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Full Paper

Design, Synthesis and *In Vitro* Antiproliferative Activity of Novel Isatin-Quinazoline Hybrids

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Using a molecular hybridization approach, a new series of isatin-quinazoline hybrids **15a–o** was designed and synthesized *via* two different synthetic routes. The target compounds **15a–o** were prepared by the reaction of quinazoline hydrazines **12a–e** with indoline-2,3-diones **13a–c** or by treating 4-chloroquinazoline derivatives **11a–e** with isatin hydrazones **14a–c**. The *in vitro* anticancer activity of the newly synthesized hybrids was evaluated against the liver HepG2, breast MCF-7 and colon HT-29 cancer cell lines. A distinctive selective growth inhibitory effect was observed towards the HepG2 cancer cell line. Compounds **15b**, **15g** and **15l** displayed the highest potency, with IC_{50} values ranging from 1.0 ± 0.2 to 2.4 ± 0.4 μM, and they were able to induce apoptosis in HepG2 cells, as evidenced by enhanced expression of the pro-apoptotic protein Bax and reduced expression of the anti-apoptotic protein Bcl-2, in addition to increased caspase-3 levels.

Keywords: Antiproliferative activity / Isatins / Molecular hybridization / Quinazolines

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Introduction

Regardless of the notable improvement in survival among cancer patients over the past three decades [1], the growing incidence of drug resistance and unwanted side effects of cancer chemotherapeutics underlie the need to develop new classes of chemotherapeutic agents for cancer treatment [2]. A combinational chemotherapeutic drug with different mechanisms of action is one method that is being adopted to treat cancer [3–6]. The pharmacophore hybrid approach is an effective and commonly used strategy for the exploration of novel active agents. Hybridization of two or more bioactive fragments, each with complementary pharmacophoric function or with different modes of action, often showed a synergistic effect and could be beneficial for the treatment of cancer [7–9]. This "merged" pharmacophore may address the active site of various targets and offers the possibility of overcoming drug resistance.

Isatin (1*H*-indole-2,3-dione) and its derivatives demonstrate a broad spectrum of biological activities [10, 11]. Cytotoxic and antineoplastic properties of isatins are widely reported [12]. These reports were substantiated by FDA approval of the tyrosine kinase inhibitor sunitinib **1** for the treatment of gastrointestinal stromal cancers and metastatic renal cell carcinoma [13, 14]. Additionally, the structurally relevant SU9516 **2** was reported to be a potential inhibitor of cyclin-

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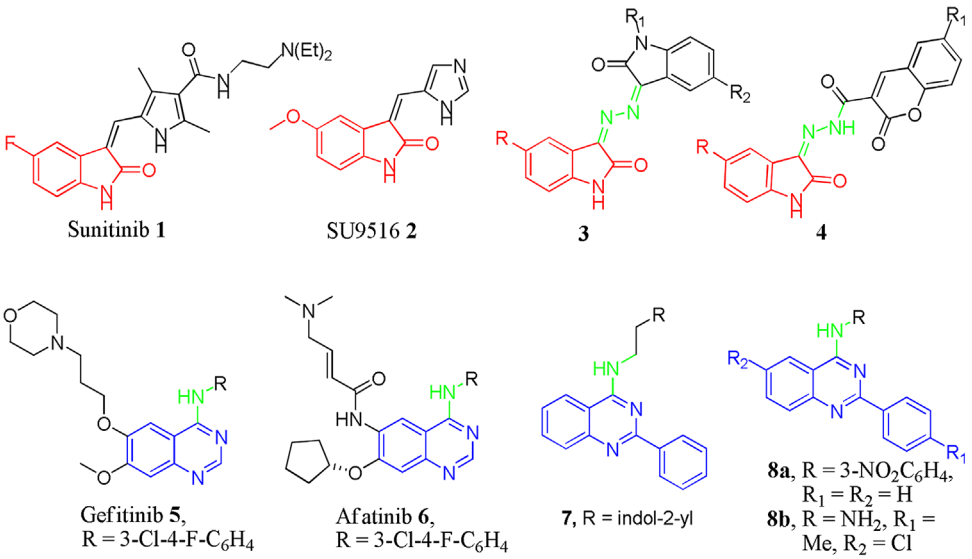

Figure 1. Structure of isatins **1–4** and quinazoline derivatives **5–9**.

dependent kinases (CDKs) that can induce apoptosis in colon carcinoma cells [15]. Moreover, isatin-3-arylhydrazones are a newly emerging class of pharmacologically important antineoplastic agents that are described as inhibitors of CDK2 [16], protein tyrosine phosphatase Shp2 [17] and c-Met kinase [18]. Other members of this class were reported to be effective against colorectal and hepatocellular carcinoma by virtue of their ability to inhibit tubulin polymerization, activate caspase-3 and induce apoptosis [19]. Furthermore, we have reported the synthesis of *N*,*N*′-hydrazino-bis-isatin derivative **3**, which has selective activity against multidrug-resistant cancer cells [20], and 2*H*-chromene-3-carbohydrazide-isatins **4** with *in vitro* antiproliferative activity and a good growth inhibitory profile against colon HT-29 cells [21] (Fig. 1).

On the other hand, quinazoline derivatives have attracted immense interest as novel class of cancer chemotherapeutic agents with significant therapeutic efficacy against solid tumors [22–24]. Quinazolines serve as the backbone for a range of tyrosine kinase inhibitors including the reversible EGFR inhibitor gefitinib **5** [23] and the dual EGFR-ErbB2 inhibitor afatinib **6** [24].

Quinazoline also forms the basis for compounds that inhibit serine/threonine kinase CDKs [25] and thymidylate synthase [26] or modulate aurora kinase activity [27]. In addition, 2-aryl quinazolines were recently identified as potential antitumor agents. For example, quinazoline derivative **7** enhanced the inhibition of NF-κB function and initiated apoptosis in the HepG2 liver cancer cell line [28]. Another analog, 2-phenyl-4-anilinoquinazoline **8a** proved to be a potent and selective breast cancer resistance protein (BCRP) inhibitor [29]. Meanwhile, the 2-*p*-tolylquinazoline derivative **8b** exhibited broad-spectrum antiproliferative activity toward HepG2, MCF-7 and HeLa human cancer cells [30] (Fig. 1).

Encouraged by the previous reports and in continuation to our efforts to develop effective anticancer agents based on the isatin scaffold [20, 21, 31], a new series of isatin-quinazoline conjugates **15a–o** was designed *via* a hybrid pharmacophore approach (Fig. 2).

Thus, the present study reports the synthesis and growth inhibition evaluation of the new hybrids **15a–o** against liver HepG2, breast MCF-7, and colon HT-29 cancer cell lines, in addition to providing some mechanistic insights for the anticancer activity of the promising derivatives.

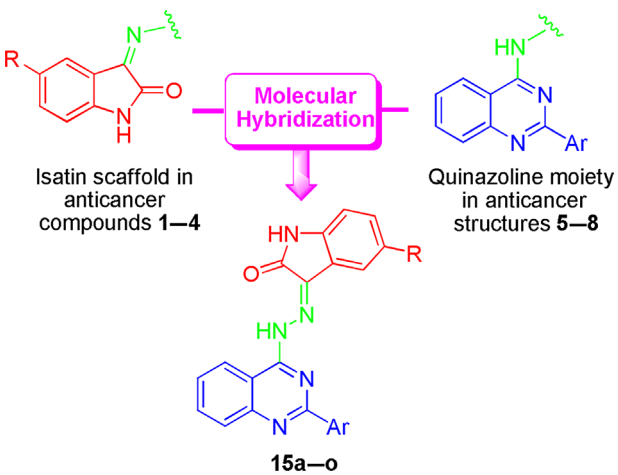

Figure 2. Design of the targeted isatin-quinazoline conjugates **15a–o**.

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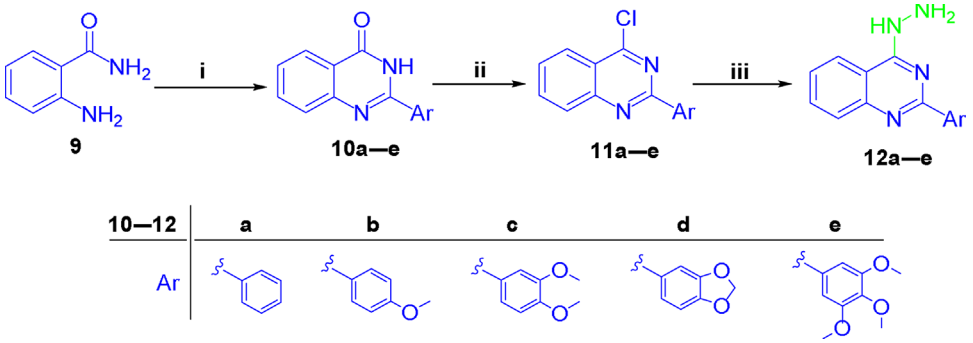


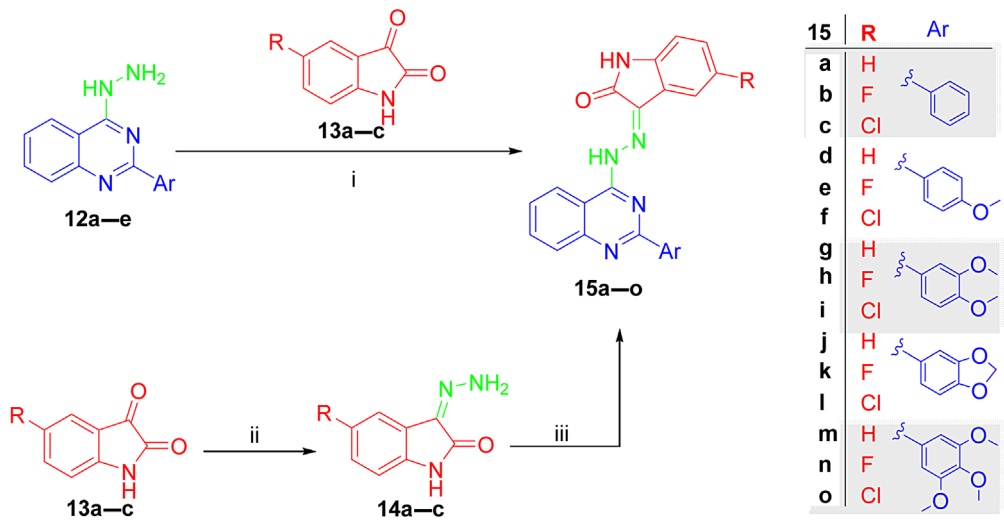
Scheme 1. Synthesis of compounds **12a–e**. Reagents and conditions: (i) Ar-CHO/DMF/I_2/K_2CO_3/70–90°C/4–5 h. (ii) $POCl_3$/*N*,*N*-dimethylaniline/reflux 6 h. (iii) $NH_2NH_2.H_2O$/EtOH/reflux 4 h.

Results and discussion

Chemistry

The synthetic pathway employed to obtain the target 3-(2-(2-phenylquinazolin-4-yl)hydrazono)indolin-2-one derivatives **15a–o** started by the preparation of 4-quinazolinones **10a–e** from 2-amino-benzamide (**9**) using a modified Niementowski reaction [29] (Scheme 1). The key intermediates 4-chloroquinazolines **11a–e** were obtained *via* chlorination of the 2-arylquinazolinone derivatives **10a–e** with phosphorous oxychloride in the presence of a catalytic amount of *N*,*N*-dimethylaniline as an acid binder [32–34]. Subsequent reaction of compounds **11a–e** with refluxing hydrazine hydrate led to the 4-hydrazinyl-2-substituted quinazoline derivatives **12a–e** with 70–79% yield (Scheme 1). IR spectra of the latter products showed the absorption bands due to the NH and NH_2 groups in the region δ 3273–3302 cm^{-1}. The ^1H NMR of compounds **12a–e** revealed two D_2O-exchangeable singlet signals of NH and NH_2 groups in the regions δ 9.56–9.62 and 4.86–4.91 ppm, respectively.

Preparation of the targeted compounds **15a–o** was achieved *via* two synthetic routes. First, the appropriate quinazoline hydrazines **12a–e** reacted with indoline-2,3-diones **13a–c**, in refluxing ethanol in the presence of a catalytic amount of glacial acetic acid to give **15a–o** with 79–91% yield (Scheme 2). Alternatively, indoline-2,3-diones **13a–c** were refluxed with hydrazine hydrate in absolute methanol to afford the corresponding hydrazine derivatives **14a–c** [31a], which, upon reaction with the appropriate 4-chloroquinazoline derivatives **11a–e** in refluxing isopropanol, furnished the target compounds **15a–o** with 60–82% yield (Scheme 2). The IR spectra of **15a–o** revealed the stretching band of NH around 3400 cm^{-1}. Furthermore, ^1H NMR spectra of these compounds showed a D_2O-exchangeable signal of NH proton of the isatin



Scheme 2. Synthesis of compounds **15a–o**. Reagents and conditions: (i) EtOH/AcOH (catalytic)/reflux 0.5 h. (ii) $NH_2NH_2.H_2O$/CH_3OH/reflux 0.5 h; (iii) **11a–e**/isopropanol/reflux 5 h.

moiety in the region δ 11.25–11.48 ppm whereas the proton of the hydrazine function (=N–NH–) appeared as two singlet signals resonating in the regions δ 10.61–10.79 and 13.75–13.90 ppm due to the hydrogen bonding.

Biological evaluations

In vitro antiproliferative activity

The newly synthesized isatin-quinazoline hybrids **15a–-o** were evaluated for their growth inhibition potential against three aggressive cancer cell lines, namely HepG2 hepatocellular carcinoma, MCF-7 breast cancer and HT-29 colon cancer cells using the sulforhodamine B (SRB) colorimetric assay [35]. Doxorubicin (Dox) was used as positive control as it is one of the most potent anticancer agents that possesses broad-spectrum against wide range of solid tumors and is known to exhibit potent pro-apoptic effects [36, 37]. Moreover, the reported activity of sunitinib **1** was included for comparison [38, 39]. The antiproliferative activities are expressed as the median growth inhibitory concentration (IC_{50}) and are provided in Table 1. From the results, it is evident that most of the tested compounds displayed potent to fair growth inhibitory activity against the HepG2 cancer cell line. Compounds **15b**, **15g**, and **15l** showed concentration-dependent cytotoxicity with IC_{50} values of 1.0 ± 0.2, 1.8 ± 0.4, and 2.4 ± 0.4 μM, respectively. The tested compounds were found to be more potent and efficacious than doxorubicin ($IC_{50} = 2.9 \pm 0.5$ μM). Moreover, compounds **15a**, **15c**, **15d**, **15e**, **15h**, and **15i** exhibited high potency at a low micromolar level. These results are in line with a previous study indicating that bis-Schiff base derivatives of isatin have antiproliferative activity against HepG2 cells [40].

Table 1. *In vitro* antiproliferative activities of the synthesized compounds against hepatocellular carcinoma (HepG2), colon cancer (HT-29), and breast cancer (MCF-7) cell lines.

	IC_{50} (μM)[a)]		
Compound 15	**HepG2**	**HT-29**	**MCF-7**
a	18.5 ± 1.7	>100	>100
b	1.0 ± 0.2	14.3 ± 1.1	7.4 ± 0.5
c	15.3 ± 1.1	>100	3.3 ± 0.4
d	13.5 ± 1.3	>100	>100
e	13.0 ± 2	>100	>100
f	28.2 ± 1.4	79 ± 6.1	>100
g	1.8 ± 0.4	26 ± 2.3	9.8 ± 0.8
h	6.4 ± 0.8	>100	>100
i	17.3 ± 1.5	>100	2.1 ± 0.19
j	25.0 ± 2.1	>100	>100
k	59.5 ± 4.9	>100	>100
l	2.4 ± 0.4	>100	>100
m	44.3 ± 3.7	>100	>100
n	>100	>100	5.5 ± 0.42
o	23.6 ± 1.9	>100	>100
Dox	2.9 ± 0.5	7.7 ± 0.6	0.24 ± 0.03
Sunitinib [38, 39]	12.0	1.6	5

[a)] IC_{50} values are the mean $\pm$ S.D. of three separate experiments.

Growth inhibition evaluation in MCF-7 cell line revealed that compounds **15b**, **15c**, **15g**, **15i**, and **15n** were the most potent ones, with IC_{50} of 7.4 ± 0.5, 3.3 ± 0.4, 9.8 ± 0.8, 2.1 ± 0.19, and 5.5 ± 0.42 μM, respectively. IC_{50} values of the positive control Dox was 0.24 ± 0.03 μM. Investigations of the antiproliferative activity against HT-29 cells indicated that this cell line was the least sensitive to the cytotoxicity of the new hybrids. Only compounds **15b** and **15g** were moderately active, with IC_{50} of 14.3 ± 1.1 and 26.0 ± 2.3 μM, respectively, while the analog **15f** exhibited weak potency ($IC_{50} = 79.0 \pm 6.1$ μM) relative to the reference drug Dox ($IC_{50} = 7.7 \pm 0.6$ μM) in this cell type.

From the aforementioned results, it was clear that the evaluated hybrids displayed distinctive selectivity towards HepG2 cancer cells, and only compounds **15b** and **15g** displayed broad spectrum anticancer activity against the three tested cell lines but with preferential potency in the HepG2 cell line. Also, it can be observed that the new isatin-quinazoline hybrids **15b**, **15g**, and **15l** demonstrated enhanced cytotoxic activity against HepG2 cells compared to sunitinib **1** and could be considered as good candidates for future investigation.

Effects on mitochondrial apoptosis pathway proteins Bax and Bcl-2

Hepatocellular carcinoma (HCC) is the fifth most frequent cancer worldwide and the third most common cause of cancer-related death [41]. Screening of the synthesized compounds in HepG2 cancer cells revealed that compounds **15b**, **15g**, and **15l** showed the highest potency for cytotoxicity compared to the other tested compounds. Previous studies indicated that induction of apoptosis is one of the main mechanisms behind the anticancer activities of several isatin and quinazoline derivatives [42–46]. Therefore, we investigated the potential pro-apoptotic effects of these promising isatin-quinazoline hybrids in an attempt to define the underlying mechanism for their antiproliferative activity.

Exposure of HepG2 hepatocellular carcinoma cells to compounds **15b**, **15g**, or **15l** for 48 h resulted in a significant increase in the expression of the pro-apoptotic protein Bax by approximately 2.6-, 1.8-, and 2.2-fold, respectively, compared to the control (Fig. 3A). However, the positive control Dox significantly boosted Bax level by about 3.6 compared to untreated cells. Bax is a member of the Bcl-2 family but has pro-apoptotic effects. Reduced expression of Bax is known to be associated with resistance to chemotherapy in many types of cancers including liver cancer [47].

Moreover, treatment of HepG2 cells with compounds **15b**, **15g**, or **15l** significantly reduced the expression levels of the anti-apoptotic protein Bcl-2 by approximately 57, 69, and 60%, respectively, compared to the control (Fig. 3B). The effect of the tested agents on Bcl-2 was more potent compared to Dox that elicited 60% reduction in Bcl-2 level compared to control untreated cells. Bcl-2 family proteins have been shown to be an important factor for apoptosis

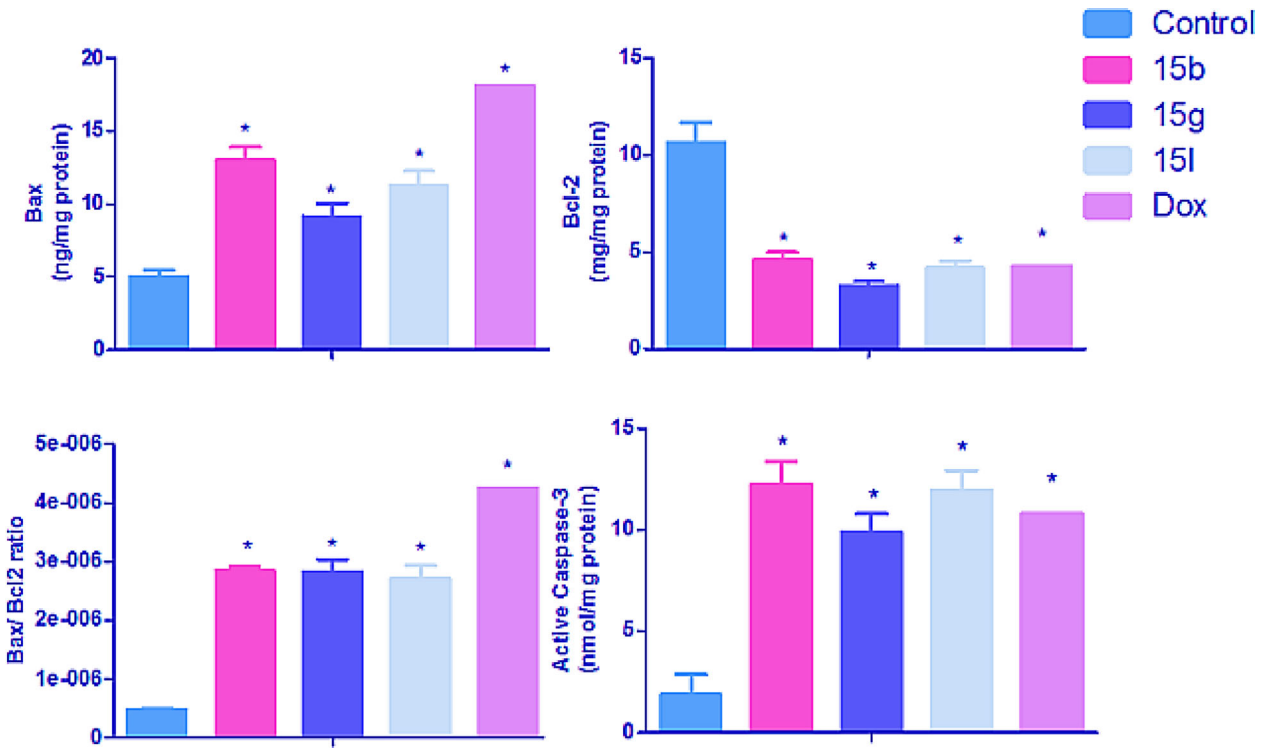


Figure 3. Effect of compounds **15b**, **15g**, and **15l** on the the expression levels of (A) Bax, (B) Bcl-2, (C) Bax/Bcl-2 ratio and (D) active caspase-3 in HepG2 cancer cells treated with the compounds at their IC_{50} concentrations. Data are the mean ± S.D. of three separate experiments. *Significantly different from control at $P < 0.05$.

resistance in HCC. Bcl-2 is known to inhibit apoptosis and to promote the process of tumorigenesis and chemoresistance [41]. The ability of the tested compounds to significantly down-regulate Bcl-2 levels indicates their potential to enhance apoptosis.

The ratio of Bax (apoptosis inducer) to Bcl-2 (apoptosis suppressor) is considered a key indicator of therapeutic response to chemotherapy [48]. The tested compounds **15b**, **15g**, or **15l** showed significant elevation in Bax/Bcl-2 ratio by about 7 folds compared to control (Fig. 3C) which supports their ability to promote the therapeutic response in HepG2 cells. The reference drug Dox triggered a significant increase in Bax/Bcl-2 ratio by about 9 folds compared to control.

Effects on the level of active caspase-3 (key executor of apoptosis)

Reduced expression of Bcl-2 leads to increased levels of free Bax, which in turn leads to activation of the caspase cascade leading to apoptosis [49]. In the current study, compounds **15b**, **15g**, and **15l** elevated the Bax/Bcl-2 ratio. Therefore, the subsequent step was to assess the levels of active caspase-3, a cysteine protease, which is a key executor of apoptosis [50]. Treatment of HepG2 cells with compounds **15b**, **15g**, and **15l** produced a significant increase in the level of active caspase-3 by approximately 6.6-, 5.3-, and 6.4-fold, respectively, compared to the control (Fig. 3D). It is noteworthy that the tested compounds resulted in more induction in active caspase-3 compared to the reference drug. Dox treatment resulted in significant increase in caspase-3 levels by about 6 folds compared to control. Active caspase-3 indicates that cells are undergoing apoptosis because this caspase **is** activated at the end of the apoptosis cascade [50].

In conclusion, compounds **15b**, **15g**, and **15l** have potent apoptotic potential through, at least in part, inducing the mitochondrial apoptotic pathway. They enhanced the expression of the pro-apoptotic protein Bax and reduced the expression of the anti-apoptotic protein Bcl-2 in addition to boosting caspase-3 levels.

Conclusion

We have presented two synthetic approaches for the synthesis of the novel isatin-quinazoline hybrids **15a–o**. The anticancer activity of the newly synthesized compounds was evaluated against liver HepG2, breast MCF-7, and colon HT-29 cancer cell lines. Most of the compounds exhibited relatively potent and selective growth inhibition against the HepG2 human HCC cell line. Compounds **15b**, **15g**, and **15l** ($IC_{50} = 1.0 \pm 0.2$, 1.8 ± 0.4,

and 2.4 ± 0.4 μM, respectively) were found to be more potent than doxorubicin (IC_{50} = 2.9 ± 0.5 μM). Compounds **15b**, **15g**, and **15l** were studied further to gain mechanistic insights into their antiproliferative activity by assessing their effect on the intrinsic apoptotic pathway in HepG2 cells. The results indicated that the tested compounds induced the activation of caspase-3. Furthermore, the Bcl-2/Bax ratio, which is crucial for the activation of the mitochondrial apoptotic pathway, was significantly reduced in cells treated with compounds **15b**, **15g**, and **15l**. These results indicate that compounds **15b**, **15g** and **15l** exert an antiproliferative effect in HepG2 liver cancer cells, which is, at least in part, *via* activation of the mitochondrial apoptotic pathway.

Experimental

Chemistry

Melting points were measured with a Gallenkamp apparatus and were uncorrected. IR spectra were recorded on Shimadzu FT-IR 8101 PC infrared spectrophotometer. The NMR spectra were recorded on a 300 NMR spectrometer. ^{1}H spectra were run at 300 MHz, and ^{13}C spectra were run at 75 or 100 MHz in deuterated dimethylsulfoxide (DMSO-d_6). Chemical shifts (δ_H) are reported relative to TMS as the internal standard. All coupling constant (*J*) values are given in Hertz. Chemical shifts (δ_C) are reported relative to DMSO-d_6 as internal standards. The abbreviations used are as follows: s, singlet; d, doublet; m, multiplet. Mass spectra were measured on a GCMS-QP1000 EX spectrometer at 70 eV. Elemental analyses were carried out at the Regional Center for Microbiology and Biotechnology, Al-Azhar University, Cairo, Egypt. Analytical thin layer chromatography (TLC) on silica gel plates containing a UV indicator was routinely employed to follow the course of reactions and to check the purity of the products. All reagents and solvents were purified and dried by standard techniques.

*General procedure for the preparation of 4-chloroquinazolines **11a–e***

2-Substituted-quinazolin-4(*3H*)-ones **10a–e** (0.01 mmol) were added portion-wise to a cooled stirred mixture of phosphorus oxychloride (15 mL) and *N*,*N*-dimethylaniline (0.5 mL). The reaction mixture was refluxed for 6 h. Excess phosphorus oxychloride was removed under vacuum, and the residue was cooled, triturated with ice/water and alkalinized with 2 N NaOH. The aqueous solution was extracted three times with DCM. The combined DCM extracts were dried over Na_2SO_4 and filtered, and the solvent was evaporated under reduced pressure. The obtained solid was crystallized from ethanol to give **11a–e** [32–34].

*General procedure for the preparation of 4-hydrazinyl-2-substituted quinazolines **12a–e***

A solution of 99% hydrazine hydrate (1 mL, 20 mmol) in ethanol (5 mL) was added to a stirred solution of 4-chloroquinazolines **11a–e** (5 mmol) in refluxing ethanol (20 mL). The reaction mixture was refluxed for 4 h and left to cool. The precipitated solid was filtered, dried and recrystallized from acetonitrile to give **12a–e**, respectively.

*4-Hydrazinyl-2-phenylquinazoline (**12a**)*

The physical properties and spectral data of this compound are identical with those that have been reported [51].

*4-Hydrazinyl-2-(4-methoxyphenyl)quinazoline (**12b**)*

Pale yellow powder; yield (73%); m.p. 292−294°C; IR ν 3300 (NH, NH_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 3.84 (s, 3H, -OCH_3), 4.90 (s, 2H, NH_2, D_2O exchangeable), 7.00 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.37–7.42 (m, 1H, Ar-H), 7.70 (m, 2H, Ar-H), 8.14–8.21 (m, 1H, Ar-H), 8.5 (d, *J* = 8.7 Hz, 2H, Ar-H), 9.57 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 55.7, 113.9, 122.7, 125.3, 126.3, 127.8, 129.9, 130.2, 131.4, 132.9, 135.0, 159.3, 161.5; Anal. calcd. for $C_{15}H_{14}N_4O$ (266.30): C, 67.65; H, 5.30; N, 21.04. Found: C, 67.89; H, 5.37; N, 21.18.

*2-(3,4-Dimethoxyphenyl)-4-hydrazinylquinazoline (**12c**)*

Pale yellow powder; yield (77%); m.p. 180−182°C; IR ν 3302 (NH, NH_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 3.84 (s, 3H, -OCH_3), 3.89 (s, 3H, -OCH_3), 4.88 (s, 2H, NH_2, D_2O exchangeable), 7.04 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.39–7.42 (m, 1H, Ar-H), 7.72–7.73 (m, 2H, Ar-H), 8.15–8.19 (m, 3H, Ar-H), 9.56 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 55.5 (2C), 111.1, 111.4, 112.6, 121.4, 122.2, 124.8, 127.4, 131.2, 132.5, 148.3, 149.5, 150.8, 159.0, 159.7; MS *m/z* (Rel. Int.) 297 [$(M+1)^+$, 17.6%], 296 [M^+, 100.0%]; Anal. calcd. for $C_{16}H_{16}N_4O_2$ (296.33): C, 64.85; H, 5.44; N, 18.91. Found: C, 65.04; H, 5.49; N, 19.09.

*2-(Benzo[d][1,3]dioxol-5-yl)-4-hydrazinylquinazoline (**12d**)*

Pale yellow fibers; yield (70%); m.p. 230−232°C; IR ν 3302, 3277 (NH, NH_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 4.86 (s, 2H, NH_2, D_2O exchangeable), 6.10 (s, 2H, -CH_2), 7.00 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.39–7.42 (m, 1H, Ar-H), 7.71–7.72 (m, 2H, Ar-H), 8.07 (s, 1H, -Ar-H), 8.15–8.20 (m, 2H, Ar-H), 9.56 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 101.8, 108.3, 108.5, 113.1, 122.7, 123.2, 125.4, 128.0, 133.0, 133.4, 147.9, 149.5, 149.9, 159.2, 160.3; Anal. calcd. for $C_{15}H_{12}N_4O_2$ (280.29): C, 64.28; H, 4.32; N, 19.99. Found: C, 64.53; H, 4.31; N, 20.17.

*4-Hydrazinyl-2-(3,4,5-trimethoxyphenyl)quinazoline (**12e**)*

Pale yellow powder; yield (79%); m.p. 235−237°C; IR ν 3273–3307 (NH, NH_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 3.74 (s, 3H, -OCH_3), 3.91 (s, 6H, -OCH_3), 4.91 (s, 2H, NH_2, D_2O exchangeable), 7.43–7.54 (m, 1H, Ar-H), 7.57 (s, 1H, Ar-H), 7.73–7.86 (m, 2H, Ar-H), 7.91 (s, 1H, Ar-H), 8.14–8.19 (m, 1H, Ar-H), 9.62 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 56.4, 60.6, 105.7, 113.2, 121.29, 125.6, 127.9, 128.1, 133.0, 140.0, 149.9, 152.2, 159.3, 160.3; Anal. calcd. for

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$C_{17}H_{18}N_4O_3$ (326.36): C, 62.57; H, 5.56; N, 17.17. Found C, 62.82; H, 5.63; N, 17.45.

*General procedure for the preparation of 3-hydrazonoindolin-2-ones **14a–c***

To a stirred solution of **13a–c** (10 mmol) in methanol (20 mL), 99% hydrazine hydrate (2.5 mL, 50 mmol) was added. Stirring was continued at the refluxing temperature for 0.5 h. The crystals were filtered, washed with methanol, dried and recrystallized from the glacial acetic acid to afford **14a–c** [52].

*General procedure for the preparation of target compounds **15a–o***

Route A: Indoline-2,3-dione derivative **13a–c** (1 mmol) was added to a suspension of the appropriate 4-hydrazinyl-2-substituted-quinazoline **12a–e** (1 mmol) in absolute ethanol (10 mL), and glacial acetic acid (0.5 mL) was added to the mixture. The reaction mixture was refluxed for 0.5 h. The precipitate formed was collected by filtration while hot, washed with hot ethanol, dried and crystallized from EtOH/DMF to afford compounds **15a–o** with 79–91% yield.

Route B: The appropriate 3-hydrazonoindolin-2-one **14a–c** (1 mmol) was added to a stirred solution of 4-chloroquinazoline **11a–e** (1 mmol) in refluxing 2-propanol (20 mL). The reaction mixture was refluxed for 5 h. The obtained product was filtered, washed with hot ethanol, dried and crystallized from EtOH/DMF to afford compounds **15a–o** with 60–82% yield. The yields from route A (79–91%) are considered for **15a–o**.

*3-(2-(2-Phenylquinazolin-4-yl)hydrazono)indolin-2-one (**15a**)*

Orange powder; yield (81%); mp 263−265°C; IR ν 3421 (NH), 1707 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ ppm: 6.89 (d, $J = 7.2$ Hz, 1H, Ar-H), 7.02–7.12 (m, 1H, Ar-H), 7.32–7.40 (m, 1H, Ar-H), 7.56–8.08 (m, 8H, Ar-H), 8.45–8.57 (m, 2H, Ar-H), 10.67 (s, 1H, NH, D_2O exchangeable, *E* form), 11.37 (s, 1H, NH of isatin, D_2O exchangeable), 13.9 (s, 1H, NH, D_2O exchangeable, *Z* form); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 110.7, 118.1, 122.5, 125.4, 127.9, 128.5 (2C), 129.4, 130.6, 132.2, 133.2, 134.5, 135.0, 144.1, 146.2, 152.5, 154.0, 155.5, 156.0, 166.1; Anal. calcd. for $C_{22}H_{15}N_5O$ (365.40): C, 72.32; H, 4.14; N, 19.17. Found: C, 72.49; H, 4.18; N, 19.32.

*5-Fluoro-3-(2-(2-phenylquinazolin-4-yl)hydrazono)-indolin-2-one (**15b**)*

Red fibers; yield (79%); mp 286−288°C; IR ν 3417 (NH), 1695 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ ppm: 6.85–6.92 (m, 1H, Ar-H), 7.11–7.20 (dd, $J = 9.3$ and 9.3, 1H, Ar-H), 7.43–7.57 (m, 3H, Ar-H), 7.60 (d, $J = 7.5$ Hz, 1H, Ar-H), 7.69 (d, $J = 7.5$ Hz, 1H, Ar-H), 7.77–7.96 (m, 1H, Ar-H), 8.04 (d, $J = 8.4$ Hz, 1H, Ar-H), 8.37 (m, 2H, Ar-H), 8.50 (s, 1H, Ar-H), 10.67 (s, 1H, NH, *E* form), 11.38 (s, 1H, NH of isatin), 13.85 (s, 1H, NH, D_2O exchangeable, *Z* form); ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 107.6 ($^2J_{F\text{-}C} = 25.3$ Hz), 112.2 ($^3J_{F\text{-}C} = 6.6$ Hz), 117.9, 120.9 ($^3J_{F\text{-}C} = 9.15$ Hz), 123.9 ($^1J_{F\text{-}C} = 163.7$ Hz), 127.5 ($^2J_{F\text{-}C} = 23.1$ Hz), 128.5, 128.8, 131.5, 134.3, 134.8, 138.4, 138.9, 154.9, 156.3, 156.8, 158.3, 159.9, 163.2, 165.4; Anal. calcd. for $C_{22}H_{14}FN_5O$ (383.39): C, 68.92; H, 3.68; N, 18.27. Found: C, 69.04; H, 3.65; N, 18.48.

*5-Chloro-3-(2-(2-phenylquinazolin-4-yl)hydrazono)-indolin-2-one (**15c**)*

Orange powder; yield (88%); mp >300°C; IR ν 3433 (NH), 1710 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ ppm: 6.90–7.03 (m, 1H, Ar-H), 7.41–7.78 (m, 6H, Ar-H), 7.99–8.10 (m, 2H, Ar-H), 8.40–8.62 (m, 3H, Ar-H), 10.67 (s, 1H, NH, D_2O exchangeable, *E* form), 11.48 (s, 1H, NH of isatin, D_2O exchangeable), 13.87 (s, 1H, NH, D_2O exchangeable, *Z* form); ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 111.5, 112.7, 119.5, 120.9, 121.7, 123.6, 126.7, 127.3, 128.8, 130.3, 130.6, 131.7, 134.0, 137.3, 140.4, 151.8, 156.0, 158.9, 163.0, 165.3; MS *m/z* (Rel. Int.) 401 [$(M+2)^+$, 4.5], 399 [M^+, 9.78], 294 [100]; Anal. calcd. for $C_{22}H_{14}ClN_5O$ (399.84): C, 66.09; H, 3.53; N, 17.52. Found: C, 66.25; H, 3.50; N, 17.61.

*3-(2-(2-(4-Methoxyphenyl)quinazolin-4-yl)hydrazono)-indolin-2-one (**15d**)*

Orange powder; yield (88%); mp 277−278°C; IR ν 3410 (NH), 1716 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ ppm: 3.85 (s, 3H, -OCH_3, *E* form), 3.87 (s, 3H, –OCH_3, *Z* form), 6.88 (d, $J = 8.4$ Hz, 1H, Ar-H), 6.98 (d, $J = 7.8$ Hz, 1H, Ar-H), 7.10–7.42 (m, 3H, Ar-H), 7.67–7.82 (m, 3H, Ar-H), 7.93–8.44 (m, 2H, Ar-H), 8.51 (d, $J = 8.7$ Hz, 2H, Ar-H)), 10.69 (s, 1H, NH, D_2O exchangeable, *E* form), 11.38 (s, 1H, NH of isatin, D_2O exchangeable), 13.85 (s, 1H, NH, D_2O exchangeable, *Z* form); ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 55.2, 110.1, 111.1, 112.4, 113.8, 114.3, 120.4, 122.5, 126.6, 128.3, 129.9, 130.9, 133.8, 141.7, 151.8, 156.0, 158.9, 161.4, 162.2, 163.3, 165.2; MS *m/z* (Rel. Int.) 396 [$(M+1)^+$, 2.86], 395 [M^+, 51.4], 205 [100]. Anal. calcd. for $C_{23}H_{17}N_5O2$ (395.42): C, 69.86; H, 4.33; N, 17.71. Found: C, 69.97; H, 4.35; N, 17.93.

*5-Fluoro-3-(2-(2-(4-methoxyphenyl)quinazolin-4-yl)-hydrazono)indolin-2-one (**15e**)*

Orange powder; yield (83%); mp 293−295°C; IR ν 3412 (NH), 1716 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ ppm: 3.86 (s, 3H, -OCH_3, *E* form), 3.87 (s, 3H, -OCH_3, *Z* form), 6.85–7.24 (m, 4H, Ar-H), 7.55–8.05 (m, 5H, Ar-H), 8.36–8.51 (m, 2H, Ar-H), 10.65 (s, 1H, NH, D_2O exchangeable, *E* form), 11.33 (s, 1H, NH of isatin, D_2O exchangeable), 13.79 (s, 1H, NH, D_2O exchangeable, *Z* form); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 55.7, 107.8 ($^2J_{F\text{-}C} = 25.0$ Hz), 111.2, 112.5 ($^3J_{F\text{-}C} = 8.0$ Hz), 112.9, 114.2, 114.7, 117.4 ($^2J_{F\text{-}C} = 24.0$ Hz), 121.8 ($^3J_{F\text{-}C} = 9.0$ Hz), 124.0, 124.9 ($^1J_{F\text{-}C} = 170.0$ Hz), 127.2, 130.2, 130.3, 138.4, 134.3, 152.3, 156.2, 159.2, 161.9, 163.8; Anal. calcd. for $C_{23}H_{16}FN_5O_2$ (413.41): C, 66.82; H, 3.90; N, 16.94. Found: C, 67.03; H, 33.87; N, 17.07.

*5-Chloro-3-(2-(2-(4-methoxyphenyl)quinazolin-4-yl)-hydrazono)indolin-2-one (**15f**)*

Yellow powder; yield (91%); mp 290−292°C; IR ν 3460 (NH), 1693 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ ppm: 3.84 (s, 3H, -OCH_3, *E* form), 3.85 (s, 3H, -OCH_3, *Z* form), 6.84 (d, $J = 8.1$ Hz,

1H, Ar-H), 6.90 (d, $J = 8.1$ Hz, 1H, Ar-H), 7.04 (d, $J = 7.8$ Hz, 2H, Ar-H), 7.11 (d, $J = 8.7$ Hz, 1H, Ar-H), 7.31–7.37 (m, 1H, Ar-H), 7.53–8.43 (m, 5H, Ar-H), 10.75 (s, 1H, NH, *E* form), 11.44 (s, 1H, NH of isatin), 13.75 (s, 1H, NH, *Z* form); ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 55.3, 111.5, 112.0, 112.6, 113.9, 114.2, 120.1, 121.4, 125.4, 126.8, 127.2, 129.1, 130.6, 134.5, 140.6, 142.1, 155.9, 158.2, 162.0, 162.2, 165.2. Anal. calcd. for $C_{23}H_{16}ClN_5O_2$ (429.86): C, 64.27; H, 3.75; N, 16.29. Found: C, 64.35; H, 3.71; N, 16.44.

*3-(2-(2-(3,4-Dimethoxyphenyl)quinazolin-4-yl)hydrazono)-indolin-2-one (**15g**)*
Orange powder; yield (81%); mp 233−234°C; IR ν 3327 (NH), 1699 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 3.86 (s, 3H, -OCH_3, *E* form), 3.88 (s, 3H, -OCH_3, *Z* form), 3.90 (s, 3H, -OCH_3, *E* form), 3.92 (s, 3H, -OCH_3, *Z* form), 6.89 (d, $J = 7.8$ Hz, 1H, Ar-H), 6.99 (d, $J = 7.8$ Hz, 1H, Ar-H), 7.1–7.16 (m, 1H, Ar-H), 7.22 (d, $J = 8.4$ Hz, 2H, Ar-H), 7.35 (m, 1H, Ar-H), 7.73 (s, 1H, Ar-H), 7.84–7.97 (m, 2H, Ar-H), 8.14–8.47 (m, 2H, Ar-H), 10.69 (s, 1H, NH, D_2O exchangeable, *E* form), 11.39 (s, 1H, NH of isatin, D_2O exchangeable), 13.88 (s, 1H, NH, D_2O exchangeable, *Z* form); ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 55.4, 55.7, 110.1, 111.3, 117.6, 120.1, 121.6, 122.0, 122.5, 124.8, 126.7, 128.0, 128.3, 130.0, 131.0, 131.9, 133.9, 141.8, 145.5, 148.8, 152.0, 158.9, 162.7, 163.3; Anal. calcd. for $C_{24}H_{19}N_5O_3$ (425.45): C, 67.76; H, 4.50; N, 16.46. Found: C, 67.93; H, 4.58; N, 16.62.

*3-(2-(2-(3,4-Dimethoxyphenyl)quinazolin-4-yl)-hydrazono)-5-fluoroindolin-2-one (**15h**)*
Orange powder; yield (84%); mp 280−282°C; IR ν 3412 (NH), 1716 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 3.87 (s, 3H, -OCH_3, *E* form), 3.88 (s, 3H, -OCH_3, *Z* form), 3.90 (s, 3H, OCH_3, *E* form), 3.92 (s, 3H, -OCH_3, *Z* form), 6.90 (m, 1H, Ar-H), 7.22 (d, $J = 8.7$ Hz, 1H, Ar-H), 7.64–7.86 (m, 4H, Ar-H), 7.98 (s, 1H, Ar-H), 8.13 (s, 1H, Ar-H), 8.21–8.45 (m, 2H, Ar-H), 10.70 (s, 1H, NH, D_2O exchangeable, *E* form), 11.43 (s, 1H, NH of isatin, D_2O exchangeable), 13.88 (s, 1H, NH, D_2O exchangeable, *Z* form). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 55.4, 55.7, 107.2 ($^2J_{F\text{-}C} = 25.4$ Hz), 110.7 ($^3J_{F\text{-}C} = 17.3$ Hz), 111.4, 112.4, 116.9 ($^2J_{F\text{-}C} = 36.5$ Hz), 121.0 ($^3J_{F\text{-}C} = 15.4$ Hz), 125.8, 126.0, 126.8 ($^1J_{F\text{-}C} = 238$ Hz), 127.3, 128.2, 129.9, 133.8, 134.5, 148.5, 151.2, 151.8, 158.8, 159.9, 162.3, 163.3, 165.3; MS *m/z* (Rel. Int.) 445 [(M+2)$^+$, 46.02%], 444 [(M+1)$^+$, 60.18%], 443 [M$^+$, 83.19%], 315 [100%]; Anal. calcd. for $C_{24}H_{18}FN_5O_3$ (443.44): C, 65.01; H, 4.09; N, 15.79. Found: C, 65.17; H, 4.13; N, 15.88.

*5-Chloro-3-(2-(2-(3,4-dimethoxyphenyl)quinazolin-4-yl)-hydrazono)indolin-2-one (**15i**)*
Orange powder; yield (90%); mp >300°C; IR ν 3435 (NH), 1716 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 3.86 (s, 3H, -OCH_3, *E* form), 3.88 (s, 3H, -OCH_3, *Z* form), 3.90 (s, 3H, -OCH_3, *E* form), 3.92 (s, 3H, -OCH_3, *Z* form), 6.90 (d, $J = 8.4$ Hz, 1H, Ar-H), 7.00 (d, $J = 8.7$ Hz, 1H, Ar-H), 7.14 (d, $J = 8.7$ Hz, 1H, Ar-H), 7.21 (d, $J = 8.4$ Hz, 1H, Ar-H), 7.41–7.45 (m, 1H, Ar-H), 7.72–7.85 (m, 1H, Ar-H), 7.95 (s, 1H, Ar-H), 8.12 (s, 1H, Ar-H), 8.17–8.57 (m, 2H, Ar-H), 10.76 (s, 1H, NH, D_2O exchangeable, *E* form), 11.45 (s, 1H, NH of isatin, D_2O exchangeable), 13.79 (s, 1H, NH, D_2O exchangeable, *Z* form); Anal. calcd. for $C_{24}H_{18}ClN_5O_3$ (459.89): C, 62.68; H, 3.95; N, 15.23. Found: C, 62.79; H, 3.91; N, 15.47.

*3-(2-(2-(Benzo[d][1,3]dioxol-5-yl)quinazolin-4-yl)-hydrazono)indolin-2-one (**15j**)*
Orange powder; yield (80%); mp 269−271°C; IR ν 3396 (NH), 1697 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 6.13 (s, 2H, -CH_2-, *E* form), 6.17 (s, 2H, -CH_2-, *Z* form), 6.88 (d, $J = 7.8$ Hz, 1H, Ar-H), 6.97 (d, $J = 7.5$ Hz, 1H, Ar-H), 7.07–7.13 (m, 1H, Ar-H), 7.15 (d, $J = 7.8$ Hz, 1H, Ar-H), 7.31–7.42 (m, 1H, Ar-H), 7.55–7.81 (m, 2H, Ar-H), 7.92 (d, $J = 3.9$ Hz, 1H, Ar-H), 7.99 (s, 1H, Ar-H), 8.15 (d, $J = 9.9$ Hz, 1H, Ar-H), 8.40 (d, $J = 7.8$ Hz, 1H, Ar-H), 10.69 (s, 1H, NH, D_2O exchangeable, *E* form), 11.38 (s, 1H, NH of isatin, D_2O exchangeable), 13.85 (s, 1H, NH, D_2O exchangeable, *Z* form); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 102.6, 108.0, 108.9, 109.0, 110.6, 118.1, 122.4, 125.0, 126.6, 127.0, 129.3, 130.4, 132.0, 134.5, 137.4, 138.6, 145.5, 146.1, 148.3, 150.9, 158.6, 163.0, 166.1; MS *m/z* (Rel. Int.) 410 [(M+1)$^+$, 6.12], 409 [M$^+$, 12.33], 260 [100]; Anal. calcd. for $C_{23}H_{15}N_5O_3$ (409.41): C, 67.48; H, 3.69; N, 17.11. Found: C, 67.69; H, 3.72; N, 17.30.

*3-(2-(2-(Benzo[d][1,3]dioxol-5-yl)quinazolin-4-yl)-hydrazono)-5-fluoroindolin-2-one (**15k**)*
Red powder; yield (86%); mp 272–274°C; IR ν 3460 (NH), 1722 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 6.10 (s, 2H, -CH_2-, *E* form), 6.15 (s, 2H, -CH_2-, *Z* form), 6.86–6.93 (m, 1H, Ar-H), 7.00 (d, $J = 8.4$ Hz, 1H, Ar-H), 7.10–7.17 (m, 1H, Ar-H), 7.45 (d, $J = 7.8$ Hz, 1H, Ar-H), 7.53–7.78 (m, 2H, Ar-H), 7.86 (s, 1H, Ar-H), 7.92 (s, 1H, Ar-H), 8.10–8.45 (m, 2H, Ar-H), 10.60 (s, 1H, NH, D_2O exchangeable, *E* form), 11.25 (s, 1H, NH of isatin, D_2O exchangeable), 13.70 (s, 1H, NH, D_2O exchangeable, *Z* form); ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 101.5, 107.2 ($^2J_{F\text{-}C} = 28.2$ Hz), 108.0, 111.9 ($^3J_{F\text{-}C} = 7.7$ Hz), 112.0, 112.4, 116.9 ($^2J_{F\text{-}C} = 24.2$ Hz), 121.2 ($^2J_{F\text{-}C} = 9.1$ Hz), 122.9, 126.7, 127.0, 128.1 ($^1J_{F\text{-}C} = 260$ Hz), 131.6, 133.7, 137.8, 147.5, 151.7, 155.62, 156.8, 158.3, 163.3, 165.5, 170.9; Anal. calcd. for $C_{23}H_{14}FN_5O_3$ (427.40): C, 64.64; H, 3.30; N, 16.39. Found: C, 64.78; H, 3.28; N, 16.52.

*3-(2-(2-(Benzo[d][1,3]dioxol-5-yl)quinazolin-4-yl)-hydrazono)-5-chloroindolin-2-one (**15l**)*
Orange powder; yield (89%); mp 291−293°C; IR ν 3423 (NH), 1716 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ ppm: 6.13 (s, 2H, -CH_2-, *E* form), 6.17 (s, 2H, -CH_2-, *Z* form), 6.87 (d, $J = 8.1$ Hz, 1H, Ar-H), 6.95 (d, $J = 8.4$ Hz, 1H, Ar-H), 7.05 (d, $J = 7.8$ Hz, 1H, Ar-H), 7.14 (d, $J = 8.1$ Hz, 1H, Ar-H), 7.35–7.41 (m, 1H, Ar-H), 7.56–7.90 (m, 1H, Ar-H), 7.92 (s, 1H, Ar-H), 7.95 (s, 1H, Ar-H), 8.12 (d, $J = 8.4$ Hz, 1H, Ar-H), 8.46 (d, $J = 9.3$ Hz, 1H, Ar-H), 10.79 (s, 1H, NH, D_2O exchangeable, *E* form), 11.46 (s, 1H, NH of isatin, D_2O exchangeable), 13.76 (s, 1H, NH, D_2O exchangeable, *Z* form); ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 102.0, 108.1, 108.6, 112.0, 112.9, 113.0, 120.4, 122.2, 123.5, 125.9, 127.2, 127.4, 128.7, 130.7, 132.1, 134.4, 140.8, 148.1, 150.0, 152.2, 156.2, 158.9, 163.5; Anal. calcd. for $C_{23}H_{14}ClN_5O_3$ (443.85): C, 62.24; H, 3.18; N, 15.78. Found C, 62.37; H, 3.12; N, 15.94.

*3-(2-(2-(3,4,5-Trimethoxyphenyl)quinazolin-4-yl)-hydrazono)indolin-2-one (**15m**)*
Yellow powder; yield (81%); mp 278–280°C; IR ν 3340 (NH), 1726 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ ppm: 3.75 (s, 3H, -OCH_3, *E* form), 3.78 (s, 3H, -OCH_3, *Z* form), 3.90 (s, 3H, -OCH_3, *Z* form), 3.92 (s, 6H, -OCH_3, *E* form), 3.95 (s, 3H, -OCH_3, *Z* form), 6.89 (d, $J = 7.5$ Hz, 1H, Ar-H), 6.99 (d, $J = 7.5$ Hz, 1H, Ar-H), 6.07–7.17 (m, 1H, Ar-H), 7.38 (s, 1H, Ar-H), 6.38–7.86 (m, 3H, Ar-H), 7.91 (s, 1H, Ar-H), 7.96–7.99 (m, 1H, Ar-H), 8.46 (d, $J = 6.3$ Hz, 1H, Ar-H), 10.64 (s, 1H, NH, D_2O exchangeable, *E* form), 11.33 (s, 1H, NH of isatin, D_2O exchangeable), 13.88 (s, 1H, NH, D_2O exchangeable, *Z* form); ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 55.8, 60.0, 105.2, 111.0, 112.5, 120.1, 122.4, 123.0, 126.8, 128.3, 130.8, 132.8, 135.0, 141.7, 148.6, 151.5, 152.9, 155.7, 158.5, 162.2, 163.2, 171.9; Anal. calcd. for $C_{25}H_{21}N_5O_4$ (455.47): C, 65.93; H, 4.65; N, 15.38. Found C, 66.17; H, 4.72; N, 15.47.

*5-Fluoro-3-(2-(2-(3,4,5-trimethoxyphenyl)quinazolin-4-yl)-hydrazono)indolin-2-one (**15n**)*
Orange powder; yield (88%); mp 287–288°C; IR ν 3396 (NH), 1726 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ ppm: 3.78 (s, 3H, -OCH_3), 3.91 (s, 6H, -OCH_3, *E* form), 3.93 (s, 6H, -OCH_3, *Z* form), 6.90–6.99 (m, 1H, Ar-H), 7.18 (dd, $J = 5.4$, 2.4 Hz, 1H, Ar-H), 7.24 (d, $J = 2.4$ Hz, 1H, Ar-H), 7.36 (s, 1H, Ar-H), 7.47 (dd, $J = 5.4$, 2.4 Hz, 1H, Ar-H), 7.68–7.73 (m, 1H, Ar-H), 7.87 (s, 1H, Ar-H), 7.97 (s, 1H, Ar-H), 8.52 (m, 1H, Ar-H), 10.61 (s, 1H, NH, D_2O exchangeable, *E* form), 11.32 (s, 1H, NH of isatin, D_2O exchangeable), 13.81 (s, 1H, NH, D_2O exchangeable, *Z* form); Anal. calcd. for $C_{25}H_{20}FN_5O_4$ (473.46): C, 63.42; H, 4.26; N, 14.79. Found C, 63.64; H, 4.23; N, 15.03.

*5-Chloro-3-(2-(2-(3,4,5-trimethoxyphenyl)quinazolin-4-yl)-hydrazono)indolin-2-one (**15o**)*
Yellow powder; yield (90%); mp >300°C; IR ν 3385 (NH), 1722 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ ppm: 3.78 (s, 3H, -OCH_3), 3.92 (s, 6H, -OCH_3, *E* form), 3.94 (s, 6H, -OCH_3, *Z* form), 6.90–7.02 (m, 1H, Ar-H), 7.40 (dd, $J = 6.3$, 2.4 Hz, 1H, Ar-H), 7.69 (s, 1H, Ar-H), 7.69–7.88 (m, 2H, Ar-H), 7.88 (s, 1H, Ar-H), 7.99 (s, 1H, Ar-H), 8.38–8.60 (m, 2H, Ar-H), 10.64 (s, 1H, NH, D_2O exchangeable, *E* form), 11.33 (s, 1H, NH of isatin, D_2O exchangeable), 13.88 (s, 1H, NH, D_2O exchangeable, *Z* form); MS *m/z* (Rel. Int.) 492 [$(M+2)^+$, 21.05], 491 [$(M+1)^+$, 58.25], 490 [$(M)^+$, 46.08], 90 [100]. Anal. calcd. for $C_{25}H_{20}ClN_5O_4$ (489.92): C, 61.29; H, 4.11; N, 14.30. Found C, 61.47; H, 4.15; N, 14.45.

Biological evaluation

In vitro antiproliferative activity
HepG2 liver cancer, MCF-7 breast cancer and HT-29 colon cancer cell lines were obtained from the National Cancer Institute (Cairo, Egypt). HepG2 cells were grown in DMEM while MCF-7 and HT-29 were grown in RPMI-1640. Media were supplemented with 10% heat-inactivated FBS, 50 units/mL of penicillin and 50 g/mL of streptomycin and maintained at 37°C in a humidified atmosphere containing 5% CO_2. The cells were maintained as a "monolayer culture" by serial subculturing. Growth inhibition was determined using the SRB method as previously described by Skehan *et al*. [35]. Exponentially growing cells were collected using 0.25% trypsin-EDTA and seeded in 96-well plates at 1000–2000 cells/well in supplemented DMEM medium. After 24 h, cells were incubated for 72 h with various concentrations of the tested compounds as well as doxorubicin as the reference compound. Following 72 h of treatment, the cells were fixed with 10% trichloroacetic acid for 1 h at 4°C. Wells were stained for 10 min at room temperature with 0.4% SRB dissolved in 1% acetic acid. The plates were air dried for 24 h, and the dye was solubilized with Tris-HCl for 5 min on a shaker at 1600 rpm. The optical density (OD) of each well was measured spectrophotometrically at 564 nm with an ELISA microplate reader (ChroMate-4300, FL, USA). The IC_{50} values were calculated according to the equation for Boltzmann sigmoidal concentration–response curve using the nonlinear regression models (GraphPad Prism, version 5). The results reported are means of at least three separate experiments. Significant differences were analyzed by one-way ANOVA wherein the differences were considered to be significant at $P < 0.05$.

Cell culture and lysate collection
Hepatocellular carcinoma human HepG2 cells were cultured in RPMI + 10% FBS + 5% penicillin/streptomycin and were incubated at humidity 5% CO_2 and maintained at 37°C. HepG2 cells were treated with the IC_{50} concentration of **15b**, **15g**, and **15l** ($IC_{50} = 1.0$, 1.8, and 2.4 μM, respectively). HepG2 cells were cultured as a monolayer in T-25 flasks and were seeded to attain 30% confluency prior to treatment. The main 100 mM stock dissolved in DMSO was diluted with cell culture medium in order to reach the previously determined IC_{50} concentration of each of the three preparations. After 72 h of treatment with **15b**, **15g**, and **15l**, HepG2 cells were collected via trypsinization and centrifuged at 10,000 rpm. The pellet was then rinsed with PBS and lysed in RIPA lysis buffer at 4°C for 45 min, then centrifuged at 14,000 rpm for 20 min to remove the cellular debris. Lysates were then collected and stored at −80°C for later protein determination and immunoassays.

ELISA immunoassay
The levels of the anti-apoptotic marker Bcl-2 as well as the apoptotic markers Bax and caspase-3 were assessed using ELISA colorimetric kits per the manufacturer's instructions. The main principle of sandwich ELISA is the quantification of a specific protein through its containment in a sandwich of specific antibodies conjugated to the colorimetric TMB substrate, whose intensity is proportional to the protein quantity and is measured spectrophotometrically.

The cell lysate was diluted 10 times, and 100 μL (50 μg protein) was added to the wells of three separate microtiter plates for the three ELISA kits that were precoated with primary antibodies specific to caspase-3, Bax and Bcl-2,

respectively. A secondary biotin-linked antibody specific to the protein captured by the primary antibody was added to bind the captured protein, forming a "sandwich" of specific antibodies around the desired protein in the cell lysate. The streptavidin-HRP complex was then used to bind the biotin-linked secondary antibody through its streptavidin portion. The HRP domain reacted with the added TMB substrate, forming a colored product that was measured at 450 nm by a plate reader (ChroMate-4300, FL, USA) after the reaction was terminated by the addition of stop solution.

Statistical analysis

The data are presented as the mean $\pm$ S.D. Comparisons were carried out using one-way analysis of variance followed by Tukey–Kramer's test for *post hoc* analysis. Statistical significance was considered to be $P < 0.05$. All statistical analyses were performed using GraphPad InStat software, version 3.05 (GraphPad Software, La Jolla, CA).

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