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

Hossa F. Al-Shareef¹, Heba A. Elhady¹,², Amany H. Aboellili³ and Essam M. Hussein¹,⁴*

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, insecticidal, 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 (NH₄Cl) 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; Fortenberry et al. 2013; Foroughifar 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.

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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) 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

| Table 1 Synthesis of the Schiff bases 5a–l using NH4Cl (10 mol%) |
|----------------|-----------------|---------------|----------------|----------------|
| Entry | Product| R1, R2 | R3 | Yielda (%) |
|-------|--------|--------|-----|------------|
| 1     | 5a     | (C6H5)2 2-OH | 92  |
| 2     | 5b     | (C6H5)2 4-OCH3 | 82  |
| 3     | 5c     | (C6H5)2 4-Cl | 78  |
| 4     | 5d     | (C6H5)2 4-NO2 | 75  |
| 5     | 5e     | (C6H5)2 2-OH | 88  |
| 6     | 5f     | (C6H5)2 4-OCH3 | 84  |
| 7     | 5g     | (C6H5)2 4-Cl | 80  |
| 8     | 5h     | (C2H5)2 4-NO2 | 77  |
| 9     | 5i     | 1-Piperidinyl 2-OH | 90  |
| 10    | 5j     | 1-Piperidinyl 4-OCH3 | 86  |
| 11    | 5k     | 1-Piperidinyl 4-Cl | 84  |
| 12    | 5l     | 1-Piperidinyl 4-NO2 | 74  |

* Reaction conditions: spiro[indoline-3,4′-pyran]-3′-carbonitrile derivatives 3a–c (10 mmol), aromatic aldehydes 4a–d (10 mmol), and NH4Cl (10 mol%) in 10 mL ethanol/reflux, 2 h
* Isolated yields

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/Et2N, EtOH/piperidine, dioxane/NH4Cl, DMF/NH4Cl, MeOH/NH4Cl, and EtOH/NH4Cl (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/Et2N 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 (NH4Cl) 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 NH4Cl 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 NH4Cl (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% NH4Cl 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
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
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 2 The effect of reaction condition on the synthesis of 5a

| Entry | Solvent<sup>a</sup> | Catalyst<sup>b</sup> | Yield<sup>c</sup> (%) |
|-------|----------------------|----------------------|----------------------|
| 1     | AcOH                 | –                     | 47                   |
| 2     | MeOH                 | –                     | 40                   |
| 3     | EtOH                 | –                     | 44                   |
| 4     | DMF                  | AcOH                 | 52                   |
| 5     | EtOH                 | AcOH                 | 61                   |
| 6     | EtOH                 | Et<sub>3</sub>N       | 71                   |
| 7     | EtOH                 | Piperidine            | 71                   |
| 8     | Dioxan               | NH<sub>4</sub>Cl       | 73                   |
| 9     | DMF                  | NH<sub>4</sub>Cl       | 75                   |
| 10    | MeOH                 | NH<sub>4</sub>Cl       | 87                   |
| 11    | EtOH                 | NH<sub>4</sub>Cl       | 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) in 10 mL ethanol at refluxing temperature/2 h.

### Table 3 Evaluation of catalytic activity of NH<sub>4</sub>Cl in the synthesis of 5a

| Entry | Amount of NH<sub>4</sub>Cl (mol%) | Yield<sup>a</sup> (%) |
|-------|-----------------------------------|----------------------|
| 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<sub>4</sub>Cl in 10 mL ethanol at refluxing temperature/2 h.

<sup>a</sup> 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<sub>50</sub> in the concentrations used. Viewing of the results, the IC<sub>50</sub> 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<sub>50</sub> may be located on the curve. Statistically the range of lethal concentrations IC<sub>50</sub> may be at about 70 µg/mL, concentration that’s when kills ninety percent of the living cells.

**Scheme 3** Model reaction
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 IC₅₀ exceed 50 µg/mL but antimicrobial activities did not detect by 5g and 5f only. IC₅₀ 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. ¹H NMR spectra (in DMSO-d₆) were recorded on Bruker Ac-400 ultra-shield NMR spectrometer at 400 MHz, using TMS as internal standard. The ¹³C NMR (100 MHz) spectra were run in dimethylsulfoxide (DMSO-d₆). 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.

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

| Compounds | R₁ | R₂ | R₃ | IC₅₀ | Inhibition zone (mm) *B. subtilis* | Inhibition zone (mm) *F. moniliforme* |
|-----------|----|----|----|------|-------------------------------|-------------------------------------|
| 3a        | (C₆H₅)₂ | –   | >50 | 12 ± 2 | Nill                          | Nill                                    |
| 3b        | (C₂H₅)₂ | –   | >50 | 24 ± 2 | 15 ± 2                       |                                       |
| 3c        | Piperidinyl | –   | 18.8 | 19 ± 4 | 2 ± 1                       |                                       |
| 5a        | (C₆H₅)₂ | 2-OH | 44.5 | 20 ± 2 | 12 ± 1                       |                                       |
| 5b        | (C₆H₅)₂ | 4-OCH₃ | 23.9 | Nill | Nill                         |                                       |
| 5c        | (C₆H₅)₂ | 4-Cl | >50 | 24 ± 2 | 10 ± 2                       |                                       |
| 5d        | (C₆H₅)₂ | 4-NO₂ | 25.0 | 18 ± 3 | 6 ± 1                       |                                       |
| 5e        | (C₆H₅)₂ | 2-OH | 25.0 | 18 ± 1 | 4 ± 1                       |                                       |
| 5f        | (C₂H₅)₂ | 4-OCH₃ | >50 | Nill | Nill                         |                                       |
| 5g        | (C₂H₅)₂ | 4-Cl | 11.9 | Nill | Nill                         |                                       |
| 5h        | Piperidinyl | 4-NO₂ | 20.9 | 25 ± 3 | 10 ± 1                       |                                       |
| 5i        | Piperidinyl | 2-OH | >50 | 19 ± 3 | Nill                         |                                       |
| 5j        | Piperidinyl | 4-OCH₃ | 18.8 | 16 ± 2 | 4 ± 2                       |                                       |
| 5k        | Piperidinyl | 4-Cl | >50 | 12 ± 1 | Nill                         |                                       |
| 5l        | Piperidinyl | 4-NO₂ | 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 |
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 acetocetate (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 (NH2), 2185 (CN), 1724 (C=O), 1670 (C=O). 1H NMR: δ = 1.25 (t, 3H, CH3), 2.29 (s, 3H, CH3), 3.98–4.00 (q, 2H, CH2), 5.41 (s, 2H, CH2), 6.80 (s, 2H, NH2, D2O-exchangeable), 6.78–7.71 (m, 14H, Ar–H) ppm. 13C NMR: δ = 13.6 (CH3), 18.6 (CH3), 49.0 (C-spiro), 56.57, 60.3 (CH2), 76.7 (CH2), 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 C30H26N4O4 (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 (NH2), 2190 (CN), 1724 (C=O), 1660, (C=O). 1H NMR: δ = 1.10 (t, 6H, CH2), 1.22 (t, 3H, CH3), 1.72 (s, 3H, CH3), 2.48 (q, 4H, 2CH2), 4.18–4.20 (q, 2H, OCH2), 4.39 (s, 2H, CH2), 6.79 (s, 2H, NH2, D2O-exchangeable), 6.92–7.76 (m, 4H, Ar–H) ppm. MS: m/z (%) = 410.19 (M+, 23), 133 (100). Anal. Calcd. For C22H26N4O4 (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 (NH2), 2190 (CN), 1715 (C=O), 1665 (C=O). 1H NMR: δ = 1.30 (t, 3H, CH3), 1.57–1.59 (m, 6H, 3CH2), 1.74 (s, 3H, CH3), 2.60 (t, 4H, 2CH2), 4.19–4.21 (q, 2H, CH2), 4.31 (s, 2H, CH2), 6.85 (s, 2H, NH2, D2O-exchangeable), 6.87–7.26 (m, 4H, Ar–H) ppm. 13C NMR: δ = 13.9 (CH3), 14.1 (CH3), 26.0, 26.3, 48.1 (C-spiro), 52.8 (C-pipredine), 53.9, 61.6 (CH2), 76.8 (CH2), 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 C23H26N4O4 (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**

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-
hydrate (1 mmol) was added. Then NH₄Cl (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’-((diphe nylamino)methyl)-2-methyl-2’-oxo-4H-spiro[pyran-4,3’-indol one]-3-carboxylate (5a)** As yellow crystals (ethanol), mp 215–217 °C. IR (KBr): 3356 (br. OH), 2210 (CN), 1735 (C=O), 1620 (C=O). ¹H NMR (DMSO-d₆): δ = 0.85 (t, 3H, CH₃), 2.35 (s, 3H, CH₃), 3.80 (s, 2H, CH₂), 3.82–3.85 (q, 2H, CH₂), 6.80–7.21 (m, 18H, Ar–H), 10.27 (s, 1H, OH), 10.38 (s, 1H, N=CH) ppm. ¹³C NMR (DMSO-d₆): δ = 12.9 (CH₃), 18.5 (CH₃), 48.9 (C-spiro), 60.2 (CH₂), 74.9 (CH₇), 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. Calcld. For C₃₇H₃₂N₅O₆ (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’-((diphe nylamino)methyl)-2-methyl-2’-oxo-4H-spiro[pyran-4,3’-i ndoline]-3-carboxylate (5b)** As pale yellow crystals (ethanol), mp 212–214 °C. IR (KBr): 2180 (CN), 1740 (C=O), 1625 (C=O). ¹H NMR (DMSO-d₆): δ = 0.80 (t, 3H, CH₃), 2.33 (s, 3H, CH₃), 3.32 (s, 3H, OCH₃), 3.81–3.83 (q, 2H, CH₂), 3.89 (s, 2H, CH₂), 6.80–7.90 (m, 18H, Ar–H), 10.38 (s, 1H, N=CH) ppm. ¹³C NMR (DMSO-d₆): δ = 12.9 (CH₃), 18.5 (CH₃), 48.9 (C-spiro), 55.6 (CH₂), 56.6, 60.2 (CH₂), 75.4 (CH₇), 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-pyrene), 164.6 (N=CH), 169.4 (C=O), 178.5 (C=O). MS: m/z (%) = 624.20 (M⁺, 13), 252.51 (100). Anal. Calcld. For C₃₈H₃₀N₄O₅ (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’-((diphe nylamino)methyl)-2-methyl-2’-oxo-4H-spiro[pyran-4,3’-ind ole]-3-carboxylate (5c)** As pale brown crystals (ethanol), mp 230–232 °C. IR (KBr): 2180 (CN), 1742 (C=O), 1635 (C=O). ¹H NMR (DMSO-d₆): δ = 0.80 (t, 3H, CH₃), 2.33 (s, 3H, CH₃), 3.80 (s, 2H, CH₂), 3.82–3.85 (q, 2H, CH₂), 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. Calcld. For C₃₇H₂₉ClN₄O₅ (629.10): C, 70.64; H, 4.65; Cl, 6.61; N, 8.93. Found: C, 70.75; H, 4.48; Cl, 5.50; N, 8.93.

**Ethyl-6-(4-nitrobenzylidenamino)-5-cyano-1’-((diphe nylamino)methyl)-2-methyl-2’-oxo-4H-spiro[pyran-4,3’-ind ole]-3-carboxylate (5d)** As pale yellow crystals (ethano-
**Ethyl-6-(4-nitrobenzylidenamino)-5-cyano-1′-((diethylamino)2-methyl-2′-oxo-4H-spiro[pyran-4,3′-indoline]-3-carboxylate (S1)** As pale yellow crystals (diethyl ether), mp 85–87 °C. IR (KBr): 3435 (br. OH), 2210 (CN), 1753 (C=O), 1634 (C=O). 1H NMR (DMSO-d6): δ = 0.79 – 0.90 (m, 6H, 2CH3), 1.16 (t, 3H, CH3), 2.67 (t, 4H, CH2), 3.46 (s, 3H, CH3), 3.64 (s, 2H, CH2), 7.57 (s, 3H, OCH3), 4.15 – 4.30 (q, 2H, CH2), 6.86 – 7.88 (m, 8H, Ar–H), 9.96 (s, 1H, N=CH). 13C NMR (DMSO-d6): δ = 26.3, 26.6 (CH2), 76.7 (CH2), 100.1, 117.3 (CN), 123.1, 123.4, 123.7, 124.3, 125.7, 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 C30H30N4O5 (526.58): C, 64.82; H, 5.28; N, 12.59. Found: C, 64.04; H, 5.64; N, 12.84.

**Ethyl-6-(2-hydroxybenzylidenamino)-5-cyano-1′-(piperidin-1-ylmethyl)-2-methyl-2′-oxo-4H-spiro[pyran-4,3′-indoline]-3-carboxylate (S5)** As pale yellow crystals (diethyl ether), mp 110–112 °C. IR (KBr): 3485 (br. OH), 2210 (CN), 1753 (C=O), 1634 (C=O). 1H NMR (DMSO-d6): δ = 0.89 – 0.91 (m, 6H, 2CH3), 1.16 (t, 3H, CH3), 2.67 (t, 4H, CH2), 3.46 (s, 3H, CH3), 3.64 (s, 2H, CH2), 7.57 (s, 3H, OCH3), 4.15 – 4.30 (q, 2H, CH2), 6.86 – 7.88 (m, 8H, Ar–H), 9.96 (s, 1H, N=CH). 13C NMR (DMSO-d6): δ = 26.3, 26.6 (CH2), 76.7 (CH2), 100.1, 117.3 (CN), 123.1, 123.4, 123.7, 124.3, 125.7, 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 C30H30N4O5 (526.58): C, 64.82; H, 5.64; N, 12.59.

**Biological screening**

**Antibacterial activity**

The newly synthesized spiro-indoline derivatives 3a–c and 5a–1 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 cultures (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–1 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 kept 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-multitwell 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 % CO2. 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 (IC50) 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

HFle-5 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.

Author details

1 Department of Chemistry, Faculty of Applied Sciences, Umm Al-Qura University, Makkah, Saudi Arabia. 2 Department of Chemistry, Faculty of Science (Girl’s), Al-Azhar University, Cairo, Egypt. 3 Department of Botany and Microbiology, Faculty of Science, Cairo University, Giza, Egypt. 4 Department of Chemistry, Faculty of Science, Assiut University, Assuit 71516, Egypt.

Competing interests

The authors declare that they have no competing interests.

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References

Abbate F, Casini A, Owa T, Scozzafava A, Supuran CT (2004) Carbonic anhydrase inhibitors: E7070, a sulfonamide anticancer agent, potently inhibits cystosolic isozymes I and II, and transmembrane, tumor-associated isozyme IX. Bioorg Med Chem Lett 14:217–223

Abdel-Mohsen ShiA, Hussein EM (2014) A green synthetic approach to the synthesis of Schiff bases from 4-amino-2-thioxo-1,3-diazaspiro[5.5]undec-4-ene-5-carbonitrile as potential anti-inflammatory agents. Russ J Bioorg Chem 40:343–349

Azam F, Alkskas IA, Khokra SL, Prakash O (2009) Synthesis of some novel \( \text{N}_2\text{N}'\)-naphthyl[1,2-d]thiazol-2-yl]semicarbazides as potential anticonvulsants. Eur J Med Chem 44:203–211

Boyd AE (1988) Sulfonylurea receptors, ion channels, and fruit flies. Diabetes 37:847–850

Dabiri M, Bahramnejad M, Baghbanzadeh M (2009) Ammonium salt catalyzed multicomponent transformation: simple route to functionalized spirochromenes and spiroacridines. Tetrahedron 65:9443–9447

Drews J (2000) Drug discovery: a historical perspective. Science 287:1960–1964

El-Zohry MF, Elossaily YA, Mohamed ThA, Hussein EM (2008a) Synthesis and reactions of some new spiropyranthiazoline derivatives. Phosphorus, Sulfur Silicon Relat Elem 183:2095–2107

El-Zohry MF, Mohamed ThA, Hussein EM (2008b) A facile synthesis of some new 7,8-dihydropyrimido[1,2-a]pyridine-7,3″-indoline-2″-one deriva-
tives. Heterocycles 75:2791–2802

El-Zohry MF, Elossaily YA, Mohamed ThA, Hussein EM (2008c) Synthesis and reactions of some new spiro[indenol(1,2-b)]pyran-4,3″-indolines). Heterocycles 75:955–963

El-Zohry MF, Mohamed ThA, Hussein EM (2009) Novel syntheses of some new 3,4-dihydropyrimido[1,2-a]pyridine-3,3″-indoline-2″-one deriva-
tives. Monatsch Chem 140:265–272

Foroughifar A, Mohimbikakale A, Moghariana H, Mozafraria R, Esfahanib HRM (2011) Ammonium chloride–catalyzed one-pot synthesis of tetrahydrobenzo[α]xanthen-11-one derivatives under solvent-free condi-
tions. Synth Commun 41:2663–2673

Fortenberry C, Nammalwara B, Buncea RA (2013) Ammonium chloride–cata-
yzed synthesis of benzo-fused heterocycles from o-substituted anilines and orthoesters. Org Prep Proc Int 45:S7–65

Hunecck S, Joseph R, George KE (1993) Study of Polyschiff’s base as a protective moiety. Russ J Org Chem 51:54–64

Hussein EM (2013) Ultrasound-promoted efficient domino reaction for the synthesis of novel hexahydroquinolines bearing a sulfonamide moiety. Russ J Org Chem 51:54–64

Hussein EM, Abdel-Moneim MI (2011) Regioselective synthesis and anti-
inflammatory activity of novel dispiro[pyrazoline-4,3″-pyridoline-2″,3″-
indoline]-2″,3″,5-triones. Arkivoc 10:85–98

Hussein EM, El-Khawaga AM (2012) Simple and clean procedure for three-
component syntheses of spiro{pyrido[2,3-d]pyrimidines in aqueous medium. Z Naturforsch 67b:231–237

Hussein EM (2013) Ultrasound-promoted efficient domino reaction for the one-pot synthesis of spiro-5-cyanopyrimidines: a rapid procedure. Monatsh Chem 144:1691–1697

Hussein EM (2015) Ammonium chloride-catalyzed four-component sono-
chemical synthesis of novel hexahydroquinolines bearing a sulfonamide moiety. Russ J Org Chem 51:54–64

Hussein EM, Abdel-Moneim MI (2011) Regioselective synthesis and anti-
inflammatory activity of novel dispiro[pyrazoline-4,3″-pyridoline-2″,3″-
indoline]-2″,3″,5-triones. Arkivoc 10:85–98

Hussein EM, El-Khawaga AM (2012) Simple and clean procedure for three-
component syntheses of spiro{pyrido[2,3-d]pyrimidines in aqueous medium. Z Naturforsch 67b:231–237

Hussein EM, Masareet GhS, Khairou KhS (2015a) Efficient synthesis and anti-
microbial evaluation of some Mannich bases from 2-arylidine-1-thia-4-
azaaspiro[4,5]decane-3-ones. Chem Cent J 9:925

Hussein EM, Al-Shareef HF, Aboellil AH, Elhady HA (2015b) Synthesis of some novel 6′-[4-chlorophenyl]-3′,4′-bipyridine-3′-carbonitriles: assessment of their antimicrobial and cytotoxic activity. Z Naturforsch 70b:783–795
Kascheres CM (2003) The chemistry of enaminoles, diazocarbonyls and small rings: our contribution. J Braz Chem Soc 14:945–969
Maleki B, Salehabadi H (2010) Ammonium chloride, as a mild and efficient catalyst for the synthesis of some 2-arylbenzothiazoles and bisbenzothiazole derivatives. Eur J Chem 1:377–380
Maen TH (1976) Relations between structure and biological activity of sulphonamides. Annu Rev Pharmacol Toxicol 16:309–327
Olomola TO, Bada DA (2009) Synthesis and antibacterial activity of two spiro[indole]thiazole derivatives. Toxicol Environ Chem 91(5):941–946
Philip S, Ritsa S, Dominic S, Anne M, James M, David V, Jonathan TW, Heidi B, Susan K, Michael RB (1990) New colorimetric cytotoxicity assay for anticancer-drug screening. J Nat Cancer Inst 82:1107–1112
Ragavendran JV, Siriram D, Patel SK, Reddy IV, Bhathwajan N (2007) Design and synthesis and anticonvulsant activity from a combined phthalimide–GABA–anilide and hydrazone pharmacophore. Eur J Med Chem 42:146–151
Rahman AHA, Keshk EM, Hanna MA (2004) Synthesis and evaluation of some new spirothonolobaline based heterocycles as potentially active antimicrobial. Bioorg Med Chem 12:2483–2488
Shaabani A, Bazgir A, Teimouri F (2003) Ammonium chloride-catalyzed one-pot synthesis of 3,4-dihydroxyindazolin-2(1H)-ones under solvent-free conditions. Tetrahedron Lett 44:857–859
Shaabani A, Rezaeezadeh F, Soleimani E (2008) Ammonium chloride catalyzed one-pot synthesis of imidazo[1,2-a]pyridines. Monatsh Chem 139:931–933
Shams HZ, Mohareb RM, Helal MH, Mahmoud AES (2011) Design and synthesis of novel antimicrobial acyclic and heterocyclic dyes and their precursors for dyeing and/or textile finishing based on 2-N-acylamino-4,5,6,7-tetrahydro-benzo[b]thiophene systems. Molecules 16:6271–6305
Simunek P, Macháček V (2010) The structure and tautomerism of azo coupled β-enaminoles. Dyes Pigments 86:197–205
Simunek P, Svobodová M, Bertolasi V, Presto L, Lycka A, Macháček V (2007) Structure and tautomerism of azo coupling products from N-alkylaminoles derived from acetylacetone and benzoylaceton in solid phase and in solution. New J Chem 31:429–438
Singare MS, Ingle DB (1976) Synthesis of pyrimidine Schiff bases as anticancer agents. J Indian Chem Soc 53:1036–1037
Singh GS, Luntha P (2009) Synthesis and antimicrobial activity of new 1-alkyl/cyclohexyl-3,3-diaryl-1′-methylspiro[azetidine-2,3′-indoline]-2′,4-diones. Eur J Med Chem 44:2265–2269
Solomon VR, Hu C, Lee H (2009) Hybrid pharmacophore design and synthesis of isatin–benzothiazole analogs for their anti-breast cancer activity. Bioorg Med Chem 17:7585–7592
Sridhar SK, Pandeyya SN, Stables JP, Ramesh A (2002) Anticonvulsant activity of hydrazones, Schiff and Mannich bases of isatin derivatives. Eur J Pharm Sci 16:129–132
Sriram D, Yogeeswari P, Gopal G (2005) Synthesis, anti-HIV and antitubercular activities of lamivudine produgs. Eur J Med Chem 40:1373–1376
Supuran CT, Scozzafava A (2000) Carbonic anhydrase inhibitors and their therapeutic potential. Expert Opin Ther Pat 10:575–600
Vine KL, Locke JM, Ronson M (2007) In vitro cytotoxicity evaluation of some substituted isatin derivatives. Bioorg Med Chem 15:931–938
Wee XX, Yeo WK, Zhang B, Tan VBC (2009) Synthesis and evaluation of functionalized isodindigo as antiproliferative agents. Bioorg Med Chem 17:7561–7562

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