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Novel 3-((2-chloroquinolin-3-yl)methylene)indolin-2-one derivatives produce anticancer efficacy in ovarian cancer *in vitro*

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ABSTRACT

A novel series of 3-((2-chloroquinolin-3-yl)methylene)indolin-2-ones were synthesized, using the ‘molecular hybridization approach’ and evaluated for anticancer efficacy. Eleven 3-((2-chloroquinolin-3-yl)methylene) indolin-2-ones (**LM01** to **LM11**) were synthesized and evaluated for *in vitro* cytotoxic efficacy in cancer (ovarian, prostate and colon) and two non-cancerous cell lines. Among the 3-((2-chloroquinolin-3-yl)methylene)indolin-2-one derivatives, **LM08,** with a 6-Cl substitution in the 3-quinolinyl moiety, had selective and potent cytotoxic efficacy in the ovarian cancer cell line A2780. Further mechanistic investigations indicated that **LM08** significantly inhibited the clonogenic survival of A2780 cancer cells, which was mediated by inducing apoptosis.

1. Introduction

Despite several decades of intensive research, cancer still remains the leading cause of human deaths worldwide, accounting for an estimated 8.2 million deaths (around 13% of all deaths) in 2012 [1]. There are more than 200 types of cancer, making therapy extremely complicated and often inefficient [2]. Although chemotherapy is the mainstay for cancer treatment, the use of available chemotherapeutics is often limited due to severe or problematic adverse effects [3] and the development of multidrug resistance [4]. Consequently, there is an urgent need for novel, efficacious anticancer compounds with reduced toxicity.

1*H*-indole-2,3-dione (also known as isatin) is a scaffold of significant interest because of its broad spectrum biological properties [5], and its cytotoxic and antineoplastic efficacies have been widely investigated [6]. 1*H*-indole-2,3-dione and its derivatives inhibit cancer cell proliferation and tumor growth by interacting with a variety of intracellular targets such as DNA, telomerase, tubulin, P-glycoprotein, protein kinases and phosphatases [6,7]. The aryl/heteroarylidene indolin-2-ones scaffold has recently become a versatile template for discovering novel kinase inhibitors for clinical use in cancer therapy (Fig. 1) [6,8,9,10]. The success of aryl/heteroarylidene indolin-2-one as a new class of antineoplastic drugs is further supported by the approval of the oxindole, sunitinib maleate (Sutent ®), by the United States Food and Drug Administration for the treatment of advanced renal carcinoma [11], gastrointestinal stromal tumors [12] and neuroendocrine tumors of the pancreas [7,13]. The clinical success of sunitinib has led to several synthetic efforts in the search for anticancer drugs based on the heteroarylidene indolin-2-one scaffold. Furthermore, research has primarily been focused on replacing the pyrrole ring in the **C3** position indolin-2-one core of sunitinib with different heterocyclic moieties like imidazole, dihydropyridine, indoles, etc [6]. This strategy has been shown to be successful in many instances. For example, compound **3**, an indolinone containing a dihydropyridine instead of a pyrrole ring, is cytotoxic and was 3-fold more efficacious than sunitinib in HCT-116 colon, A549 lung and HepG2 liver cancer cells [7,14].

Another class of compounds potentially suitable for anticancer drug

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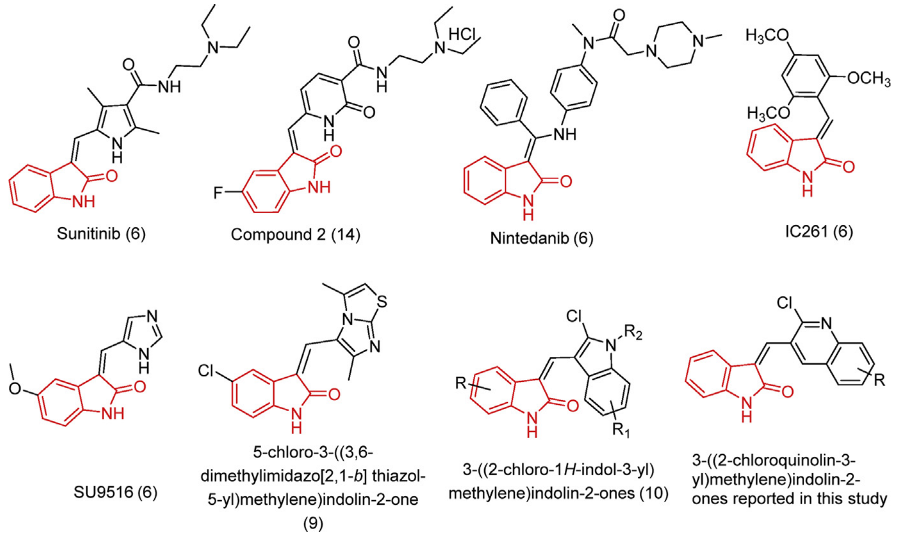


Sunitinib (6)

Compound 2 (14)

Nintedanib (6)

IC261 (6)

SU9516 (6)

5-chloro-3-((3,6-dimethylimidazo[2,1-*b*] thiazol-5-yl)methylene)indolin-2-one (9)

3-((2-chloro-1*H*-indol-3-yl) methylene)indolin-2-ones (10)

3-((2-chloroquinolin-3-yl)methylene)indolin-2-ones reported in this study

Fig. 1. Representative examples of aryl/heteroarylidene indolin-2-ones reported as potent anticancer compounds.

discovery are the quinolines and their derivatives [15]. Quinolines are inhibitors of tyrosine kinases, proteasomes, tubulin polymerization and DNA repair [15]. Furthermore, a combination of different bioactive fragments with complementary pharmacophoric functions often produced synergistic effects [16]. Based on these data and our ongoing efforts towards developing efficacious antitumor drugs through a "molecular hybridization approach" [17], we synthesized a series of novel 3-((2-chloroquinolin-3-yl)methylene)indolin-2-one derivatives, which incorporate a 2-chloroquinolinyl moiety at **C3** position indolin-2-one as novel anticancer drugs. The present study represents the first systematic study on the synthesis and anticancer efficacy evaluation of 3-((2-chloroquinolin-3-yl)methylene)indolin-2-ones.

2. Results & discussion

2.1. Chemistry

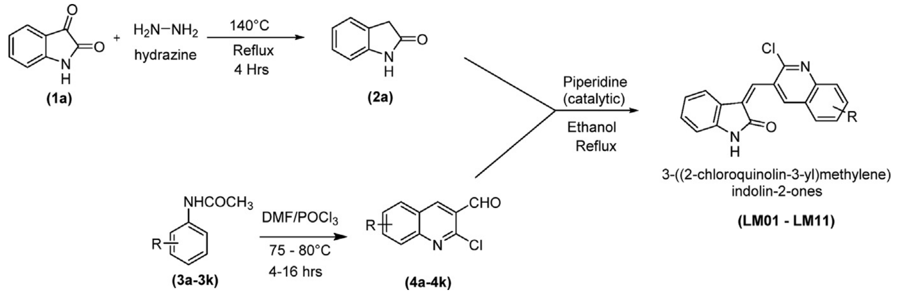
The synthetic protocol used to obtain the desired 3-((2-chloroquinolin-3-yl)methylene)indolin-2-ones (**LM01-LM11**) is illustrated in Scheme 1. The key intermediates, 2-chloroquinoline-3-carbaldehydes (**4a-4k**), were synthesized from commercially available acetanilides (**3a–3k**) *using* the Vilsmeier-Haack reaction as previously described [18, 19]. Another intermediate, indolin-2-one (**2a**), was obtained using the Wolff-Kishner reduction of isatin (**1a**) with hydrazine hydrate [20]. The reaction of indolin-2-one (**2a**) with 2-chloroquinoline-3-carbaldehydes (**4a-4k**) in ethanol, in the presence of piperidine (catalytic), yielded the target compounds, 3-((2-chloroquinolin-3-yl)methylene)indolin-2-ones (**LM01-LM11**). The structures of the newly synthesized compounds were confirmed by microanalyses, IR, MASS and NMR spectral experiments.

2.2. Anticancer efficacy evaluation

The synthesized compounds (**LM01-LM11**) were evaluated for *in vitro* cytotoxic efficacy in ovarian cancer (OV2008, A2780) colon carcinoma (HCT-116 and HT29), prostate cancer (PC3 and DU-145), human primary embryonic kidney (HEK293/pcDNA3.1) mouse fibroblast (NIH/3T3), and Chinese hamster ovarian (CHO) cell lines, using the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay [21]. All of the compounds were tested at several concentrations ranging from 0.1 to 100 μM. The concentration of the tested compounds that produces a 50% inhibition of cell growth (IC_{50}) was calculated. Table 1 summarizes the IC_{50} values obtained for the compounds evaluated in the cancer cell lines used in this study.

Among the 11 compounds tested for cytotoxic efficacy, compound **LM08,** with a 6-Cl substitution in the 3-quinolinyl moiety, had significant *in vitro* cytotoxic efficacy in the three tested ovarian tumor cell lines, with IC_{50} values ranging from 7.7 to 48.8 μM. Interestingly, compound **LM09,** a positional isomer of **LM08,** also showed a similar, but reduced efficacy profile in ovarian cancer cells, indicating that the chlorine substitution in the benzo ring of the 3-quinolinyl moiety may affect the cytotoxic efficacy of the 3-((2-chloroquinolin-3-yl)methylene)indolin-2-one derivatives on ovarian cancer cells. Other than the chloro-substituted derivatives, **LM01**, an unsubstituted derivative and compound **LM02**, a 6-methyl substituted derivative, also had cytotoxic efficacy ($IC_{50} \sim 50$ μM) in OV2008 cells, and **LM07**, an 8-methoxyl substituted derivative, had cytotoxicity ($IC_{50} = 32$ μM) in A2780 ovarian cancer cells.

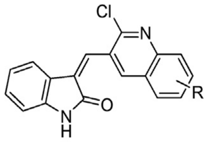
In the two prostate cancer cell lines, PC3 and DU-145, the growth of the PC3 cells was decreased by only two of the 3-((2-chloroquinolin-3-yl) methylene)indolin-2-one derivatives that had 6-OCH_3 (**LM05)** and 8-OCH_3 (**LM08**) substitutions at high concentrations ($IC_{50}\sim 90$ μM). In



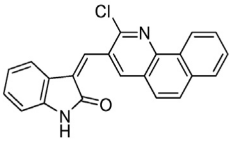
Scheme 1. Synthesis of 3-((2-chloroquinolin-3-yl)methylene)indolin-2-one derivatives (**LM01** to **LM11**).

Table 1

Cytotoxic efficacy of 3-((2-chloroquinolin-3-yl)methylene)indolin-2-ones on various cell lines (cancerous and non-cancerous).



(LM01 to LM10)



(LM11)

Compound Code	R	IC$_{50}$ (μM)[a]				
		OV2008	A2780	CHO	PC3	DU-145
		Ovarian			Prostate	
LM01	H	55.3	>100	>100	>100	77.5
LM02	6-CH$_3$	59.7	>100	80.0	>100	>100
LM03	6,7-di CH$_3$	NA	>100	NA	>100	>100
LM04	6-OCH$_3$	NA	>100	NA	>100	47.2
LM05	7-OCH$_3$	NA	>100	NA	98.1	>100
LM06	6,7-di OCH$_3$	NA	>100	NA	>100	>100
LM07	8-OCH$_3$	NA	31.9	NA	>100	11.0
LM08	6-Cl	48.8	7.7	38.5	91.1	91.2
LM09	7-Cl	49.1	22.7	39.2	>100	54.9
LM10	6-Br	NA	>100	NA	>100	>100
LM11	–	NA	>100	NA	>100	95.7

Compound Code	R	IC$_{50}$ (μM)[a]			
		HCT-116	HT-29	HEK293/pcDNA.3.1	3T3
		Colon		Normal	
LM01	H	>100	>100	64.5	>100
LM02	6-CH$_3$	52.1	>100	73.4	>100
LM03	6,7-di CH$_3$	>100	>100	97.4	>100
LM04	6-OCH$_3$	60.9	>100	39.9	>100
LM05	7-OCH$_3$	56.2	>100	>100	>100
LM06	6,7-di OCH$_3$	75.0	>100	>100	>100
LM07	8-OCH$_3$	>100	>100	80.8	>100
LM08	6-Cl	77.8	>100	64.3	>100
LM09	7-Cl	75.2	>100	63.2	>100
LM10	6-Br	41.3	>100	50.5	>100
LM11	–	>100	>100	75.3	>100

[a] IC$_{50}$ values are represented as the mean of three independent experiments performed in triplicate. A mean IC$_{50}$ value of 100 μM was the cut off.

contrast, DU-145 cells were significantly inhibited by six compounds in the series, **LM01, LM04, LM07-09,** and **LM11.** Compound **LM07,** containing an 8-methoxyl substitution in the 3-quinolinyl moiety, had the greatest cytotoxic efficacy in DU-145 cells, with an IC$_{50}$ value of 11 μM. The remaining five compounds had low to moderate cytotoxic efficacy in DU-145 cells, with IC$_{50}$ values ranging from 47 to 96 μM.

In the colon cancer cells lines, HT-29 and HCT-115, none of the synthesized compounds produced cytotoxicity in HT-29 cancer cells, even at the maximum concentration tested (100 μM). In contrast, seven of the 3-((2-chloroquinolin-3-yl)methylene)indolin-2-one derivatives had cytotoxic efficacy in HCT-115 cells. Compound **LM10,** with a 6-bromo substitution in the benzo ring of the 3-quinolinyl moiety, had the highest cytotoxic efficacy, with an IC$_{50}$ value of 41.3 μM. It is important to note that none of 3-((2-chloroquinolin-3-yl)methylene) indolin-2-ones, with the exception of **LM04 and LM10,** produced significant cytotoxicity in human embryonic kidney cells at a concentration of less than 50 μM. Furthermore, all of the 3-((2-chloroquinolin-3-yl) methylene)indolin-2-ones were non-cytotoxic in mouse fibroblast (3T3) cells at the maximum concentration used, suggesting that most of the compounds studied in this study were non-lethal in the normal cell lines.

Our results indicate that compound **LM08** may be a potential candidate for further investigations in terms of its potent and selective *in vitro* cytotoxicity in A2780 ovarian cancer cells. Subsequently, we determined the efficacy of **LM08** (10 or 20 μM) to inhibit the clonogenic survival of A2780 cells. The results (Fig. 2) indicate that **LM08**, at 10 or 20 μM, while negatively impacting the growth and survival of A2780 cells, also significantly inhibited the colony formation of A2780 cells.

A number of clinically approved anticancer drugs have been reported to produce cytotoxic effects by inducing apoptosis [22]. Therefore, we assessed the apoptosis-inducing potential of **LM08** in A2780 cells using the Alexa Fluor 488 annexin V kit for flow cytometry assay.

As shown in Fig. 3, the untreated cells (i.e. control cells) were primarily healthy, viable cells that showed little or no apoptosis (Q1: 94.1%, Q2: 5.87 %). However, there was a significant increase in annexin red fluorescence in cells incubated with **LM08,** indicating a significant shift in the number of cells showing apoptosis at 10 μM (Q1: 64.8%, Q2:

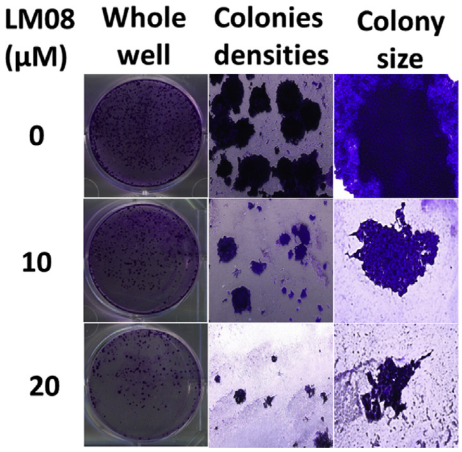


Fig. 2. LM08 inhibits colony formation in A2780 cells. The cells were incubated with different concentrations of LM08 (10 or 20 μM) or vehicle. The pictures show the effect of **LM08** on A2780 cells colony density and colony size. Each experiment was repeated independently in triplicate.

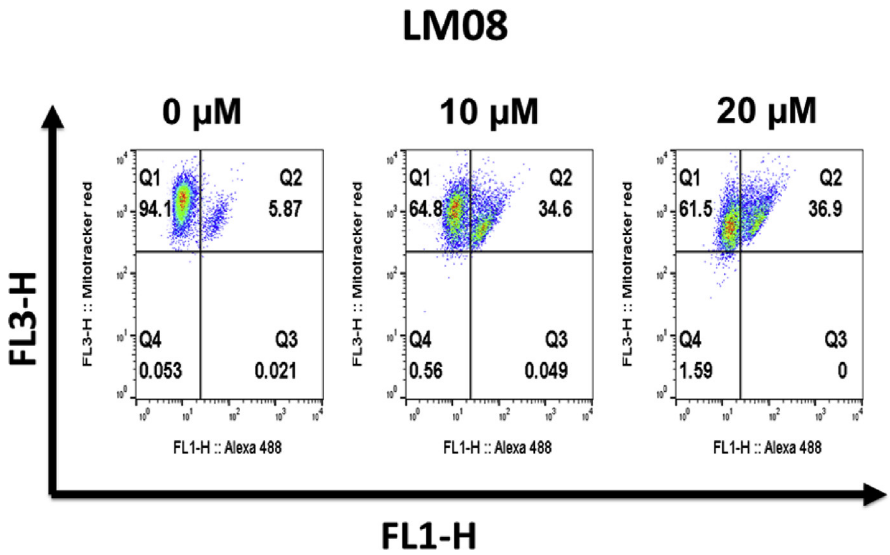


Fig. 3. **LM08** induces apoptosis in A2780 cells. A2780 cells in complete medium were incubated with **LM08** at 10 or 20 µM or vehicle for 24 h. Cells were then incubated with annexin red dye and analyzed by flow cytometry. The representative results for A2780 cells are from at least two independent experiments, each performed in triplicate.

34.6%) and 20 µM (Q1: 61.5%, Q2: 36.9%). These data indicate that apoptosis induction is one of the mechanisms by which **LM08** induces cytotoxicity in A2780 ovarian cells.

Nuclear condensation is one of the prominent hallmarks of apoptotic cell death [23]. Therefore, we determined the effects of **LM08** on the nuclear morphology of A2780 cells using DAPI staining. The nuclear changes in A2780 cells after incubation with different concentrations of **LM08** (0, 5,10 or 20 µM) for 24 and 48 h were visualized and recorded (Fig. 4**)**. The results, as shown in Fig. 4**,** confirm the development of nuclear condensation in A2780 cells incubated with 5, 10, or 20 µM of **LM08** at 24 and 48 h, whereas the control cells had a normal nuclear shape, consisting of an oval, non-condensed, shape. Thus, these results further validate the concept that **LM08**-induced cytotoxicity in A2780 cells is mediated by apoptosis.

3. Conclusion

In conclusion, a novel series of 3-((2-chloroquinolin-3-yl)methylene) indolin-2-one derivatives was designed, synthesized and evaluated for *in vitro* cytotoxic efficacy in ovarian, colon and prostate cancer cells and two non-cancerous cell lines. Among the 3-((2-chloroquinolin-3-yl)methylene)indolin-2-one derivatives, **LM08,** with a 6-Cl substitution in the 3-quinolinyl moiety, displayed selective and potent cytotoxic efficacy in the ovarian cancer cell line, A2780. Mechanistic experiments indicated that **LM08** significantly inhibited the clonogenic survival of A2780 cells, and this effect was mediated by apoptosis. Based on the results of this study, **LM08** may be an suitable molecule for the discovery of novel and selective anticancer drugs for the treatment of ovarian cancer.

4. Materials & methods

4.1. *Chemistry*

All reagents and solvents used in this study were obtained from commercial suppliers and used without further purification. The reaction progress was monitored by TLC, using silica gel 60 F-254 (0.25 mm) plates and the developed plates were visualized with UV light. Melting points were determined in open glass capillaries using a Veego digital melting point apparatus and are uncorrected.^{1}H NMR spectra were recorded using dilute solutions of $CDCl_3$ or DMSO-d_6 on a Bruker Avance II 400 spectrometer. Chemical shifts (δ) are reported in ppm relative to trimethylsilane, and coupling constants (J) are reported in Hz. Electrospray ionization mass spectra (ESI-MS) were acquired using an Applied BiosystemQtrap 3200 MS/MS system. Infrared (IR) spectral data were obtained using a Schimadzu FT-IR 8400S IR spectrophotometer with an ATR accessory. Elemental analyses were performed with a Vario Microcube CHNS analyzer (Elementar, NJ, USA).

4.1.1. *Synthesis of indolin-2-one (2a)*

Isatin (0.05 mol) was dissolved in 30 ml of hydrazine hydrate (0.6

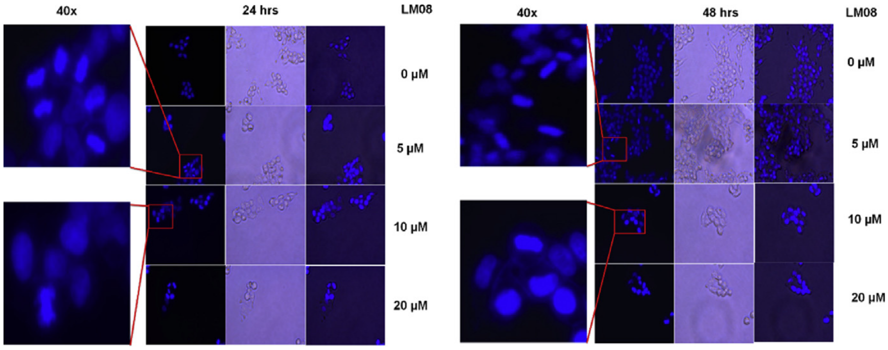


Fig. 4. The effect of **LM08** on the nuclear morphology of A2780 cells. The nuclear morphology changes in A2780 cells upon incubation with 5 or 10 µM of **LM08** or vehicle for 24 or 48 h, respectively, were detected using DAPI staining. Condensed and fragmented nuclei were observed under an EVOS fluorescent microscope at 40X.

mol) and refluxed at 140 °C for 4 hrs. The reaction mixture was poured into ice-water, acidified with 6N HCl and left at room temperature for 2 days to yield pure crystals of Indolin-2-one.

52 % yield, M.P. 127–128 °C, FT-IR (ATR) cm^{-1}: 3202 (NH), 3028 (Aromatic C–H), 2839 (Aliphatic C–H), 1689(C=O), 1616 (C=C). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.27 (br, s, 1H), 7.35–7.12 (m, 2H), 7.01 (t, *J* = 7.3 Hz, 1H), 6.91 (d, *J* = 7.9 Hz, 1H), 3.54 (s, 2H). MS-API [M + H]$^+$134 (calculated 133.05).

4.1.2. General procedure for the synthesis of 2 chloro 3 formyl quinolines (4a–4e)

Acetanilide and substituted acetanilides, **4a**–**4c** (0.05 mol) were dissolved in 9.6 ml of dimethyl formamide (0.125 mol) and 32 ml of phosphorus oxychloride (0.35 mol) was added to this solution gradually at 0 °C. The reaction mixture was placed in a round bottom flask (RBF equipped with reflux condenser fitted with a drying tube) and heated for 4–16 hrs on oil bath at 75–80 °C. The solution was cooled to room temperature and then poured on 100 ml of ice water. The precipitate was collected by filtration and recrystallized from ethyl acetate.

2-Chloroquinoline-3-carbaldehyde (**4a**)*:* 72 % yield, M.P. 148–150 °C (Lit.149 °C). FT-IR (ATR) cm^{-1}: 3044 (Aromatic C–H), 2870 (aldehyde C–H), 1684(C=O), 1574(C=N), 760 (C–Cl), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.57 (s, 1H), 8.77 (s, 1H), 8.08 (d, *J* = 8.5 Hz, 1H), 7.99 (d, *J* = 8.1 Hz, 1H), 7.90 (t, *J* = 7.7 Hz, 1H), 7.66 (t, *J* = 8.0 Hz, 1H). MS-API [M + H]$^+$192 (calculated 191.01).

2-chloro-6-methylquinoline-3-carbaldehyde(4b): 75 % yield, M.P. 122–123 °C (Lit.123 °C). IR (ATR) cm^{-1}: 3051 (Aromatic C–H), 2873 (aldehyde C–H), 1686 (C=O), 1576 (C=N), 752 (C–Cl). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.55 (s, 1H), 8.66 (s, 1H), 7.96 (d, *J* = 8.5 Hz, 1H), 7.79–7.63 (m, 2H), 2.57 (s, 3H). MS-API [M + H]$^+$206 (calculated 205.03).

2-chloro-6,7-dimethylquinoline-3-carbaldehyde (**4c**): 77 % yield, M.P. 156–157 °C (Lit. 156–157 °C). IR (ATR) cm^{-1}: 3055 (Aromatic C–H), 2893(aldehyde C–H), 1682 (C=O), 1576 (C=N), 750 (C–Cl). ^{1}H NMR (700 MHz, Chloroform-*d*) δ 10.52 (s, 1H), 8.63 (s, 1H), 7.83 (s, 1H), 7.69 (s, 1H), 2.51 (s, 3H), 2.47 (s, 3H).

2-Chloro-6-methoxyquinoline-3-carbaldehyde(4d): 63% yield, M.P. 145–146 °C (Lit.146 °C). IR (ATR) cm^{-1}: 3053 (Aromatic C–H), 2829 (aldehyde C–H), 1680(C=O), 1574(C=N), 1227, 1026 (C–O–C), 766 (C–Cl). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.53 (s, 1H), 8.63 (s, 1H), 7.95 (d, *J* = 9.2 Hz, 1H), 7.50 (ddd, *J* = 9.3, 2.9, 1.0 Hz, 1H), 7.18 (s, 1H), 3.94 (s, 3H). MS-API [M + H]$^+$222 (calculated 221.02).

2-Chloro-7-methoxyquinoline-3-carbaldehyde(4e): 78% yield, M.P. 195–196 °C (Lit 196 °C). IR (ATR) cm^{-1}: 3053 (Aromatic C–H), 2879 (aldehyde C–H), 1688 (C=O), 1583(C=N), 1240, 1043 (C–O–C), 760 (C–Cl). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.51 (s, 1H), 8.66 (s, 1H), 7.85 (d, *J* = 9.0 Hz, 1H), 7.38 (s, 1H), 7.27 (dd, *J* = 9.0, 2.5 Hz, 1H), 3.98 (s, 3H). MS-API [M + H]$^+$222 (calculated 221.02).

2-Chloro-8-methoxyquinoline-3-carbaldehyde (***4f***): 12% yield, M.P. 190–191 °C (Lit. 190 °C). IR (ATR) cm^{-1}: 3047 (Aromatic C–H), 2862 (aldehyde C–H), 1684 (C=O), 1574 (C=N), 1267, 1036 (C–O–C), 763 (C–Cl). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.57 (s, 1H), 8.72 (s, 1H), 7.64–7.46 (m, 2H), 7.32–7.17 (m, 1H), 4.10 (s, 3H). MS-API [M + H]$^+$222 (calculated 221.02).

2-chloro-6,7-dimethoxyquinoline-3-carbaldehyde (4g): 68% yield, M.P. 222°–224 °C (Lit. 222°–224 °C). IR (ATR) cm^{-1}: 3055 (Aromatic (C–H), 2893 (aldehyde C–H), 1682 (C=O), 1576 (C=N), 1248, 1051 (C–O–C), 750 (C–Cl). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.51 (s, 1H), 8.57 (s, 1H), 7.38 (s, 1H), 7.15 (s, 1H), 4.06 (s, 3H), 4.03 (s, 3H). MS-API [M + H]$^+$252.03 (calculated 251.03).

2,6-dichloroquinoline-3-carbaldehyde (4h): 12% yield, M.P. 191–192 °C (Lit.191 °C), IR (ATR) cm^{-1}: 3065 (Aromatic (C–H), 2883 (aldehyde C–H), 1686 (C=O), 1572 (C=N), 766 (C–Cl). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 10.58 (s, 1H), 8.70 (s, 1H), 8.05 (d, *J* = 9.0 Hz, 1H), 7.99 (d, *J* = 2.3 Hz, 1H), 7.84 (dd, *J* = 9.0, 2.3 Hz, 1H). MS-API [M + H]$^+$226 (calculated 224.97).

6-bromo-2-chloroquinoline-3-carbaldehyde (4i):22% yield, M.P. 187–188 °C (Lit. 188 °C), IR (ATR) cm^{-1}: 3053, 2912 (Aromatic (C–H), 1690 (C=O), 1574 (C=N), 1045 (C–Br), 760 (C–Cl). ^{1}H NMR (400 MHz, Chloroform-*d*): d (ppm) 10.55 (s, 1H), 8.73 (s, 1H), 8.07 (s, 1H), 7.93 (d, *J* = 8.5 Hz, 1H), 7.61 (d, *J* = 8.8 Hz, 1H). MS-API [M + H]$^+$270 (calculated 268.92).

2,7-dichloroquinoline-3-carbaldehyde (4j): 26% yield, M.P. 159–160 °C (Lit. 160 °C), IR (ATR) cm^{-1}: 3053 (Aromatic (C–H), 2847 (aldehyde C–H), 1676 (C=O), 1578 (C=N), 752 (C–Cl). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.53 (s, 1H), 8.72 (s, 1H), 8.06 (s, 1H), 7.91 (d, *J* = 8.8 Hz, 1H), 7.60 (dd, *J* = 8.7, 2.1 Hz, 1H). MS-API [M + H]$^+$226 (calculated 224.97).

2-chlorobenzo[h]quinoline-3-carbaldehyde (4k):82% yield, M.P. 210–211 °C (Lit. 210–212 °C), IR (ATR) cm^{-1}: 3038 (Aromatic (C–H), 2866 (aldehyde C–H), 1682 (C=O), 1574 (C=N), 752 (C–Cl). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.61 (s, 1H), 9.47–9.01 (m, 1H), 8.72 (s, 1H), 8.12–7.60 (m, 5H). MS-API [M + H]$^+$242 (calculated 241.03).

4.1.3. General procedure of 3-((2-chloroquinolin-3-yl)methylene)indolin-2-ones (LM01-LM11)

To a stirred a solution of indolin-2-one (1 mmol) in 5 ml of ethanol, 2–3 drops of piperidine and 2-chloroquinoline-3-carbaldehydes (1 mmol) were added at 0 °C. The mixture was then heated under reflux for 4–6 hrs. After the completion of the reaction, based on a single spot in TLC ($CHCl_3$: MeOH, 9:1), the precipitated solid was filtered and washed with cold ethanol to give the corresponding 3-((2-chloroquinolin-3-yl)methylene)indolin-2-ones.

3-((2-chloroquinolin-**3-yl*****)methylene)indolin-2-one*** **(LM01):**bright yellow crystals, 88 % yield, M.P. >275 °C, FT-IR (ATR) cm^{-1}: 3181 (NH), 3009 (Aromatic C–H), 1709(C=O), 1607, 1584 (C=C, C=N), 775 (C–Cl). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.56 (s, 1H), 8.13 (d, *J* = 8.4 Hz, 1H), 8.02 (br, s, 1H), 7.92 (s, 1H), 7.90–7.82 (m, 2H), 7.67 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H), 7.33–7.24 (m, 3H), 6.95 (d, *J* = 7.6 Hz, 1H), 6.84 (td, *J* = 7.7, 1.0 Hz, 1H). MRMS-API [M + H]$^+$307 (calculated 306.06), Anal. Calcd for$C_{18}H_{11}N_2$ C, 70.58; H, 3.61; N, 9.13; Found: C, 70.66; H, 3.64; N, 9.28.

3-((2-chloro-6-methylquinolin-**3-yl*****)methylene)indolin-2-one (LM02)***: 92 % yield, M.P. >275 °C, FT-IR (ATR) cm^{-1}: 3159 (NH), 3086 (Aromatic C–H), 2812 (Aliphatic C–H), 1707 (C=O), 1609, 1584 (C=C, C=N), 777 (C–Cl).^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.46 (s, 1H), 8.01 (d, *J* = 8.5 Hz, 2H), 7.92 (s, 1H), 7.68 (dd, *J* = 8.6, 1.9 Hz, 1H), 7.62 (s, 1H), 7.32–7.25 (m, 3H), 6.94 (d, *J* = 7.8 Hz, 1H), 6.84 (t, *J* = 7.7 Hz, 1H), 2.60 (s, 3H). MRMS-API [M + H]$^+$321 (calculated 320.07). Anal. Calcd for $C_{19}H_{13}N_2$ C, 71.14; H, 4.08; N, 8.73, Found: C, 71.22; H, 4.12; N, 8.82.

3-((2-chloro-6,7-dimethylquinolin-**3-yl*****)methylene)indolin-2-one (LM03)*****:** 89 % yield, M.P. >275 °C, FT-IR (ATR) cm^{-1}: 3159 (NH), 3086, 3015 (Aromatic C–H), 1709 (C=O), 1609, 1587 (C=C, C=N), 777 (C–Cl).^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.43 (s, 1H), 7.94 (d, *J* = 13.5 Hz, 2H), 7.88 (s, 1H), 7.59 (s, 1H), 7.38–7.20 (m, 4H), 6.94 (d, *J* = 7.8 Hz, 1H), 6.89–6.77 (m, 1H), 2.54 (s, 3H), 2.50 (s, 3H). MRMS-API [M + H]$^+$335.2 (calculated 334.09). Anal. Calcd for$C_{19}H_{13}N_2$ C, 71.75; H, 4.52; N, 8.37 Found: C, 71.82; H, 4.58; N, 8.42.

3-((2-chloro-6-methoxyquinolin-**3-yl*****)methylene)indolin-2-one (LM04)*****:** 84 % yield, M.P. >275 °C, FT-IR (ATR) cm^{-1}: 3161 (NH), 3094, 3015 (Aromatic C–H), 2895 (Aliphatic C–H), 1711 (C=O), 1614, 1584 (C=C, C=N), 1231, 1051 (C–O–C), 775 (C–Cl). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.42 (s, 1H), 8.01 (d, *J* = 9.3 Hz, 1H), 7.90 (s, 1H), 7.49 (dd, *J* = 9.3, 2.8 Hz, 2H), 7.32–7.25 (m, 2H), 7.10 (d, *J* = 2.8 Hz, 1H), 6.92 (d, *J* = 7.8 Hz, 1H), 6.85 (t, *J* = 7.7 Hz, 1H), 3.97 (s, 3H). MRMS-API [M + H]$^+$337.2 (calculated 336.07), Anal. Calcd for$C_{19}H_{13}N_2$ C, 67.76; H, 3.89; N, 8.32 Found: C, 67.83; H, 4.02; N, 8.39.

3-((2-chloro-7-methoxyquinolin-**3-yl*****)methylene)indolin-2-one (LM05)*****:** 81 % yield, M.P. >275 °C, FT-IR (ATR) cm^{-1}: 3184 (NH), 3084 (Aromatic C–H), 2974 (Aliphatic C–H), 1711 (C=O), 1605 (C=C, C=N), 1232, 1047 (C–O–C), 775 (C–Cl). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.47 (s, 1H), 7.91 (s, 1H), 7.74 (d, *J* = 8.9 Hz, 1H), 7.49 (s, 1H), 7.45 (d, *J* = 2.5 Hz, 1H), 7.34 (d, *J* = 7.7 Hz, 1H), 7.32–7.22 (m, 2H), 6.91 (d, *J* = 7.9 Hz, 1H), 6.85 (t, *J* = 7.6 Hz, 1H), 4.01 (s, 3H). MRMS-API [M + H]$^+$337.2 (calculated 336.07), Anal. Calcd for$C_{19}H_{13}N_2$ C, 67.76; H, 3.89; N, 8.32 Found: C, 67.82; H, 4.10; N, 8.42.

3-((2-chloro-6,7-dimethoxyquinolin-3-yl)methylene)indolin-2-one (LM06): 77 % yield, M.P. >275 °C, FT-IR (ATR) cm^{-1}: 3190 (NH), 3015 (Aromatic C–H), 1711 (C=O), 1614 (C=C, C=N), 1238, 1057 (C–O–C), 775 (C–Cl). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.38 (s, 1H), 7.92 (s, 1H), 7.53 (s, 1H), 7.45 (s, 1H), 7.35 (d, *J* = 8.4 Hz, 1H), 7.29–7.27 (m, 1H), 7.07 (s, 1H), 6.92 (d, *J* = 7.8 Hz, 1H), 6.87 (t, *J* = 7.9 Hz, 1H), 4.09 (s, 3H), 4.06 (s, 3H). MRMS-API [M + H]$^+$367.2 (calculated 366.08), Anal. Calcd for$C_{20}H_{15}N_2$; C, 65.49; H, 4.12; N, 7.64 Found: C, 65.72; H, 4.24; N, 7.73.

3-((2-chloro-8-methoxyquinolin-3-yl)methylene)indolin-2-one (LM07): 82 % yield, M.P. >275 °C, FT-IR (ATR) cm^{-1}: 3296 (NH), 3001 (Aromatic C–H), 1713 (C=O), 1612, 1564 (C=C, C=N), 1269, 1044 (C–O–C), 770 (C–Cl). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.48 (s, 1H), 8.00 (s, 1H), 7.89 (s, 1H), 7.56 (t, *J* = 7.9 Hz, 1H), 7.41 (d, *J* = 8.1 Hz, 1H), 7.23 (d, *J* = 7.2 Hz, 2H), 7.18 (d, *J* = 7.8 Hz, 1H), 6.91 (d, *J* = 7.9 Hz, 1H), 6.80 (t, *J* = 7.6 Hz, 1H), 4.12 (s, 3H). MRMS-API [M + H]$^+$337.2 (calculated 336.07), Anal. Calcd for$C_{19}H_{13}N_2$; C, 67.76; H, 3.89; N, 8.32**;** Found: C, 68.04; H, 3.99; N, 8.52.

3-((2,6-dichloroquinolin-3-yl)methylene)indolin-2-one(LM08): 87 % yield, M.P. >275 °C, FT-IR (ATR) cm^{-1}: 3144 (NH), 3026 (Aromatic C–H), 1699 (C=O), 1614, 1551 (C=C, C=N), 779 (C–Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.61 (s, 1H), 8.94 (s, 1H), 8.16 (d, *J* = 2.4 Hz, 1H), 7.98 (d, *J* = 9.0 Hz, 1H), 7.91–7.83 (m, 2H), 7.75 (d, *J* = 7.5 Hz, 1H), 7.26 (t, *J* = 7.7 Hz, 1H), 7.00 (t, *J* = 7.5 Hz, 1H), 6.82 (d, *J* = 7.7 Hz, 1H). MRMS-API [M + H]$^+$341 (calculated 340.02), Anal. Calcd for$C_{19}H_{13}N_2$; C, 63.36; H, 2.95; N, 8.21**;** Found: C, 63.52; H, 3.08; N, 8.36.

3-((2,7-dichloroquinolin-3-yl)methylene)indolin-2-one (LM09): 86 % yield, M.P. >275 °C, FT-IR (ATR) cm^{-1}: 3169 (NH), 3071 (Aromatic C–H), 1699 (C=O), 1614, 1551 (C=C, C=N), 789 (C–Cl). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.38 (s, 1H), 8.01 (s, 1H), 7.87 (t, *J* = 4.5 Hz, 2H), 7.73–7.42 (m, 4H), 7.11 (t, *J* = 7.7 Hz, 1H), 6.87 (d, *J* = 7.9 Hz, 1H). MRMS-API [M + H]$^+$341 (calculated 340.02), Anal. Calcd for$C_{19}H_{13}N_2$; C, 63.36; H, 2.95; N, 8.21**;** Found: C, 63.66; H, 3.12; N, 8.33.

3-((6-bromo-2-chloroquinolin-3-yl)methylene)indolin-2-one (LM10): 92 % yield, M.P. >275 °C, FT-IR (ATR) cm^{-1}: 3144 (NH), 3063, 3026 (Aromatic C–H), 1701 (C=O), 1614, 1553 (C=C, C=N), 785 (C–Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.61 (s, 1H), 8.93 (s, 1H), 8.32 (s, 1H), 8.06–7.81 (m, 3H), 7.75 (d, *J* = 7.2 Hz, 1H), 7.26 (t, *J* = 7.4 Hz, 1H), 7.00 (t, *J* = 7.2 Hz, 1H), 6.82 (d, *J* = 7.5 Hz, 1H). MRMS-API [M + H]$^+$385 (calculated 383.97), Anal. Calcd for$C_{18}H_{10}N_2$;**C, 56.06; H, 2.61;N, 7.26;** Found: C, 56.34; H, 2.83; N, 7.65.

3-((2-chlorobenzo[h]quinolin-3-yl)methylene)indolin-2-one (LM11): 89% yield, M.P. >275 °C, FT-IR (ATR) cm^{-1}: 3140 (NH), 3071, 3024 (Aromatic C–H), 1699 (C=O), 1616, 1583 (C=C, C=N), 787 (C–Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.62 (s, 1H), 9.12 (s, 1H), 9.09–8.96 (m, 1H), 8.33–7.67 (m, 8H), 7.26 (t, *J* = 7.7 Hz, 1H), 7.01 (t, *J* = 7.6 Hz, 1H), 6.84 (d, *J* = 7.8 Hz, 1H). MRMS-API [M + H]$^+$357.2 (calculated 356.07), Anal. Calcd for$C_{22}H_{13}N_2$; C, 74.06; H, 3.67; N, 7.85**;** Found: C, 74.57; H, 3.79; N, 7.92.

4.2. *Anticancer activity evaluation*

4.2.1. *Cell lines and culture*

Ovarian cancer (OV2008, A2780), colon carcinoma (HCT-116 and HT29), prostate cancer (PC3 and DU-145), normal human primary embryonic kidney (HEK293/pcDNA3.1) normal mouse fibroblast (NIH/3T3) and normal chinse hamster ovarian (CHO) cell lines were all used for cell cytotoxicity assay (MTT). All the cells were grown as monolayers in culture flasks using complete culture medium (DMEM) and 4.5 g of glucose, 10% FBS and 1% penicillin/streptomycin).

4.2.2. *Cell cytotoxicity by MTT assay*

The effect of the compounds on the cell survival was determined in different cancer and normal cell lines using the MTT assay. The MTT assay was conducted as previously as described [24,25]. The seeded cells in 96 well plates were incubated with different analogs at different concentrations for 72 hrs. The cells were further incubated with MTT for 4 hrs to produce the formation of the purple formazan crystals, which is indicative of viable cells. DMSO was added to dissolve the crystals and the absorbance was determined at 570 nm wavelength as described previously [26]. The IC_{50} was calculated and the selectivity of the compounds was determined by comparing survival in normal cells.

4.2.3. *Evaluation of cell cytotoxicity by colony formation assay*

The survival of ovarian cancer cells (A2780) in the presence of LM08 was further determined by colony formation assay as previously described in detail [26,27,28]. Briefly, A2780 cells were incubated with vehicle (0 μM) or LM08 (10 or 20 μM) for 24 hrs. The cells were reseeded at a very low density in six well plates to determine their efficacy to inhibit the formation of new cancer colonies over a period of 2 weeks. The colonies were then fixed and stained. The number of colonies for each treatment was counted under EVOS microscope (Themo Fisher Scientific, Wayne, MI, USA) and colony formation rate was calculated.

4.2.4. *Annexin V based apoptosis assay*

The induction of apoptosis in A2780 cells was evaluated using MitoTracker Red and Alexa Fluor 488 annexin V kits in combination with flow cytometry (Molecular Probes Inc., Invitrogen, Eugene, OR) as previously described [24] and according to manufacturer instructions. The cells were incubated with vehicle (0 μM) or LM08 (10 or 20 μM). The fluorescence of annexin V (499/521 nm) and MitoTracker Red (579/599 nm) was detected by flow cytometry (BD Accuri™ C6 flow cytometer and analyzed using FCS express 5 plus De Novo software) as shown earlier [28].

4.2.5. *Nuclear condensation-based apoptosis assay*

The detection of the nuclear changes, including nuclear condensation, to further confirm apoptosis, was conducted as previously described [29]. The A2780 were seeded and incubated with vehicle (0 μM) or LM08 (5, 10, or 20 μM) for 24 or 48 hrs. The cells were fixed and stained with DAPI nuclear staining. Finally, an EVOS microscope was used to detect the blue fluorescent staining of the nuclei and observe the nuclear changes produced by vehicle or LM08.

Declarations

Author contribution statement

Chandrabose Karthikeyan: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Haneen Amawi, Veronica Jones: Performed the experiments.

Charles Ashby, Vishwa Khare: Analyzed and interpreted the data; Wrote the paper.

Piyush Trivedi: Contributed reagents, materials, analysis tools or data. Critically edited the manuscript.

Hari Moorthy: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Amit Tiwari: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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