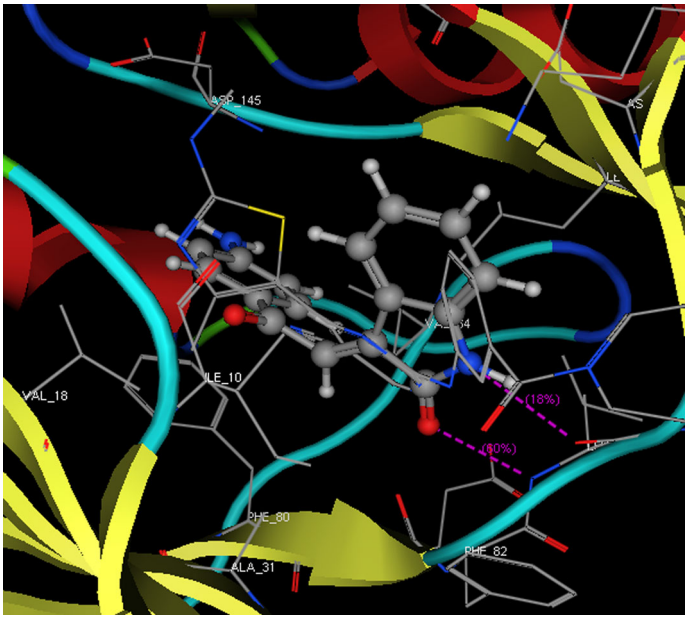


Fig. 4 3D Docking of compound **4** ($S = -10.04$ kcal mol^{-1}) in the active site of 4BCQ

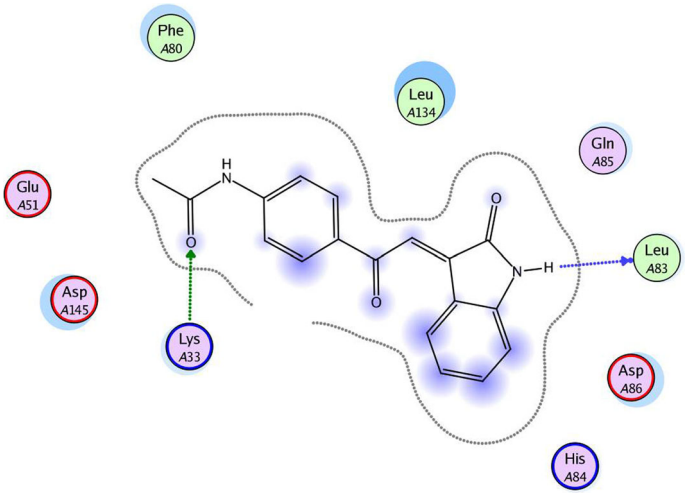


Fig. 5 2D ligand interaction of compound **5** with the active site amino acids of 4BCQ

docking score (-10.04 kcal mol^{-1}) and binding interactions, as it binds to the active site of CDK2 by three interactions: Leu 83 with a hydrogen bond of 1.94, 2.56 A° and Phe 80 with a hydrophobic bond of 2.47 A°.

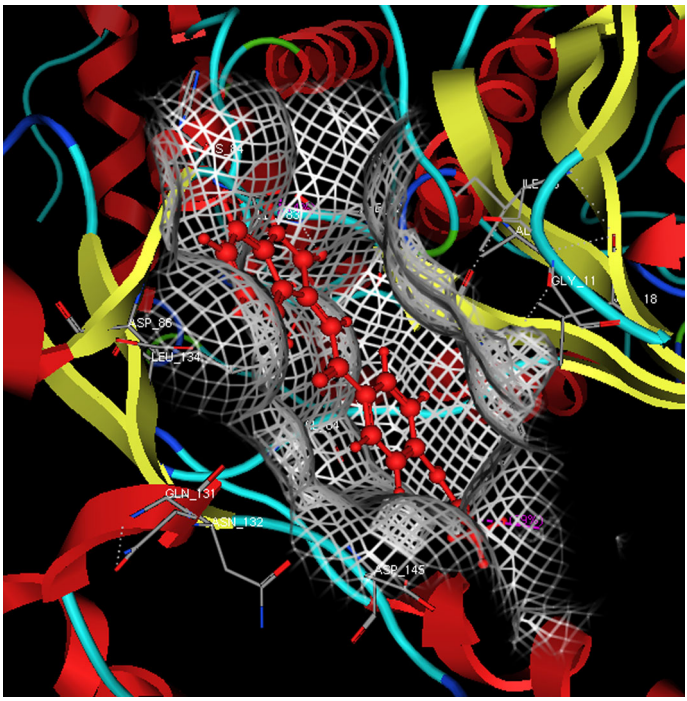


Fig. 6 3D Docking of compound **5** (*red*) ($S = -8.91$ kcal mol^{-1}) in the active site of 4BCQ showing the surface of the binding pocket to give an estimation of any possible steric hindrance. (Color figure online)

Table 5 Docking results of the most active compounds within CDK2/CyA active site

Compound	Energy score (S) (Kcal/mol)	Amino acids	Interacting groups	Length (Å)
Ligand	−10.82	Leu 83	NH	2.90
		Lys 33	CO	2.79
2	−9.15	Leu 83	NH	2.37
		Lys 33	CO	2.94
		Tyr 15	NH	2.03
3	−10.03	Leu 83	NH	2.19
		Lys 33	CO	2.65
4	−10.04	Leu 83	NH	1.94
		Leu 83	CO	2.56
		Phe 80	Phe	2.47
5	−8.91	Leu 83	NH	1.64
		Lys 33	CO	2.09

Conclusion

A new series of isatin-linked chalcones were synthesized. The target compounds were designed and synthesized as potential cyclin-dependent kinase 2 inhibitors (CDKI) and evaluated for their cytotoxic activity against three human carcinoma cell lines, MCF-7, HepG-2, and HCT-116, and MCF-12A normal breast cell line. Compounds **2–5** were found to be the most potent in this study compared to Imatinib, the reference drug. The acetyl derivative **2** showed the highest activity against MCF-7 with IC_{50} 2.88 μM followed by the acetamide derivative **5**, then, for compounds **3** and **4**, the activity was diminished but still stronger than the standard compound. The IC_{50} was 9.95 μM for the saturated compound **3** and 18.12 μM for the unsaturated compound **4**. On the other hand, the most active compound against the HepG-2 cell line was **4** with IC_{50} 5.22 μM followed by **5**, **2** and **3**. Compound **2** was found to be the most potent against MCF-7, while compound **4** was the most potent for both the HepG-2 and HCT-116 cell lines. The docking study showed that all the docked compounds exhibited similar binding interactions as those previously reported with the ligand when docked into the active site of CDK2/CyA. Compound **4** showed the best energy score and binding interactions, which suggests that these compounds may possibly act as CDK inhibitors and thus may participate in anticancer activity.

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