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Original article

Analogue-based design, synthesis and biological evaluation of 3-substituted-(methylenehydrazono)indolin-2-ones as anticancer agents

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ABSTRACT

The docking studies on CDK2 and GSK-3β inspired us to synthesis a series of indoline-2,3-dione hydrazones **10a**–**l**. Treatment of indoline-2,3-dione derivatives **7a**–**d** with hydrazine gave 3-hydrazonoindolin-2-ones **8a**–**d** which were reacted with the appropriate aldehydes **9a**–**c** to yield 3-substituted-(methylenehydrazono)indolin-2-ones **10a**–**l**. Compounds **10a**–**l** showed a significant anticancer activity against human breast cell line MCF-7. Compounds **10c**, **f**, **i** exhibited the highest activity almost the same of doxorubicin (IC_{50} = 6.10 μM) with IC_{50} = 7.75, 6.75, 6.25 μM, respectively.

1. Introduction

Nowadays, cancer is one of the main reasons of death between nations caused by different elements such as presence of mutagenic and cancer causing materials in the environment. On the other hand, 1*H*-indole-2,3-dione derivatives have aroused great attention due to their wide variety of biological activities, relevant to application in a broad range of drug therapies, including anticancer drugs [1–3]. A plethora of biologically active C3-substituted indole-2,3-dione has been generated in the literature, these due to the susceptibility of isatin to be attacked by nucleophiles at the C3 position [3]. C3-substituted indole-2,3-dione drugs such as indirubin **1a** (X = H, Y = O) and its derivatives 5-bromoindirubin **1b** (X = Br, Y = O), indirubin-3′-oxime **1c** (X = H, Y = N–OH) and 5-bromoindirubin-3′-oxime **1d** (X = Br, Y = N–OH) were found to have potent anticancer activity with excellent inhibitory of tyrosin kinases CDK2, CDK5 and GSK-3β [4–7]. Moreover, 1*H*-indole-2,3-dione drugs which contain arylidene branches in position 3 such as SU5416 (semaxanib®, **2a**) [8], SU5402 (**2b**) [9], SU6668 (**2c**) [10] and SU14813 (**2d**) [11] (Fig. 1) showed a significant cytotoxic activity. However, SU11248 (sunitinib, Sutent®, **2**) is an inhibitor of receptor tyrosine kinase (RTK) and it is the standard first-line treatment for the treatment of gastrointestinal stromal cancers and renal cell carcinoma [12] (Fig. 1).

Moreover, hydrazones of 1*H*-indole-2,3-dione **3** (Fig. 1) have been identified as inhibitors of the protein tyrosine phosphatase Shp2, which plays an important role in cell signaling, cell proliferation, differentiation and migration [13]. In recent years, the cytotoxic activity against *Artemia salina* of 1*H*-indole-2,3-dione thiosemicarbazones **4** (a hydrazone derivative containing a sulfur atom) have reported [14,15] (Fig. 1). Furthermore, thiosemicarbazones **3** have been found to display cytotoxicity against the KB-3-1 cell line (a HeLa derivative) [16,17].

Recently, we have been reported the synthesis of hydrazino derivative **5** with selective activity against multidrug-resistant cancer cells [18] and *in vitro* antiproliferative activity of synthesized 2*H*-chromene-3-carbohydrazides **6** which exhibited good antiproliferative profile against colon HT-29 [19] (Fig. 1).

In the light of the above data and through our docking studies that will be mentioned later, and in a continuation of our interest in the synthesis of hydrazone-based compounds with anticancer

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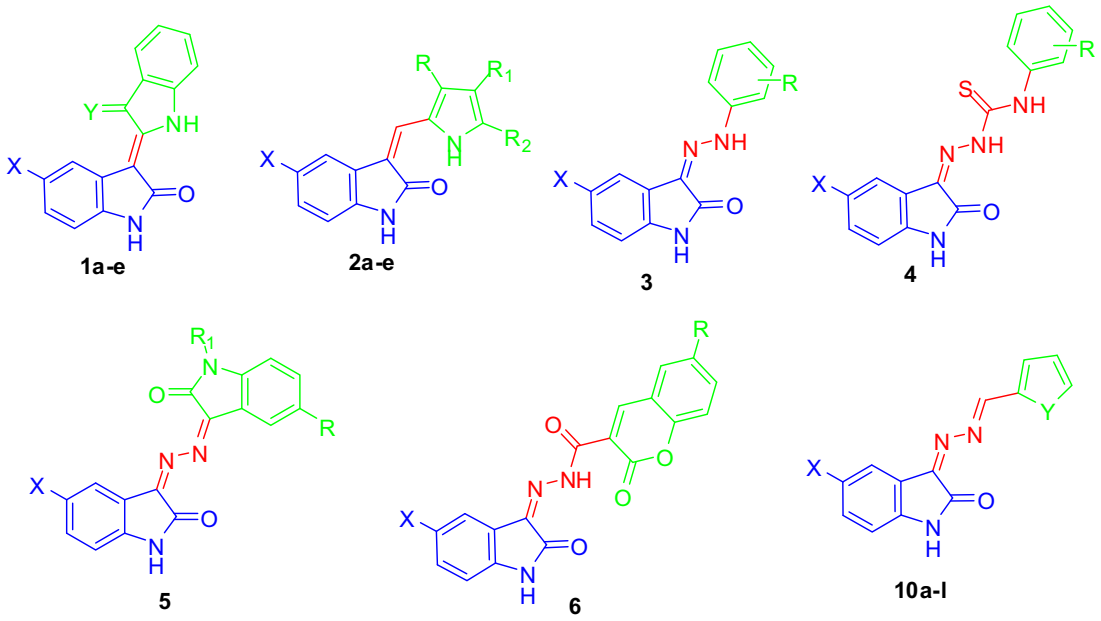


Fig. 1. Structure of **1**–**6** and **10a**–**l**.

activity [18–24], in this study, we have designed and synthesized certain derivatives of indoline-2,3-dione hydrazones **10a**–**l** (Fig. 1) that have potential utility as anticancer drugs.

2. Results and discussion

2.1. Analogue-based design through molecular docking studies

Crystallographic data of CDK2 [4,5] and GSK-3β [6] in complex with various indirubins has provided valuable information on the active site differences along with specific interactions between those kinases and indirubin analogues [7]. This mode of interaction inspired us to deduce indoline-2,3-dione hydrazone derivatives with an interaction near to indirubin analogues. During the investigation of the interaction between 5-bromoindurubin with human cyclin dependent protein kinase 2 of the ligand gave an energy score (S) value = −21.09 kcal/mol and root of mean square deviation (RMSD) value = 1.69 Å. It was found that there are three important hydrogen bond with Glu81 and Leu83 (Fig. 2a). After docking simulations on same active site, it was found that our suggested compounds **10a**–**l** gave a binding pattern near to the original ligand as in case of **10i** where the binding energy (S) was −18.69 kcal/mol and RMSD value was 1.55 Å (Fig. 2b).

Similarly, docking analysis of GSK-3β receptor for both indirubin-3′-oxime and our suggested compounds **10a**–**l**, taking **10i** as representative example, showed that they interacted through hydrogen bonds with Val135 and Asp133 (Fig. 2c, d). Furthermore, **10i** recorded an excellent binding energy score (S) = −18.08 kcal/mol when compared with that of ligand (−27.89 kcal/mol) under suitable RMSD value = 0.41 Å and 1.50 Å for indirubin-3′-oxime and **10i**, respectively. These results motivated us to synthesis a series of indoline-2,3-dione hydrazones **10i**–**l**. Keeping in consideration the structure of compounds **1**–**6**, hybridization structure of compounds **10i**–**l** (Fig. 3) showed an interesting combination of the structure features of compounds **1**–**6**.

The isosteric replacement of nitrogen in pyrrole moiety by oxygen or sulfur in furan and thiophene moieties in compounds **10a**–**d** and **10e**–**h**, respectively, supported the structure activity relationship (SAR) in the anticancer activity screening for targeted compounds. In addition, variation of substitution in position 5 of indoline-2,3-dione moiety was considered as a strategic key for functionalization of synthesized compounds due to its importance in the inhibition process as reported [25].

2.2. Chemistry

Indoline-2,3-diones **7a**–**d** were reacted with hydrazine hydrate in absolute methanol to afford the corresponding hydrazine derivatives **8a**–**d** [26]. The reaction of compounds **8a**–**d** with aldehydes **9a**–**c** gave hydrazones **10a**–**l** (Scheme 1). The IR spectra of **10a**–**l** showed bands due to isatin NH in the region 3256–3125 cm^{-1} in addition to a carbonyl band in the region 1737–1704 cm^{-1}. ^1H NMR spectra of these compounds revealed D_2O-exchangeable signal in the region δ 10.59–10.97 which were assigned to NH isatin proton, in addition to the signal of methine proton (−CH═N−) in the region δ 8.54–8.99. However, ^1H NMR spectra showed additional NH signal appeared around 12 due to pyrrole NH in compounds **10i**–**l**.

2.3. Anticancer activity

Treatment of human breast cancer cell line MCF-7 with different concentrations of synthesized compounds **10a**–**l** resulted in an inhibition of the growth by different IC_{50} values comparing with reference drug doxorubicin (Table 1). From IC_{50} values of **10a**–**l**, we can deduce that the presence of pyrrole ring, as well as indoline-2,3-dione hydrazone, gave the highest activity where compound **10i** gave IC_{50} = 6.25 μM almost the same of doxorubicin (IC_{50} = 6.10 μM). Considering the substitutions in position 5 of indoline-2,3-dione moiety, we found that furan derivative **10c**, with Cl in position 5 of indoline-2,3-dione, showed IC_{50} = 7.75 μM while thiophene derivative **10f**, with methyl substitution in position 5 of indoline-2,3-dione, exhibited IC_{50} = 6.75 μM (Table 1).

3. Conclusion

Docking studies were done to predict the possible anticancer activity of a series of 2-oxindolin-3-hydrazones **10a**–**l**. Synthesis and

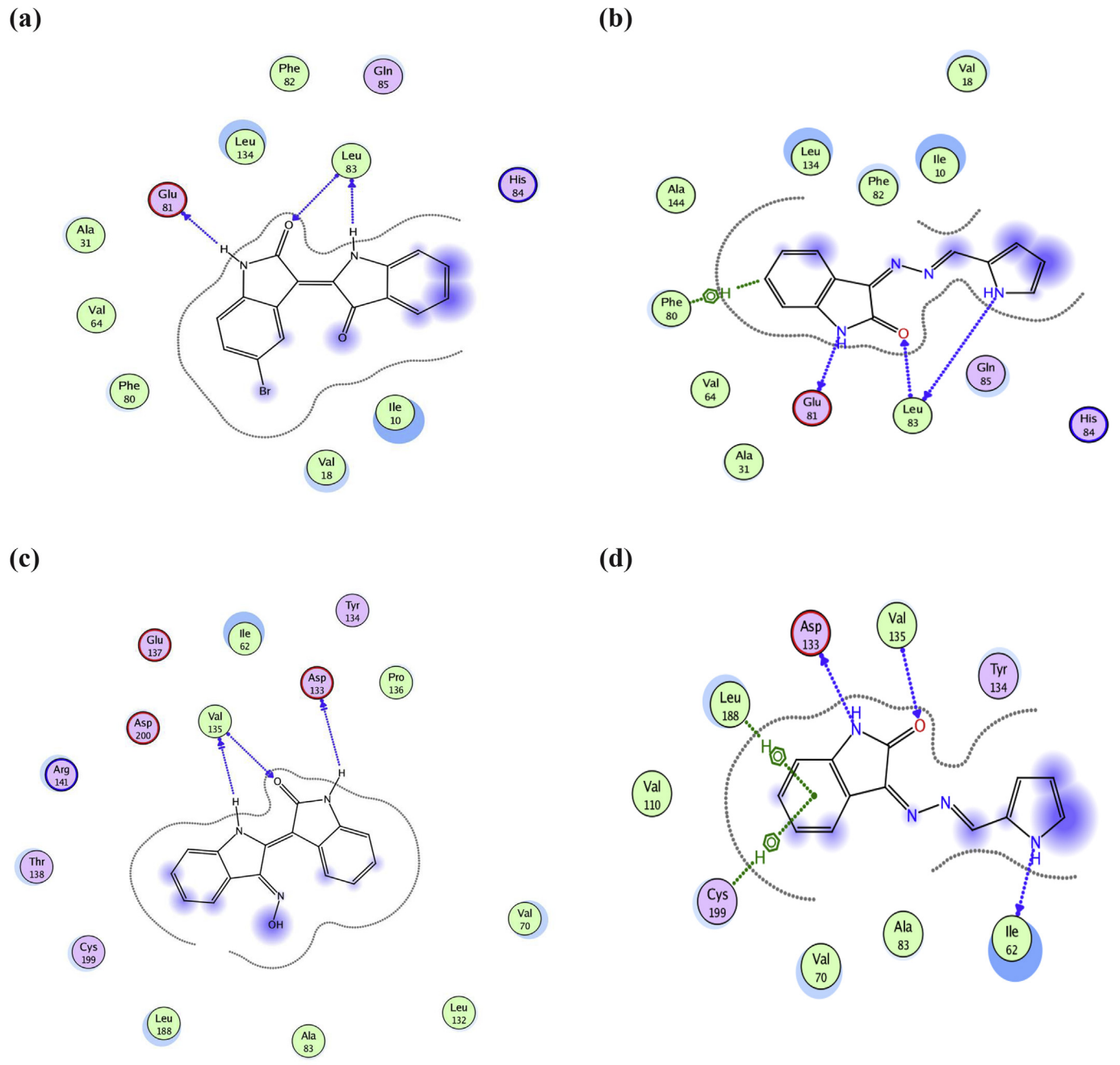


Fig. 2. a, b. 2D representations of the binding patterns of 5-bromoindirubin and **10i** with CDK2, respectively. **c**, **d**. 2D representations of the binding patterns of indirubin-3′-oxime and **10i** with GSK-3β, respectively.

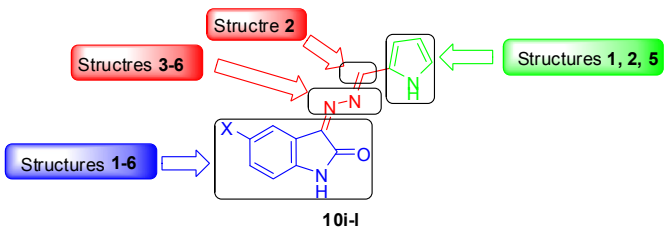


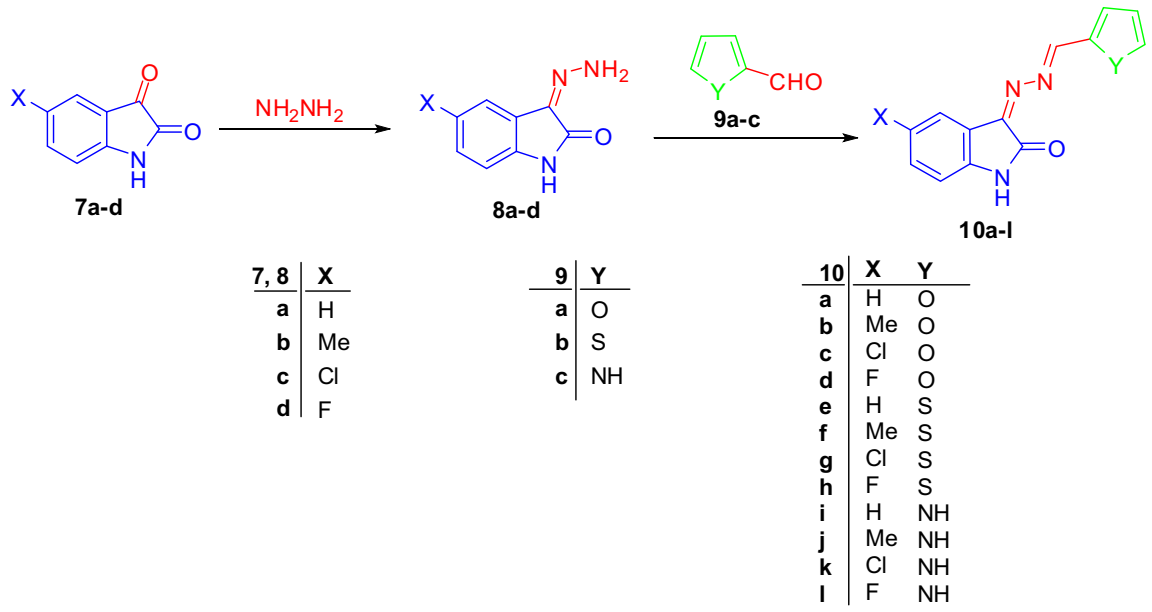
Fig. 3. Structure features of compounds **10i**–**l** comparing with compounds **1**–**6**.

in vitro anticancer activity **10a**–**l** toward MCF-7 cell line were studied to support our hypothesis. From the biological assay results, compounds **10a**–**l** showed a significant activity and we found that 3-(((1*H*-pyrrol-2-yl)methylene)hydrazono)indolin-2-one (**10i**) gave the maximum activity with IC_{50} = 6.25 μM. This result give a new promising venue for further investigations of the targeted compounds.

4. Experimental

4.1. Molecular docking studies

The molecular modeling studies were carried out using Molecular Operating Environment (MOE 2008.10; Chemical Computing



7, 8	X
a	H
b	Me
c	Cl
d	F

9	Y
a	O
b	S
c	NH

10	X	Y
a	H	O
b	Me	O
c	Cl	O
d	F	O
e	H	S
f	Me	S
g	Cl	S
h	F	S
i	H	NH
j	Me	NH
k	Cl	NH
l	F	NH

Scheme 1. Synthesis of compounds **10a**–**l**.

Group, Canada). All the minimizations were performed with MOE until a RMSD gradient of 0.05 kcal mol^{-1} $Å^{-1}$ with MMFF94X forcefield and the partial charges were automatically calculated. The X-ray crystallographic structure of GSK-3β and CDK2 complexes with indirubin-3′-oxime (PDB ID: 1Q41 and 2BHE respectively) was obtained from the protein data bank. The interaction mode of the ligand were investigated then the receptors were prepared for docking studies by assigning of the hydrogen bond state of the receptors and removing of water atoms. Molecular docking simulations were done to predict the interactions of the suggested compound.

4.2. Chemistry

4.2.1. General

Melting points (°C, uncorrected) were determined using a Stuart melting point apparatus. The IR spectra (KBr) were recorded on a PerkinElmer FT/IR spectrometer. NMR Spectra were scanned in DMSO-d_6 on a Brucker NMR spectrometer operating at 400 MHz for ^{1}H and 75 MHz for ^{13}C. Chemical shifts are expressed in δ-values (ppm) relative to TMS as an internal standard. Coupling constants (*J*) are expressed in Hz. D_2O was added to confirm the exchangeable protons.

4.2.2. General procedure for the synthesis of (Z)-3-hydrazonoindolin-2-ones *8a*–*d*

A mixture of isatin **7a**–**d** (1 mmol) and hydrazine hydrate (99%, 2 mmol) in absolute methanol (25 ml) was refluxed for 1 h, and then cooled to room temperature. The precipitate of hydrazone was filtered, dried and then crystallized from EtOH/DMF to afford compounds **8a**–**d** which used for next step without any further purification [26].

4.2.3. General procedure for the synthesis of hydrazones *10a*–*l*

To a mixture of hydrazone **8a**–**d** (1 mmol) and aldehyde **9a**–**c** (1 mmol) in ethanol (25 ml), 0.5 ml acetic acid was added. The reaction mixture was refluxed for 6 h, and then cooled to room temperature. The precipitate was filtered, dried and finally crystallized from EtOH/DMF to afford hydrazones **10a**–**l**.

Table 1
In vitro anticancer screening of **10a**–**l** against human breast cell line MCF-7.

Compound	Concentration (μM)				IC_{50} (μM)
	Surviving fraction (Mean ± S.E.)[a]				
	10	25	50	100	
10a	0.637 ± 0.122	0.306 ± 0.089	0.158 ± 0.035	0.333 ± 0.086	15.8
10b	0.436 ± 0.032	0.213 ± 0.036	0.195 ± 0.069	0.102 ± 0.096	8.75
10c	0.384 ± 0.096	0.274 ± 0.069	0.259 ± 0.023	0.231 ± 0.169	7.75
10d	0.667 ± 0.035	0.429 ± 0.133	0.335 ± 0.086	0.257 ± 0.068	20.3
10e	0.352 ± 0.065	0.602 ± 0.045	0.522 ± 0.162	0.546 ± 0.108	8.25
10f	0.227 ± 0.162	0.132 ± 0.025	0.099 ± 0.065	0.137 ± 0.081	6.75
10g	0.619 ± 0.023	0.310 ± 0.123	0.204 ± 0.078	0.194 ± 0.037	15.3
10h	0.616 ± 0.035	0.505 ± 0.056	0.331 ± 0.032	0.308 ± 0.161	25.8
10i	0.465 ± 0.086	0.328 ± 0.032	0.395 ± 0.152	0.357 ± 0.059	6.25
10j	0.473 ± 0.056	0.351 ± 0.065	0.221 ± 0.138	0.206 ± 0.087	9.25
10k	0.510 ± 0.032	0.392 ± 0.096	0.238 ± 0.052	0.259 ± 0.098	11.8
10l	0.671 ± 0.098	0.274 ± 0.152	0.456 ± 0.121	0.528 ± 0.087	10.8
Doxorubicin	0.257 ± 0.036	0.158 ± 0.068	0.098 ± 0.086	0.125 ± 0.075	6.10

[a] Each value is the mean of three values ± Standard Error.

*4.2.3.1. 3-((Furan-2-ylmethylene)hydrazono)indolin-2-one (**10a**).* Yield (55%); mp: 209 °C; IR (KBr) *ν* 3196 (NH), 1729 (C=O), 1622 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) *δ*: 6.77–8.09 (m, 7H, Ar-H), 8.55 (s, 1H, –CH=), 10.81 (s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) *δ*: 110.73, 113.08, 116.67, 119.67, 127.32, 129.00, 133.64, 144.87, 148.04, 149.03, 150.76, 151.31, 164.62 (C=O).

*4.2.3.2. 3-((Furan-2-ylmethylene)hydrazono)-5-methylindolin-2-one (**10b**).* Yield (52%); mp: 194 °C; IR (KBr) *ν* 3245 (NH), 1719 (C=O), 1618 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) *δ*: 2.26 (s, 3H, CH_3), 6.78–8.01 (m, 6H, Ar-H), 8.54 (s, 1H, –CH=), 10.7 (s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) *δ*: 20.65 (CH_3), 110.45, 113.09, 116.72, 119.47, 129.40, 131.01, 133.98, 142.5, 148.01, 149.07, 150.82, 151.44, 164.68 (C=O).

*4.2.3.3. 5-Chloro-3-((furan-2-ylmethylene)hydrazono)indolin-2-one (**10c**) [27].* Yield (47%); mp: 265 °C; IR (KBr) *ν* 3145 (NH), 1737 (C=O), 1616 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) *δ*: 6.81–8.15 (m, 6H, Ar-H), 8.62 (s, 1H, –CH=), 10.97 (s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) *δ*: 112.27, 113.36, 117.83, 120.91, 125.80, 128.27, 132.99, 143.61, 148.56, 148.84, 150.81, 152.21, 152.26, 164.33 (C=O).

*4.2.3.4. 5-Fluoro-3-((furan-2-ylmethylene)hydrazono)indolin-2-one (**10d**).* Yield (48%); mp: 236 °C; IR (KBr) *ν* 3256 (NH), 1732 (C=O), 1619 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) *δ*: 6.82–8.14 (m, 6H, Ar-H), 8.62 (s, 1H, –CH=), 10.86 (s, D_2O exch., 1H, NH).

*4.2.3.5. 3-((Thiophen-2-ylmethylene)hydrazono)indolin-2-one (**10e**).* Yield (67%); mp: 206 °C; IR (KBr) *ν* 3249 (NH), 1733 (C=O), 1605 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) *δ*: 6.89–8.85 (m, 7H, Ar-H), 8.90 (s, 1H, –CH=), 10.76 (s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) *δ*: 110.78, 116.63, 122.22, 127.82, 128.81, 128.91, 133.11, 133.64, 135.16, 138.42, 144.00, 157.04, 164.64 (C=O).

*4.2.3.6. 5-Methyl-3-((thiophen-2-ylmethylene)hydrazono)indolin-2-one (**10f**).* Yield (63%); mp: 228 °C; IR (KBr) *ν* 3163 (NH), 1735 (C=O), 1617 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) *δ*: 2.26 (s, 3H, CH_3), 6.78–8.46 (m, 6H, Ar-H), 8.91 (s, 1H, –CH=), 10.71 (s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) *δ*: 20.67 (CH_3), 110.51, 116.69, 128.9, 129.56, 130.98, 133.14, 133.90, 135.10, 138.51, 142.71, 151.60, 156.92, 164.72 (C=O).

*4.2.3.7. 5-Chloro-3-((thiophen-2-ylmethylene)hydrazono)indolin-2-one (**10g**).* Yield (57%); mp: 274 °C; IR (KBr) *ν* 3142 (NH), 1732 (C=O), 1667 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) *δ*: 6.91–8.07 (m, 6H, Ar-H), 8.99 (s, 1H, –CH=), 10.96 (s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) *δ*: 112.31, 117.80, 125.80, 128.39, 128.98, 132.94, 133.84, 136.12, 138.25, 143.68, 150.86, 158.59, 164.35 (C=O).

*4.2.3.8. 5-Fluoro-3-((thiophen-2-ylmethylene)hydrazono)indolin-2-one (**10h**).* Yield (50%); mp: 244 °C; IR (KBr) *ν* 3155 (NH), 1735 (C=O), 1619 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) *δ*: 6.89–8.02 (m, 6H, Ar-H), 8.99 (s, 1H, –CH=), 10.88 (s, D_2O exch., 1H, NH).

*4.2.3.9. 3-(((1H-Pyrrol-2-yl)methylene)hydrazono)indolin-2-one (**10i**).* Yield (44%); mp: 280 °C; IR (KBr) *ν* 3242 (2NH), 1714 (C=O), 1606 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) *δ*: 6.91–8.45 (m, 6H, Ar-H), 8.56 (s, 1H, –CH=), 10.95 (s, D_2O exch., 1H, NH), 11.91 (s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) *δ*: 111.04, 115.75, 122.52, 126.81, 128.16, 129.59, 132.79, 133.53, 134.36, 144.32, 144.74, 145.74, 145.16, 168.38 (C=O).

*4.2.3.10. 3-(((1H-Pyrrol-2-yl)methylene)hydrazono)-5-methylindolin-2-one (**10j**).* Yield (42%); mp: 241 °C; IR (KBr) *ν* 3300, 3144 (2NH), 1706 (C=O), 1616 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) *δ*: 2.32 (s, 3H, CH_3), 6.26–8.22 (m, 6H, Ar-H), 8.55 (s, 1H, –CH=), 10.59 (s, D_2O exch., 1H, NH), 11.96 (s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) *δ*: 20.50 (CH_3), 109.99, 111.00, 116.98, 119.28, 126.62, 127.18, 129.66, 131.02, 133,18, 142.03, 150.21, 155.86, 165.25 (C=O).

*4.2.3.11. 3-(((1H-Pyrrol-2-yl)methylene)hydrazono)-5-chloroindolin-2-one (**10k**).* Yield (45%); mp: 248 °C; IR (KBr) *ν* 3303, 3125 (2NH), 1704 (C=O), 1613 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) *δ*: 6.35–8.43 (m, 6H, Ar-H), 8.59 (s, 1H, –CH=), 10.83 (s, D_2O exch., 1H, NH), 12.18 (s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) *δ*: 111.30, 111.73, 117.95, 120.31, 126.00, 127.08, 127.48, 128.29, 132.25, 142.97, 148.85, 157.27, 164.89 (C=O).

*4.2.3.12. 3-(((1H-Pyrrol-2-yl)methylene)hydrazono)-5-fluoroindolin-2-one (**10l**).* Yield (37%); mp: 195 °C; IR (KBr) *ν* 3176 (2NH), 1706 (C=O), 1620 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) *δ*: 6.35–8.39 (m, 6H, Ar-H), 8.61 (s, 1H, –CH=), 10.73 (s, D_2O exch., 1H, NH), 12.13 (s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) *δ*: 109.65, 111.31, 116.31, 117.39, 119.16, 120.76, 127.02, 127.49, 140.54, 141.84, 149.60, 150.54, 156.23, 157.56, 159, 37, 165.24 (C=O).

4.3. Anticancer activity

4.3.1. Materials and methods

4.3.1.1. Chemicals. Dimethylsulfoxide (DMSO), Doxorubicin and Sulfo-Rhodamine-B stain (SRB) were purchased from Merck (Darmstadt, Germany). All other chemicals and reagents used in this study were of analytical grade and purchased from Sigma–Aldrich chemical Co. (St. Louis, MO, USA).

4.3.1.2. Cell culture. The MCF-7 cancer cell line was obtained from the American Type Culture Collection (Rockville, MD, USA). The tumor cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal calf serum (GIBCO), penicillin (100 U/ml) and streptomycin (100 μg/ml) at 37 °C in humidified atmosphere containing 5% CO_2. MCF-7 cells at a concentration of 0.50×10^6 were grown in a 25 cm^2 flask in 5 ml of complete culture medium.

4.3.2. In vitro cytotoxic assay

The cytotoxic activity was determined *in vitro* for all the synthesized compounds and the reference drug using the Sulfo-Rhodamine-B stain (SRB) colorimetric assay according to Skehan et al. [28] This assay was performed in National Cancer Institute, Cairo, Egypt. Cells were inoculated in 96-well microliter plate (10^4 cells/well) for 24 h before treatment with the compounds to allow attachment of cell to the wall of the plate. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the compound under test (10, 25, 50, and 100 μM) were added to the cell monolayer. Triplicate wells were prepared for each individual concentration. Monolayer cells were incubated with the compound(s) for 48 h at 37 °C and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained for 30 min with 0.4% (wt/vol) SRB dissolved in 1% acetic acid. Excess unbound dye was removed by four washes with 1% acetic acid and attached stain was recovered with Tris–EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve for breast tumor cell line after the specified time. The molar concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and compared with the reference drug doxorubicin (CAS, 25316-40-9).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.03.058.

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