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Ammonium chloride catalyzed synthesis of novel Schiff bases from spiro[indoline-3,4′-pyran]-3′-carbonitriles and evaluation of their antimicrobial and anti-breast cancer activities

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Abstract

Background: Indolinone and spiro-indoline derivatives have been employed in the preparation of different important therapeutic compounds required for treatment of anticonvulsants, antibacterial, Antitubercular, and anticancer activities. Schiff bases have been found to possess various pharmacological activities such as antitubercular, plant growth inhibiting, insecticsidal, central nerve system depressant, antibacterial, anticancer, anti-inflammatory, and antimicrobial. Mannich bases have a variety of biological activities such as antibacterial and antifungal activities.

Results: In this study, a green, rapid and efficient protocol for the synthesis of a new series of Schiff bases from spiro[indoline-3,4′-pyran]-3′-carbonitrile derivatives using ammonium chloride as a very inexpensive and readily available reagent. The prepared compounds were assessed in vitro for their antimicrobial activity. Also, the cytotoxic activity of the prepared compounds was assessed in vitro against human cells line MCF7 breast cancer.

Conclusion: Good activity was distinguished for Schiff bases from spiro[indoline-3,4′-pyran]-3′-carbonitriles, with some members recorded higher antimicrobial and anti-breast cancer activities.

Keywords: Ammonium chloride, Schiff bases, Spiro[indoline-3,4′-pyran]-3′-carbonitriles, Antimicrobial, Anti-breast cancer

Background

The development of eco-friendly and environmentally benign catalytic systems is one of the main themes of modern organic synthesis. Ammonium chloride (NH_4Cl) is a very inexpensive and readily available catalyst; it has been reported as a catalyst for the synthesis of various heterocyclic compounds (Shaabani et al. 2003; Dabiri et al. 2009; Fortenberrya et al. 2013; Foroughifarab et al. 2011; Maleki and Salehabadi 2010; Shaabani et al. 2008; Hussein 2015). There are many bioactive molecules which possess various heteroatoms such as nitrogen, sulfur and oxygen, always taken the attention of chemists over the years mainly because of their biological significance. Pyrano derivatives have well-known biological effects, such as analgesic and anti-inflammatory activities (El-Zohry et al. 2008a). Indolinone and spiro-indoline derivatives have possessed broad-spectrum therapeutic activities such as anticonvulsants (Ragavendran et al. 2007; Azam et al. 2009; Sridhar et al. 2002), antibacterial (Rahman et al. 2004; Singh and Luntha 2009; Olomola and Bada 2009), Antitubercular (Sriram et al. 2005), and anticancer activities (Vine et al. 2007; Solomon et al. 2009; Wee et al. 2009). Schiff bases are important compounds owing to their wide range of biological activities and industrial applications. They have been found to possess various pharmacological activities such as antitubercular (Kascheres 2003), plant growth inhibiting (Simunek and Machácek 2010), insecticsidal (Shams et al. 2011; Boyd 1988), central nerve system (CNS) depressant (Drews 2000), antibacterial (Maren 1976; Supuran

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and Scozzafava 2000), anticancer (Simunek et al. 2007), anti-inflammatory, and antimicrobial (Abbate et al. 2004; Abdel-Mohsen and Hussein 2014). Moreover, Mannich bases are reported to show a variety of biological activities, such as antibacterial and antifungal activities (Singare and Ingle 1976; Huneck et al. 1993; Hussein et al. 2015a). Based on these prior observations, we postulated that a Schiff base containing both indoline and pyran pharmacophores could be very effective for antimicrobial and anticancer activity. In this paper and as a consequence of our previous work on the green synthesis of different spiroheterocyclic (Hussein 2013; Hussein and El-Khawaga 2012; Hussein 2012; El-Zohry et al. 2008b, c, 2009), and bioactive heterocyclic compounds (Hussein et al. 2015b; Hussein and Abdel-Monem 2011), we investigated a novel green and efficient protocol that was developed for the synthesis of some Schiff bases (**5a**–**l**) by the condensation of spiro[indoline-3,4′-pyran]-3′-carbonitrile derivatives (**3a**–**c**) with aromatic aldehydes (**4a**–**d**) using ammonium chloride (10 mol%) in refluxing ethanol as shown in Scheme 2 and Table 1. The antimicrobial and cytotoxic properties of the prepared compounds were screened.

Results and discussion

Chemistry

Synthesis of spiro[indoline-3,4′-pyran]-3′-carbonitrile derivatives (3a–c)

The spiro[indoline-3,4′-pyran]-3′-carbonitrile derivatives **3a**–**c** described in this study were prepared as outlined in Scheme 1. The isatin Mannich bases **2a**–**c** were prepared by condensing the active hydrogen atom of istain with formaldehyde and secondary amine namely diphenylamine, diethylamine, and piperidine in ethanol at room temperature as previously reported procedure (Solomon et al. 2009). Compounds **3a**–**c** were obtained in good yield via three-component condensation of **2a**–**c**, malononitrile and ethyl acetoacetate in refluxed ethanol in presence of catalytic amount of piperidine.

Table 1 Synthesis of the Schiff bases 5a–l using NH_4Cl (10 mol%)

Entry	Product[a]	R_1, R_2	R_3	Yield[b] (%)
1	**5a**	$(C_6H_5)_2$	2-OH	92
2	**5b**	$(C_6H_5)_2$	4-OCH_3	82
3	**5c**	$(C_6H_5)_2$	4-Cl	78
4	**5d**	$(C_6H_5)_2$	4-NO_2	75
5	**5e**	$(C_2H_5)_2$	2-OH	88
6	**5f**	$(C_2H_5)_2$	4-OCH_3	84
7	**5g**	$(C_2H_5)_2$	4-Cl	80
8	**5h**	$(C_2H_5)_2$	4-NO_2	77
9	**5i**	1-Piperidinyl	2-OH	90
10	**5j**	1-Piperidinyl	4-OCH_3	86
11	**5k**	1-Piperidinyl	4-Cl	84
12	**5l**	1-Piperidinyl	4-NO_2	74

[a] Reaction conditions: spiro[indoline-3,4′-pyran]-3′-carbonitrile derivatives **3a**–**c** (10 mmol), aromatic aldehydes **4a**–**d** (10 mmol), and NH_4Cl (10 mol%) in 10 mL ethanol/reflux, 2 h

[b] Isolated yields

Synthesis of target compounds

The Schiff bases **5a**–**l** were obtained by the condensation of spiro[indoline-3,4′-pyran]-3′-carbonitrile derivatives **3a**–**c** with aromatic aldehydes **4a**–**d** using ammonium chloride (10 mol%) in refluxing ethanol (Scheme 2; Table 1).

To find out the suitable conditions for the reaction, a series of experiments were performed with the standard reaction of ethyl 2′-amino-3′-cyano-1-((diphenylamino) methyl)-6′-methyl-2-oxospiro[indoline-3,4′-pyran]-5′-carboxylate (**3a**), salicylaldehyde (**4a**) as a model reaction (Table 2; Scheme 3).

Effect of the reaction conditions

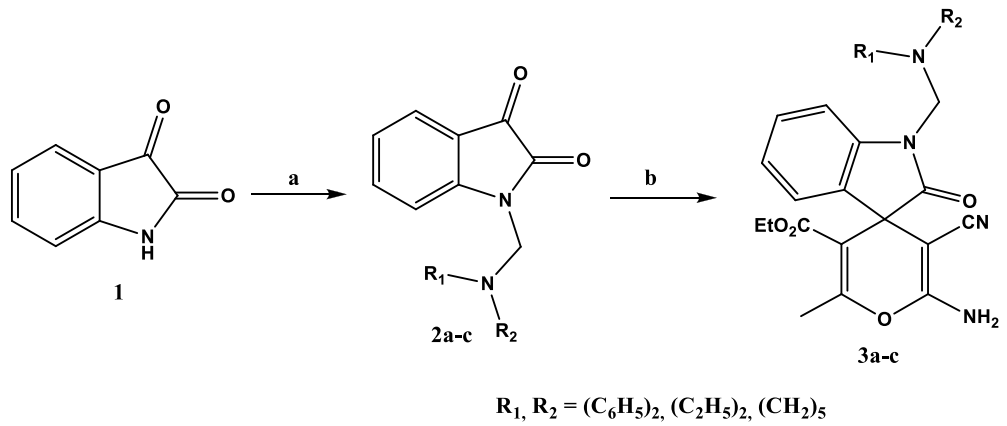
In our initial study, we tried to optimize the model procedure mentioned above by detecting the efficiency of different reaction conditions in the absence and presence of catalysts, such as AcOH, MeOH, EtOH, DMF/AcOH, EtOH/AcOH, EtOH/Et_3N, EtOH/piperidine, dioxane/NH_4Cl, DMF/NH_4Cl, MeOH/NH_4Cl, and EtOH/NH_4Cl (Scheme 3).

In each case, the reactants (10 mmol) were allowed together in 10 mL solvent at reflux temperature for 2 h. In the absence of catalyst, the reaction proceeded with comparatively lower reaction yield (Table 2, entries 1–3). DMF/AcOH, EtOH/AcOH, EtOH/Et_3N and EtOH/piperidine can push the reaction towards the formation of product in yields of 52, 61, 71, and 71 %, respectively (Table 2, entries 4–7). In the presence of ammonium chloride (NH_4Cl) the reaction was possible and the product (**5a**) was obtained in good yields. Ammonium chloride was used in different reaction media such as dioxane, DMF, methanol and ethanol (Table 2, entries 8–11). The best results were obtained when NH_4Cl was used as catalyst in ethanol as reaction medium, which provided a yield of 92 %.

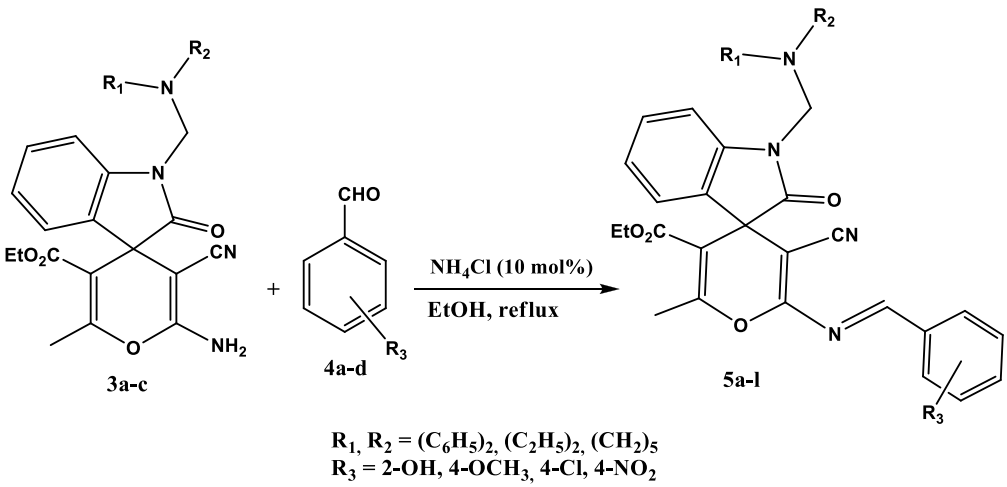
Evaluation of catalytic activity of ammonium chloride

To determine the appropriate concentration of the catalyst used, we investigated the model reaction at different concentrations of NH_4Cl (5, 10, 15, 20, and 25 mol%). The product was formed in 80, 92, 92, 89, and 85 % yield, respectively (Table 3). This indicates that 10 mol% NH_4Cl is sufficient to carry out the reaction smoothly.

The structures of the isolated new products **5a–l** were deduced by analyzing their physical and spectroscopic



Scheme 1 Synthesis of spiro[indoline-3,4′-pyran]-3′-carbonitrile derivatives **3a**–**c**. Reagents and conditions: *a* Mannich reaction (HCHO and secondary amine in ethanol, rt, 2–4 h, *b* malononitrile and ethyl acetoacetate in ethanol/piperidine (one drop), reflux, 5–6 h



Scheme 2 Synthesis of Schiff bases **5a**–**l**

data, such as the data obtained using IR, ^{1}H NMR, and ^{13}C NMR spectroscopy. Taking **5a** as an example, broad absorption band at 3356 cm^{-1} for OH group, sharp absorption band at 2210 cm^{-1} for CN group, and two absorption band at 1735, 1620 cm^{-1} for two C=O groups were observed in the IR spectrum with absence of absorption bands at 3350, 3260 cm^{-1} which corresponding to NH_2 group. The ^{1}H NMR spectrum showed the presence of triplet and quartet signals at 1.28, and 3.85 for ethyl protons, as well as, four singlet signals at $\delta = 2.28$, 5.30, 8.15, and 10.38 ppm for the methyl, methylene, methane, and OH protons, respectively. In the ^{13}C NMR spectrum, the quaternary spiro carbon typically appeared at $\delta = 48.9$ ppm. The nitrile and two carbonyl carbons resonated at 117.4, 164.4, and 178.5 ppm, respectively.

Biological activity

Antimicrobial activity

In view of biological significance, it was studied the synthesized some spiro-indoline derivatives as previous, to get the activities of the potent compounds and evaluated their potential in vitro as antibacterial, antifungal and antitumor activities.

Antimicrobial activities of all the synthesized Schiff bases **5a**–**l** were done by cup-plate agar diffusion method. The compounds were prepared in DMSO and evaluated them for their in vitro antibacterial and antifungal activities against *Bacillus subtilis* and *Fusarium moniliforme* respectively. The bacterial isolate was grown on nutrient agar (37 °C, 24 h) the fungus was grown on potato dextrose agar plates (26 °C, 48–72 h). The results were noted

Table 2 The effect of reaction condition on the synthesis of 5a

Entry	Solvent[a]	Catalyst[b]	Yield[c] (%)
1	AcOH	–	47
2	MeOH	–	40
3	EtOH	–	44
4	DMF	AcOH	52
5	EtOH	AcOH	61
6	EtOH	Et_3N	71
7	EtOH	Piperidine	71
8	Dioxane	NH_4Cl	73
9	DMF	NH_4Cl	75
10	MeOH	NH_4Cl	87
11	EtOH	NH_4Cl	92

The reaction was carried out with ethyl 2′-amino-3′-cyano-1-((diphenylamino) methyl)-6′-methyl-2-oxospiro[indoline-3,4′-pyran]-5′-carboxylate (**3a**) (10 mmol) and 2-hydroxybenzaldehydes (**4a**) (10 mmol)

[a] 10 mL solvent/reflux, 2 h

[b] 10 mol%

[c] Isolated yields

by the presence of clear zone of inhibition around the active compounds (Table 4).

All the synthesized compounds **3a–c** and **5a–l** were tested for in vitro antibacterial activity by inhibition zone method against the reference compound amoxicillin (20 mm). It has been observed that all the compounds tested showed mild to moderate activity against tested bacterium but **5f**, **5g** and **3a**. The antifungal activity of the compounds was studied with *F. Moniliforme*. The results are summarized in Table 4. Fluconazole has been used as reference for inhibitory activity (18 mm) against fungi and some tested compounds showed lesser activity to standard against the tested fungi. While the others showed no antifungal activities against the fungus.

Table 3 Evaluation of catalytic activity of NH_4Cl in the synthesis of 5a

Entry	Amount of NH_4Cl (mol%)	Yield[a] (%)
1	5	80
2	10	92
3	15	92
4	20	89
5	25	85

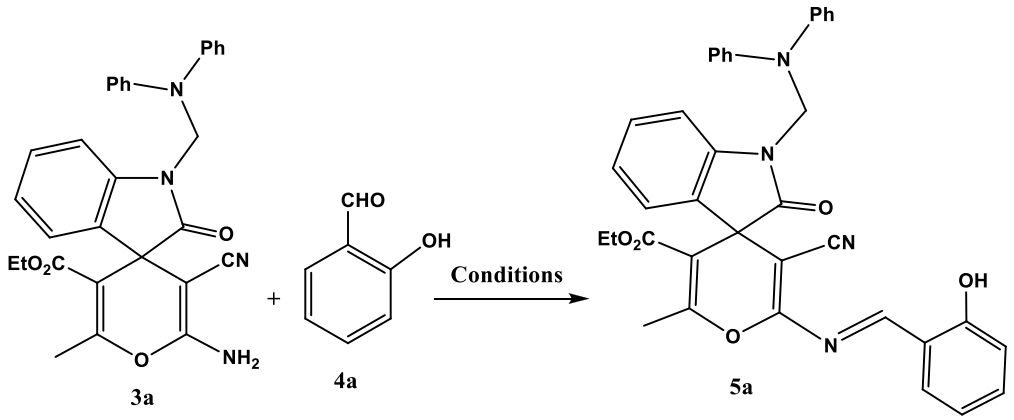
The reaction was carried out with ethyl 2′-amino-3′-cyano-1-((diphenylamino) methyl)-6′-methyl-2-oxospiro[indoline-3,4′-pyran]-5′-carboxylate (**3a**) (10 mmol) and 2-hydroxybenzaldehydes (**4a**) (10 mmol), and NH_4Cl in 10 mL ethanol at refluxing temperature/2 h

[a] Isolated yield of (**5a**)

In vitro anticancer activity

Antitumor activities were found moderate effective as screened for in vitro cytotoxicity activity against human cancer cells line MCF7 breast cancer (Table 5). Although the positive impact of each of the synthesized compound conducted toxicity in cells, some lost IC_{50} in the concentrations used. Viewing of the results, the IC_{50} required was higher than that of the reference compound (3.8 μg/mL) used in the analysis. There are no significant differences between the results of the synthetic chemical compounds compared to the reference compound, where statistically significant differences is the numerical value, and therefore all synthesized compounds located with reference drug in one hand.

It is worth mentioning, that the curve of the compound **5c** only showed clear a straight line. A high concentration compared to the control has shown. Theoretically an expectation of the IC_{50} may be located on the curve. Statistically the range of lethal concentrations IC_{50} may be at about 70 μg/mL, concentration that's when kills ninety percent of the living cells.



Scheme 3 Model reaction

Table 4 Biological activities of the synthesized spiro-indoline derivatives 3a–c and 5a–l

Compounds	R_1, R_2	R_3	IC_{50}	Inhibition zone (mm) *B. subtilis*	Inhibition zone (mm) *F. moniliforme*
3a	$(C_6H_5)_2$	–	>50	12 ± 2	Nill
3b	$(C_2H_5)_2$	–	>50	24 ± 2	15 ± 2
3c	Piperidinyl	–	18.8	19 ± 4	2 ± 1
5a	$(C_6H_5)_2$	2-OH	44.5	20 ± 2	12 ± 1
5b	$(C_6H_5)_2$	4-OCH_3	23.9	Nill	Nill
5c	$(C_6H_5)_2$	4-Cl	>50	24 ± 2	10 ± 2
5d	$(C_6H_5)_2$	4-NO_2	25.0	18 ± 3	6 ± 1
5e	$(C_2H_5)_2$	2-OH	25.0	18 ± 1	4 ± 1
5f	$(C_2H_5)_2$	4-OCH_3	>50	Nill	Nill
5g	$(C_2H_5)_2$	4-Cl	11.9	Nill	Nill
5h	$(C_2H_5)_2$	4-NO_2	20.9	25 ± 3	10 ± 1
5i	Piperidinyl	2-OH	>50	19 ± 3	Nill
5j	Piperidinyl	4-OCH_3	18.8	16 ± 2	4 ± 2
5k	Piperidinyl	4-Cl	>50	12 ± 1	Nill
5l	Piperidinyl	4-NO_2	38.3	4 ± 1	Nill

Table 5 Cytotoxicity activity of spiro-indoline derivatives 3a–c and 5a–l at different concentrations (0.0, 5.0, 12.5, 25 and 50 µg/mL) of some synthesized compounds and reference drug against human cancer cells line MCF7 breast cancer

CONC	DOX	3a	5a	5b	5c	5d	3b	5e	5f	5g	5h	3c	5i	5j	5k	5l
0.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
5.0	0.361	0.836	0.758	0.867	0.930	0.977	0.786	0.911	0.764	0.853	0.978	0.799	0.974	0.688	0.824	0.906
12.5	0.385	0.738	0.479	0.633	0.837	0.740	0.542	0.876	0.643	0.728	0.669	0.642	0.752	0.637	0.588	0.803
25.0	0.332	0.653	0.340	0.498	0.698	0.619	0.462	0.467	0.547	0.560	0.628	0.437	0.829	0.498	0.414	0.725
50.0	0.299	0.631	0.353	0.502	0.442	0.619	0.444	0.416	0.608	0.453	0.564	0.526	0.772	0.479	0.368	0.829
SD	0.024	0.009	0.013	0.025	0.020	0.016	0.034	0.039	0.120	0.022	0.020	0.025	0.010	0.013	0.058	0.009

The biological activities of **3a–c** and its derivatives **5a–l** were summarized in (Fig. 1). Only cell toxicity of **3a** did not record (>50 µg/mL) and antimicrobial activities of **5b** and antifungal activity of **5c** were not detected. The compounds **3b** and **5f** just showed IC_{50} exceed 50 µg/mL but antimicrobial activities did not detect by **5g** and **5f** only. IC_{50} did not show in the tested concentrations of **5i** and **5k**. Also antifungal activities did not record in **5i**, **5k** and **5l** as shown in (Fig. 1).

Conclusion

The authors have developed a green, rapid and efficient protocol for the synthesis of a new series of Schiff bases from spiro[indoline-3,4′-pyran]-3′-carbonitrile derivatives using ammonium chloride as a very inexpensive and readily available reagent. The prepared compounds were assessed in vitro for their antibacterial activity against *B. subtilis* as well as antifungal activity against *F. moniliforme.* Also, the cytotoxic activity of the prepared compounds was assessed in vitro against human cells line MCF7 breast cancer.

Experimental

Chemistry

General methods

The IR spectra of the synthesized compounds were taken on a Shimazu FT spectrometer with a device of singly perturbed internal reflection. ^{1}HNMR spectra (in DMSO-d_6) were recorded on Bruker Ac-400 ultra-shield NMR spectrometer at 400 MHz, using TMS as internal standard. The ^{13}C NMR (100 MHz) spectra were run in dimethylsulfoxide (DMSO-d_6). Chemical shifts were related to that of the solvent. Mass spectra were obtained on a Joel JMSD-300 spectrometer operating at 70 eV. The elemental analysis was carried out on a perkin-Elmer C, H, N analyzer. Melting points were determined in open capillaries on a Gallenkemp melting point apparatus and are uncorrected.

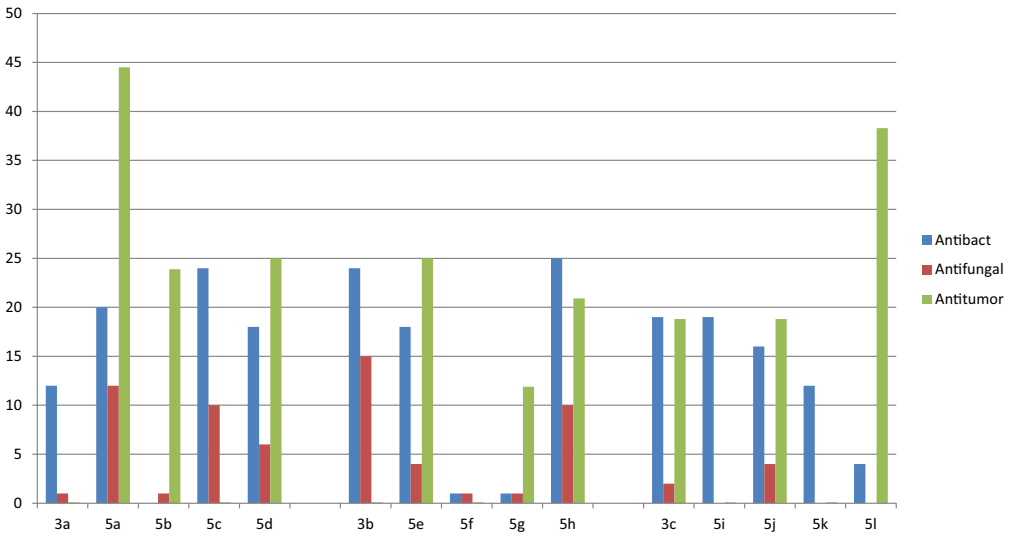


Fig. 1 Biological activities of spiro-indoline derivatives of **3a**–**c** and **5a**–**l**

Synthesis of spiro[indoline-3,4′-pyran]-3′-carbonitrile derivatives **3a**–**c**

General procedure A mixture of 1-((diphenylamino) methyl)indoline-2,3-dione (**2a**) (3.28 g, 10 mmol) and malononitrile (0.66 g, 10 mmol) was dissolved in 20 mL absolute ethanol and stirred for 30 min. Then ethyl acetoacetate (1.30 g, 10 mmol) was added in the presence of piperidine (one drop) and the reaction mixture was heated under reflux with stirring for 6 h. Then cooled and the formed crystals was collected by filtration. Dried and recrystallized for a proper solvent.

*Ethyl-6-amino-5-cyano-1′-((diphenylamino)methyl)-2-methyl-2′-oxo-4H-spiro[pyran-4,3′-indoline]-3-carboxylate (**3a**)* White crystals (ethanol), yield 75 %, mp 225–227 °C. IR (KBr): 3260, 3150 (NH_2), 2185 (CN), 1724 (C=O), 1670 (C=O). ^{1}H NMR: δ = 1.25 (t, 3H, CH_3), 2.29 (s, 3H, CH_3), 3.98–4.00 (q, 2H, CH_2), 5.41 (s, 2H, CH_2), 6.80 (s, 2H, NH_2, D_2O-exchangeable), 6.78–7.71 (m, 14H, Ar–H) ppm. ^{13}C NMR: δ = 13.6 (CH_3), 18.6 (CH_3), 49.0 (C-spiro), 56.57, 60.3 (CH_2), 76.7 (CH_2), 100.4, 118.5 (CN), 121.5, 121.9, 123.0, 123.4, 125.1, 128.6, 128.7, 142.1, 151.0, 156.5, 159.2, 164.6 (C=O), 166.7 (C=O) ppm. MS: *m*/*z* (%) = 506.05 (M^+, 45), 169.11 (100). Anal. Calcd. For $C_{30}H_{26}N_4O_4$ (506.55): C, 71.13; H, 5.17; N, 11.06. Found: C, 71.17; H, 5.08; N, 10.89.

*Ethyl-6-amino-5-cyano-1′-((diethylamino)methyl)-2-methyl-2′-oxo-4H-spiro[pyran-4,3′-indoline]-3-carboxylate (**3b**)* As pale yellow crystals (dioxane), yield 90 %, mp 140–142 °C. IR (KBr): 3270, 3190 (NH_2), 2190 (CN), 1722 (C=O), 1660, (C=O). ^{1}H NMR: δ = 1.10 (t, 6H, CH_3), 1.22 (t, 3H, CH_3), 1.72 (s, 3H, CH_3), 2.48 (q, 4H, $2CH_2$), 4.18–4.20 (q, 2H, OCH_2), 4.39 (s, 2H, CH_2), 6.79 (s, 2H, NH_2, D_2O-exchangeable), 6.92–7.76 (m, 4H, Ar–H) ppm. MS: *m*/*z* (%) = 410.19 (M^+, 23), 133 (100). Anal. Calcd. For $C_{22}H_{26}N_4O_4$ (410.47): C, 64.37; H, 6.38; N, 13.65. Found: C, 64.42; H, 6.37; N, 13.59.

*Ethyl-6-amino-5-cyano-1′-(piperidin-1-ylmethyl)-2-methyl-2′-oxo-4H-spiro[pyran-4,3′-indoline]-3-carboxylate (**3c**)* As pale yellow crystals (ethanol), yield 87 %, mp 189–190 °C. IR (KBr): 3240, 3100 (NH_2), 2170 (CN), 1715 (C=O), 1665 (C=O). ^{1}H NMR: δ = 1.30 (t, 3H, CH_3), 1.57–1.59 (m, 6H, $3CH_2$), 1.74 (s, 3H, CH_3), 2.60 (t, 4H, $2CH_2$), 4.19–4.21 (q, 2H, CH_2), 4.31 (s, 2H, CH_2), 6.85 (s, 2H, NH_2, D_2O-exchangeable), 6.87–7.26 (m, 4H, Ar–H) ppm. ^{13}C NMR: δ = 13.9 (CH_3), 14.1 (CH_3), 26.0, 26.3, 48.1 (C-spiro), 52.8 (C-pipredine), 53.9, 61.6 (CH_2), 76.8 (CH_2), 106.1, 120.0 (CN), 122.9, 123.8, 126.5, 127.5, 131.2, 138.0, 151.5, 156.2, 165.44 (C=O), 167.3 (C=O). MS: *m*/*z* (%) = 422.15 (M^+, 23), 142 (100). Anal. Calcd. For $C_{23}H_{26}N_4O_4$ (422.48): C, 65.39; H, 6.20; N, 13.26. Found: C, 65.36; H, 6.18; N, 13.19.

General procedure for the synthesis of the Schiff bases **5a**–**l**

General procedure To a solution of spiro[indoline-3,4′-pyran]-3′-carbonitrile derivative 3a (0.51 g, 1 mmol) in absolute ethanol (10 mL), corresponding aromatic alde-

hyde (1 mmol) was added. Then NH_4Cl (5.35 mg, 10 mol %) was added and the reaction mixture was refluxed for 2 h (monitored by TLC). After completion of the reaction, cold water (15–25 mL) was added to the reaction mixture. The solid product was filtered, washed with cold water, dried, and recrystallized from proper solvents.

*Ethyl-6-(2-hydroxybenzylidenamino)-5-cyano-1′-((diphenylamino)methyl)-2methyl-2′-oxo-4H-spiro[pyran-4,3′-indoline]-3-carboxylate (**5a**)* As yellow crystals (ethanol), mp 215–217 °C. IR (KBr): 3356 (br. OH), 2210 (CN), 1735 (C=O), 1620 (C=O). ^{1}H NMR (DMSO-d_6): δ = 0.85 (t, 3H, CH_3), 2.53 (s, 3H, CH_3), 3.80 (s, 2H, CH_2), 3.82–3.85 (q, 2H, CH_2), 6.80–7.21 (m, 18H, Ar–H), 10.27 (s, 1H, OH), 10.38 (s, 1H, N=CH) ppm. ^{13}C NMR (DMSO-d_6): δ = 12.9 (CH_3), 18.5 (CH_3), 48.9 (C-spiro), 60.2 (CH_2), 74.9 (CH_2), 104.6, 111.0, 117.4 (CN), 123.3, 125.7, 127.6, 127.9, 128.4, 129.0, 131.1, 131.6, 134.5, 142.1, 144.2, 158.4, 158.9, 163.7 (N=CH), 164.4 (C=O), 178.5 (C=O). MS: *m*/*z* (%) = 610.08 (M^+, 10), 262.10 (100). Anal. Calcd. For $C_{37}H_{30}N_4O_5$ (610.66): C, 72.77; H, 4.95; N, 9.17. Found: C, 72.82; H, 4.76; N, 9.14.

*Ethyl-6-(4-methoxybenzylidenamino)-5-cyano-1′-((diphenylamino)methyl)-2-methyl-2′-oxo-4H-spiro[pyran-4,3′-indoline]-3-carboxylate (**5b**)* As pale yellow crystals (ethanol), mp 212–214 °C. IR (KBr): 2180 (CN), 1740 (C=O), 1625 (C=O). ^{1}H NMR (DMSO-d_6): δ = 0.80 (t, 3H, CH_3), 2.33 (s, 3H, CH_3), 3.32 (s, 3H, OCH_3), 3.81–3.83 (q, 2H, CH_2), 3.89 (s, 2H, CH_2), 6.80–7.90 (m, 18H, Ar–H), 10.38 (s, 1H, N=CH) ppm. ^{13}C NMR (DMSO-d_6): δ = 12.9 (CH_3), 18.5 (CH_3), 48.9 (C-spiro), 55.6 (CH_3), 56.6, 60.2 (CH_2), 75.4 (CH_2), 104.6, 117.4 (CN), 119.3, 121.5, 122.9, 123.8, 125.1, 127.9, 128.4, 129.1, 130.3, 131.7, 134.5, 142.1 (C-aromatic), 158.3 (C-pyrane), 164.6 (N=CH), 169.4 (C=O), 178.5(C=O). MS: *m*/*z* (%) = 624.20 (M^+, 13), 252.51 (100). Anal. Calcd. For $C_{38}H_{32}N_4O_5$ (624.68): C, 73.06; H, 5.16; N, 8.97. Found: C, 72.91; H, 4.90; N, 9.02.

*Ethyl-6-(4-chlorobenzylidenamino)-5-cyano-1′-((diphenylamino)methyl)-2-methyl-2′-oxo-4H-spiro[pyran-4,3′-indoline]-3-carboxylate (**5c**)* As pale brown crystals (ethanol), mp 230–232 °C. IR (KBr): 2180 (CN), 1742 (C=O), 1635 (C=O). ^{1}H NMR (DMSO-d_6): δ = 0.80 (t, 3H, CH_3), 2.33 (s, 3H, CH_3), 3.80 (s, 2H, CH_2), 3.82–3.85 (q, 2H, CH_2), 6.80–7.71 (m, 18H, Ar–H), 10.38 (s, 1H, N=CH) ppm. MS: *m*/*z* (%) = 628.61 (M^+, 16), 262.11 (100). Anal. Calcd. For $C_{37}H_{29}ClN_4O_4$ (629.10): C, 70.64; H, 4.65; Cl, 5.64; N, 8.91. Found: C, 70.75; H, 4.48; Cl, 5.50; N, 8.93.

*Ethyl-6-(4-nitrobenzylidenamino)-5-cyano-1′-((diphenylamino)methyl)-2-methyl-2′-oxo-4H-spiro[pyran-4,3′-indoline]-3-carboxylate (**5d**)* As pale yellow crystals (ethanol), mp 235–237 °C. IR (KBr): 2200 (CN), 1740 (C=O), 1630 (C=O). ^{1}H NMR (DMSO-d_6): δ = 0.80 (t, 3H, CH_3), 2.30 (s, 3H, CH_3), 3.32 (s, 2H, CH_2), 3.81–3.83 (q, 2H, CH_2), 6.80–8.24 (m, 18H, Ar–H), 10.37 (s, 1H, N=CH) ppm. ^{13}C NMR (DMSO-d_6): δ = 12.90 (CH_3), 18.46 (CH_3), 48.91 (C-spiro), 61.12 (CH_2), 75.48 (CH_2), 104.64, 117.35 (CN), 118.45, 121.74, 123.35, 125.75, 127.95, 128.52, 129.21, 131.77, 134.47, 142.00, 158.87 (C-pyrane), 163.65 (N=CH), 166.41 (C=O), 178.45 (C=O). MS: *m*/*z* (%) = 639.20 (M^+, 13), 169.69 (100). Anal. Calcd. For $C_{37}H_{29}N_5O_6$ (639.66): C, 69.47; H, 4.57; N, 10.95. Found: C, 69.44; H, 4.48; N, 10.81.

*Ethyl-6-(2-hydroxybenzylidenamino)-5-cyano-1′-((diethylamino)methyl)-2-methyl-2′-oxo-4H-spiro[pyran-4,3′-indoline]-3-carboxylate (**5e**)* As pale yellow crystals (ethanol), mp 125–127 °C. IR (KBr): 3414 (br. OH), 2191 (CN), 1724 (C=O), 1620 (C=O). ^{1}H NMR (DMSO-d_6): δ = 1.15 (t, 6H, $2CH_3$), 1.24 (t, 3H, CH_3), 2.27 (s, 3H, CH_3), 2.39 (q, 4H, $2CH_2$), 3.86 (s, 2H, CH_2), 4.32–4.39 (m, 4H, $2CH_2$), 6.84–7.80 (m, 8H, Ar–H), 9.85 (s, 1H, N=CH), 12.50 (s, 1H, OH, exchangeable with D_2O) ppm. ^{13}C NMR (DMSO-d_6): δ = 13.8 ($2CH_3$), 14.1 (CH_3), 14.2 (CH_3), 49.0 (C spiro), 61.4 ($2CH_2$), 61.6 (CH_2), 67.7 (CH_2), 100.2, 110.9 (CN), 123.1, 123.4, 123.7, 124.3, 125.7, 127.7, 129.2, 130.4, 131.2, 134.6, 140.1, 147.8, 162.2, 166.3 (N=CH), 167.3 (C=O), 170.3 (C=O). MS: *m*/*z* (%) = 514.08 (M^+, 41), 262.14 (100). Anal. Calcd. For $C_{29}H_{30}N_4O_5$ (514.57): C, 67.69; H, 5.88; N, 10.89. Found: C, 67.42; H, 5.62; N, 11.01.

*Ethyl-6-(4-methoxybenzylidenamino)-5-cyano-1′-((diethylamino)methyl)-2-methyl-2′-oxo-4H-spiro[pyran-4,3′-indoline]-3-carboxylate (**5f**)* As pale yellow crystals (petroleum ether 60–80), mp 70–72 °C. IR (KBr): 2160 (CN), 1720 (C=O), 1650 (C=O). ^{1}H NMR (DMSO-d_6): δ = 0.84–1.24 (m, 6H, $2CH_3$), 1.41 (t, 3H, CH_3), 2.83 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 4.19–4.39 (m, 8H, $3CH_2$), 6.85–7.83 (m, 8H, Ar–H), 9.87 (s, 1H, N=CH) ppm. MS: *m*/*z* (%) = 528.21 (M^+, 20), 234.05 (100). Anal. Calcd. For $C_{30}H_{32}N_4O_5$ (528.60): C, 68.17; H, 6.10; N, 10.60. Found: C, 68.21; H, 6.01; N, 10.56.

*Ethyl-6-(4-chlorobenzylidenamino)-5-cyano-1′-((diethylamino)methyl)-2-methyl-2′-one-4H-spiro[pyran-4,3′-indoline]-3-carboxylate (**5g**)* As pale yellow crystals (diethyl ether), mp 90–92 °C. IR (KBr): 2190 (CN), 1730 (C=O), 1655 (C=O). ^{1}H NMR (DMSO-d_6): δ = 0.91–1.32 (m, 6H, $2CH_3$), 1.39 (t, 3H, CH_3), 2.83 (s, 3H, CH_3), 4.19–4.39 (m, 6H, $3CH_2$), 5.51 (s, 2H, CH_2), 7.03–7.65 (m, 8H, Ar–H), 9.88 (s, 1H, N=CH) ppm. MS: *m*/*z* (%) = 532.09 (M^+, 31), 146.00 (100). Anal. Calcd. For $C_{29}H_{29}ClN_4O_4$ (533.02): C, 65.35; H, 5.48; Cl, 6.65; N, 10.51. Found: C, 65.45; H, 5.73; Cl, 6.61; N, 10.47.

*Ethyl-6-(4-nitrobezylidenamino)-5-cyano-1′-((diethylamino)methyl)-2-methyl-2′-oxo-4H-spiro[pyran-4,3′-indoline]-3-carboxylate (**5h**)* As pale yellow crystals (diethyl ether), m.p 85–87 °C. IR (KBr): 2170 (CN), 1724 (C=O), 1650 (C=O). ^{1}H NMR (DMSO-d_6): δ = 0.88–1.31 (m, 6H, $2CH_3$), 1.41 (t, 3H, CH_3), 2.83 (s, 3H, CH_3), 4.21–4.36 (m, 6H, $3CH_2$), 4.38 (s, 2H, CH_2), 7.12–7.85 (m, 8H, Ar–H), 9.89 (s, 1H, N=CH) ppm. ^{13}C NMR (DMSO-d_6): δ = 13.9 ($2CH_3$), 14.2 (CH_3), 15.1 (CH_3), 49.1 (C spiro), 61.3 ($2CH_2$), 61.6 (CH_2), 76.7 (CH_2), 100.1, 117.3 (CN), 123.1, 123.4, 123.7, 124.3, 125.7, 127.7, 129.2, 130.4, 131.2, 134.6, 147.9, 162.2, 166.0 (N=CH), 167.3 (C=O), 170.0 (C=O). MS: *m*/*z* (%) = 543.01 (M^+, 15), 234 (100). Anal. Calcd. For $C_{29}H_{29}N_5O_6$ (543.57): C, 64.08; H, 5.38; N, 12.88. Found: C, 64.04; H, 5.64; N, 12.84.

*Ethyl-6-(2-hydroxybenzylidenamino)-5-cyano-1′-(piperidin-1-ylmethyl)-2methyl-2′-oxo-4H-spiro[pyran-4,3′-indoline]-3-carboxylate (**5i**)* As pale yellow crystals (diethyl ether), mp 110–112 °C. IR (KBr): 3455 (br. OH), 2210 (CN), 1753 (C=O), 1634 (C=O). ^{1}H NMR (DMSO-d_6): δ = 090–1.13 (m, 6H, $3CH_2$), 1.16 (t, 3H, CH_3), 2.67 (t, 4H, $2CH_2$), 3.46 (s, 3H, CH_3), 3.64 (s, 2H, CH_2), 4.15–4.31 (q, 2H, CH_2), 6.81–7.87 (m, 8H, Ar–H), 9.96 (s, 1H, N=CH), 10.51 (s, 1H, OH) ppm. ^{13}C NMR (DMSO-d_6): δ = 13.9 (CH_3), 14.1 (CH_3), 26.2, 26.6, 52.7 (CH_2-piperidine), 49.0 (C-spiro), 53.9, 61.1, 61.6 (CH_2), 76.7 (CH_2), 109.2, 117.6 (CN), 119.8, 120.1, 121.5, 123.0, 123.8, 124.6, 126.5, 131.1, 133.7, 137.0, 140.6 (C-aromatic), 156.9, 163.5 (N=CH), 165.4 (C=O), 167.2 (C=O). MS: *m*/*z* (%) = 526.09 (M^+, 5), 234.22 (100). Anal. Calcd. For $C_{30}H_{30}N_4O_5$ (526.58): C, 68.43; H, 5.74; N, 10.64. Found: C, 68.40; H, 5.71; N, 10.36.

*Ethyl-6-(4-methoxybenzylidenamino)-5-cyano-1′-(piperidin-1-ylmethyl)-2-methyl-2′-oxo-4H-spiro[pyran-4,3′-indoline]-3-carboxylate (**5j**)* As brown crystals (diethyl ether), mp 114–117 °C. IR (KBr): 2210 (CN), 1742 (C=O), 1631 (C=O). ^{1}H NMR (DMSO-d_6): δ = 0.90–1.13 (m, 6H, $3CH_2$), 1.16 (t, 3H, CH_3), 2.67 (t, 4H, $2CH_2$), 3.46 (s, 3H, CH_3), 3.64 (s, 2H, CH_2), 3.75 (s, 3H, OCH_3), 4.15–4.30 (q, 2H, CH_2), 6.86–7.89 (m, 8H, Ar–H), 9.95 (s, 1H, N=CH) ppm. ^{13}C NMR (DMSO-d_6): δ = 14.0 (CH_3), 14.1 (CH_3), 25.9, 26.1 (CH_2), 48.4 (C-spiro), 52.1 (CH_2), 53.4 (CH_3), 55.8, 61.6 (CH_2), 76.8 (CH_2), 106.0, 120.0 (CN), 121.1, 122.6, 123.3, 124.8, 125.9, 126.3, 128.3, 129.7, 131.9, 156.2, 164.3, 164.7 (N=CH), 167.5 (C=O), 169.9 (C=O). MS: *m*/*z* (%) = 540.20 (M^+, 31), 299 (100). Anal. Calcd. For $C_{31}H_{32}N_4O_5$ (540.61): C, 68.87; H, 5.97; N, 10.36. Found: C, 68.63; H, 5.69; N, 10.32.

*Ethyl-6-(4-chlorobenzylidenamino)-5-cyano-1′-(piperidin-1-ylmethyl)-2methyl-2′-oxo-4H-spiro[pyran-4,3′-indoline]-3-carboxylate (**5k**)* As pale yellow crystals (diethyl ether), mp 140–142 °C. IR (KBr): 2219 (CN), 1725 (C=O), 1622 (C=O). ^{1}H NMR (DMSO-d_6): δ = 1.13–1.17 (m, 6H, $3CH_2$), 1.25 (t, 3H, CH_3), 2.65 (t, 4H, $2CH_2$), 3.46 (s, 3H, CH_3), 3.65 (s, 2H, CH_2), 4.15–4.31 (q, 2H, CH_2), 6.81–7.85 (m, 8H, Ar–H), 9.96 (s, 1H, N=CH) ppm. ^{13}C NMR (DMSO-d_6): δ = 14.1 (CH_3), 14.2 (CH_3), 26.3, 26.6 (CH_2), 49.1 (C-spiro), 53.9 (CH_2), 61.3, 61.6 (CH_2), 76.7 (CH_2), 106.2, 120.0 (CN), 121.5, 123.0, 124.5, 125.7, 126.5, 128.3, 128.7, 130.9, 131.1, 134.7 (C-aromatic), 156.2, 163.7, 165.7 (N=CH), 167.4 (C=O), 174.1 (C=O). MS: *m*/*z* (%) = 544.10 (M^+, 11), 261.15 (100). Anal. Calcd. For $C_{30}H_{29}ClN_4O_4$ (544.03): C, 66.11; H, 5.36; Cl, 6.50; N, 10.28. Found: C, 66.13; H, 5.30; Cl, 6.47; N, 10.32.

*Ethyl-6-(4-nitrobenzylidenamino)-5-cyano-1′-(piperidin-1-ylmethyl)-2methyl-2′-oxo-4H-spiro[pyran-4,3′-indoline]-3-carboxylate (**5l**)* As pale brown crystals (ethanol), mp 160–162 °C. IR (KBr): 2210 (CN), 1732 (C=O), 1630 (C=O). ^{1}H NMR (DMSO-d_6): δ = 1.15–1.17 (m, 6H, $3CH_2$), 1.28 (t, 3H, CH_3), 2.15 (t, 4H, $2CH_2$), 3.58 (s, 3H, CH_3), 4.15–4.35 (q, 2H, CH_2), 5.50 (s, 2H, CH_2), 6.98–7.37 (m, 8H, Ar–H), 8.39 (s, 1H, N=CH) ppm. MS: *m*/*z* (%) = 555.12 (M^+, 25), 205 (100). Anal. Calcd. For $C_{30}H_{29}N_5O_6$ (555.58): C, 64.85; H, 5.26; N, 12.61. Found: C, 64.82; H, 5.28; N, 12.59.

Biological screening

Antibacterial activity

The newly synthesized spiro-indoline derivatives **3a**–**c** and **5a**–**l** were screened for their antibacterial activity against bacterial isolate namely *B. subtilis* by inhibition zone method against the reference compound amoxicillin (20 mm). The bacterial subcultures (18–24 h grown) were added to sterilize nutrient agar medium and shaken thoroughly to ensure uniform distribution of organism throughout the medium. In sterilized Petri dishes containing about 20 mL of the medium, wells were made with a sterile cork borer and were filled with 0.1 mL of respective solution. Then, the Petri dishes were kept for incubation in an inverted position for 24–48 h at 37 °C in an incubator. When growth inhibition zones were developed, diameter (in mm) was measured and compared with that of amoxicillin.

Antifungal activity

The newly synthesized spiro-indoline derivatives **3a**–**c** and **5a**–**l** were screened for their antifungal activity against fungus *F. moniliforme* at the concentration levels of 50 μg/mL by inhibition zone method. Fluconazole has been used as reference for inhibitory activity (18 mm) against fungi. To the sterilized potato dextrose agar medium, subculture of fungus were added and shaken thoroughly to ensure uniform distribution and incubated

for 72 h. Then, this was poured into sterilized and labeled Petri dishes and allowed to solidify. Wells were made in each plate by a cork borer. Each well was filled with 0.1 mL of test solution and the other with respective concentrations of standard dilutions. The plates were left 2–3 h for diffusion and incubated at 37 °C for 24 h. The diameter of the zones of growth inhibition was measured and compared with that of standard. The solutions of required concentration (50 µg/mL) of test compounds were prepared by dissolving the compounds in DMSO.

Anticancer activity

Breast cancer cell line (MCF7) as human tumor was used in this study. The cytotoxicity was measured in vitro for the newly synthesized compounds assay using the method of Philip et al. (1990). The in vitro anticancer.

Screening was done by the pharmacology unit at Pharmacology unit, Cancer biology department, the National Cancer Institute, Cairo University. Cells were plated in 96-multiwell plate (104 cells/well) for 24 h before treatment with the compound(s) to allow attachment of cell to the wall of the plate. Tested compounds were dissolved in dimethyl sulfoxide (DMSO). Different concentrations of the compound under test (0.0, 5.0, 12.5, 25.0 and 50.0 µg/mL) 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 % (W/V) 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 to the reference drug Doxorubicin (CAS, 25316-40-9). The surviving fractions were expressed as means and the results are given in Table 5.

Authors' contributions

HFA-S analyzed the data and shared in experimental section; HAE analyzed the data, shared in the experimental section and shared in writing the manuscript; AHA performed the biological activity and shared in writing manuscript. EMH designed the research, shared in the experimental work, shared in writing the manuscript, and revised the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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