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Synthesis, Characterization and *in vitro* Antibacterial Evaluation of New Oxindoles and Spiro-Oxindoles Derivatives

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ARYLIDENES oxindole **3** and **5** were synthesized via the reactions of isatin **1** with malononitrile dimmer **2** and 5-amino-3-(cyanomethyl)-1H-pyrazole-4-carbonitrile **4.** Compound **3** react by Michael addition with malononitrile, ethyl 2-cyanoacetate and acetyl acetone to give 6'-amino-2'-(dicyanomethylene)-2-oxo-2',3'-dihydro-1'H-spiro[indoline-3,4'-pyridine]-3',5'-dicarbonitrile **6a**, 2'-(dicyanomethylene) 2,6'-dioxospiro[indoline-3,4'-piperidine]-3',5'-dicarbonitrile **6b** and 2-(5'-acetyl-3'-cyano-6'-methyl-2-oxo-1'H-spiro[indoline-3,4'-pyridin]-2'(3'H) ylidene) malononitrile **7**, respectively. compound **3** also, reacted with dimethylformamide dimethyl acetal to give N,N-dimethyl-N'-(1,1,3-tricyano-3-((Z)-2-oxoindolin-3-ylidene)prop-1-en-2-yl)formimidamide **7** which cyclized by ammonium acetate to give 4-amino-6-(cyano(2-oxoindolin-3-ylidene)methyl)pyrimidine-5-carbonitrile **8**. Compound **5** reacted with dimedone **10** and acetyl acetone to give 3-(cyano(2-oxoindolin-3-ylidene)methyl)-5-((5,5-dimethyl-3-oxocyclohex-1-en-1-yl)amino)-1H-pyrazole-4-carbonitrile **11** and 2-(cyano(2-oxoindolin-3-ylidene)methyl)-5,7dimethylpyrazolo[1,5-a] pyrimidine-3-carbonitrile **12.** Moreover, compound **3** and **5** reacted with hydrazine to give 3,3'-(hydrazine-1,2-diylidene)bis(indolin-2-one) **15**. The antibacterial activities of the synthesized compounds were evaluated.

Keywords: Isatin, Malononitrile dimmer, Arylidenes oxindole, Spiro-oxindole, Antibacterial.

Introduction

Isatin is an oxindole derivative and used as important intermediates in organic synthesis [1-3]. Isatin is an important component of many alkaloids, [4] drugs, [5] dyes, [6, 7] pesticides, and analytical reagents. Various activities such as antibacterial, [8] antifungal, [9] antiviral, [10] anti-HIV, [11] anti- mycobacterial, [12] anticancer, [13] anti-inflammatory [14] and anticonvulsant activities [15] are possessed by derivatives of isatin. Oxindoles are biologically [16-19] active and naturally occurring molecules found in both human organs and plants [20-22]. Recently, several oxindole derivatives have been reported as anticancer agents [23, 24]. Oxindoles have a broad range of pharmacological actions, being described as anxiogenic and sedative agents [25, 26] or antagonists of guanylate cyclase-coupled atrial natriuretic peptide receptor [27].

The heterocyclic spiro-oxindole ring system is a widely distributed structural framework present in a number of pharmaceuticals and natural products [28] with potent biological activities. [29-31] Among these, the spirooxindole cores fused nitrogen heterocyclic rings at the quaternary C3-spirostereocenters are the most widely distributed because of their frequent occurrence in biologically active compounds as well as in applicable synthetic intermediates for the synthesis of clinical pharmaceuticals. [29, 32-35].

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Experimental

All analyses were done at faculty of science, Sohag University, Sohag (Egypt). All melting points are uncorrected. IR spectra (KBr) were recorded on a FTIR 5300 spectrometer (ν, cm^{-1}). The ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-*d6* at 400MHz on a Varian Gemini NMR spectrometer (δ, ppm) using TMS as an internal standard. Microbiology screening was carried out in Botany Department, Faculty of Science, Al-Azhar University, Assiut

Synthesis of 2-amino-3-(2-oxoindolin-3-ylidene) prop-1-ene-1,1,3 tricarbonitrile 3:

A mixture of isatin (1.47g, 0.01 mole) and malononitrile dimmer (1.32g, 0.01mole) in absolute ethanol was heated under reflux for 3 hours. After cooling, the precipitate was filtered and recrystallized from ethanol/ DMF (3:1) as brown crystals, Yield: (2.2g, 84.3%), m.p. over 300 °C; IR (KBr, cm^{-1}): 3325, 3275 (NH_2, NH), 3050 (CH aromatic), 2215 (CN) and 1725 (C=O); ^{1}HNMR (DMSO-d_6, ppm): 6.95 (s, 2H, NH_2), 7.18 (d, 1H, Ar-H), 7.4 (m, 2H, Ar-H), 8.68 (d, 1H, Ar-H) and 10.45 (s, 1H, NH); Anal. Calcd. For $C_{14}H_7N_5O$: C, 64.37; H, 2.70; N, 26.81. Found: C, 64.12; H, 2.54; N, 26.65.

Synthesis of 5-amino-3-(cyano(2-oxoindolin-3-ylidene)methyl)-1H-pyrazole-4-carbonitrile 5.

A mixture of isatin (1.47g, 0.01 mole) and 5-amino-3-(cyanomethyl)-1H-pyrazole-4-carbonitrile **4** (1.47g, 0.01mole) in absolute ethanol was heated under reflux for 3 hours. After cooling, the precipitate was filtered and recrystallized from ethanol/ DMF (3:1) as brown crystals, Yield: (2.25g, 81.5%), m.p. 216-218 °C; IR (KBr, cm^{-1}): 3387, 3324, 3232 (NH_2, NH), 2213 (CN) and 1707 (C=O); ^{1}HNMR (DMSO-d_6, ppm): 6.08 (s, 2H, NH_2), 6.75 (d, 1H, Ar-H), 7.25 (m, 2H, Ar-H), 8.56 (d, 1H, Ar-H) and 10.35 and 12.28 (2s, 2H, 2NH); Anal. Calcd. For $C_{14}H_8N_6O$: C, 60.87; H, 2.92; N, 30.42.. Found: C, 60.65; H, 2.80; N, 30.36.

General procedure for synthesis of 2-oxo-1'H-spiro indoline-3,4'-pyridine derivatives 6a,b and 7

To a mixture of compound **3** (2.61g, 0.01mole) and the appropriate active methylene compound (0.01mole) in absolute ethanol, a few drops triethylamine was added. The reaction mixture was refluxed for 3 h. After cooling, the solid product, formed, was collected by filtration and recrystallized from an appropriate solvent.

Synthesis of 6'-amino-2'-(dicyanomethylene)-2-oxo-2',3'-dihydro-1'H-spiro[indoline-3,4'-pyridine]-3',5'-dicarbonitrile 6a:

Use of malononitrile (0.66g, 0.01mole) gave deep brown crystals, recrystallized from ethanol, Yield: (2.6g, 79.5%), m.p. over 300 °C; IR(KBr, cm-1): 3395, 3320, 3295 (NH_2, 2NH), 3035 (CH aromatic), 2920 (CH aliphatic), 2236 (CN) and 1728 (C=O); ^{1}HNMR (DMSO-d_6, ppm): 4.12 (s, 1H, CH), 6.65 (s, 2H, NH_2), 7.1 (d, 1H, Ar-H), 7.26 (m, 2H, Ar-H), 7.82 (d, 1H, Ar-H) and 9.08, 10.2 (2s, 2H, 2NH); ^{13}CNMR (DMSO-d_6, ppm): 51.2, 54.6, 64.2. 69.5, 109.6, 115.8, 119.4, 123, 128.4, 132.3, 140.5, 149, 160.3, 172; Anal. Calcd. For $C_{17}H_9N_7O$: C, 62.38; H, 2.77; N, 29.96. Found: C, 62.16; H, 2.48; N, 29.78.

Synthesis of ethyl 6'-amino-3'-cyano-2'-(dicyanomethylene)-2-oxo-2',3'-dihydro-1'H-spiro[indoline-3,4'-pyridine]-5'-carboxylate 6b:

Use of ethyl 2-cyanoacetate (1.12ml, 0.01mole) give brown crystals, recrystallized from ethanol, Yield: (2.5g, 76.2%), m.p. over 300 °C; IR(KBr, cm-1): 3375, 3324, 3315, (NH_2, 2NH), 3085 (CH aromatic), 2942 (CH aliphatic), 2185 (CN) and 1734, 1685 (2 C=O); ^{1}HNMR (DMSO-d_6, ppm): 1.21(t, 3H, CH_3), 4.1 (s, 1H, CH), 4.32 (q, 2H, CH_2), 6.64 (s, 2H, NH_2), 6.85 (d, 1H, Ar-H), 7.28 (m, 2H, Ar-H), 7.75 (d, 1H, Ar-H) and 9.65, 10.34 (2s, 2H, 2NH); Anal. Calcd. For $C_{19}H_{14}N_6O_3$: C, 60.96; H, 3.77; N, 22.45. Found: C, 60.78; H, 3.54; N, 22.26**.**

Synthesis of 2-(5'-acetyl-3'-cyano-6'-methyl-2-oxo-1'H-spiro[indoline-3,4'-pyridin]-2'(3'H) ylidene) malononitrile 7:

Use of acetyl acetone (1.03ml, 0.01mole) gave brown crystals, recrystallized from ethanol, Yield: (2.45g, 71.4%), m.p. over 300 °C; IR(KBr, cm^{-1}): 3310, 3285 (2NH), 3015 (CH aromatic), 2895 (CH aliphatic), 2212 (CN) and 1715, 1690 (2 C=O); ^{1}HNMR (DMSO-d_6, ppm): 2.25 (s, 3H, CH_3), 3.02 (s, 3H, CH_3CO), 4.16 (s, 1H, CH), 7.1 (d, 1H, Ar-H), 7.2 (m, 2H, Ar-H), 7.75 (d, 1H, Ar-H) and 9.24, 10.23 (2s, 2H, 2NH); Anal. Calcd. For $C_{19}H_{13}N_5O_2$: C, 66.47; H, 3.82; N, 20.40 Found: C, 66.29; H, 3.72; N, 20.28**.**

Synthesis of N,N-dimethyl-N'-(1,1,3-tricyano-3-((Z)-2-oxoindolin-3-ylidene)prop-1-en-2-yl) formimid amide 8:

With a mixture of compound **3** (2.61g, 0.01mole) and DMFDMA (1.32ml, 0.01mole) in dry dioxane was refluxed for 4 h. After cooling, the precipitate was filtered and recrystallized from

ethanol as brown crystals, Yield: (2.3g, 72.7%), m.p. over 300 °C; IR(KBr, cm^{-1}): 3295 (NH), 3062 (CH aromatic), 2942 (CH aliphatic), 2220 (CN) and 1725 (C=O); ^{1}HNMR (DMSO-d_6, ppm): 3.25 (s, 6H, 2CH3), 6.95 (d, 1H, Ar-H), 7.3 (m, 2H, Ar-H), 8.52 (d, 1H, Ar-H), 8.8 (s, 1H, CH) and 10.55 (s, 1H, NH); ^{13}CNMR (DMSO-d_6, ppm): 40.6, 65.2, 113.6, 116.8, 124.2, 128.4, 134.7, 142.4, 159.5, 170.6; Anal. Calcd. For $C_{17}H_{12}N_6O$: C, 64.55; H, 3.82; N, 26.57 Found: C, 64.34; H, 3.74; N, 26.38**.**

Synthesis of 4-amino-6-(cyano(2-oxoindolin-3-ylidene)methyl)pyrimidine-5-carbonitrile 9:

To a mixture of compound **7** (1.58g, 0.005mole) and ammonium acetate in acetic acid was refluxed for 2 h. After cooling, the precipitate was filtered and recrystallized from ethanol as brown crystals, Yield: (1g, 69.4%), m.p. over 300 °C; IR (KBr, cm^{-1}): 3420, 3326 (NH_2, NH), 3014 (CH aromatic), 2228 (CN) and 1719 (C=O); ^{1}HNMR(DMSO-d_6, ppm): 6.9 (s, 2H, NH_2), 7.12 (d, 1H, Ar-H), 7.34 (m, 2H, Ar-H), 8.26 (d, 1H, Ar-H), 8.68 (s, 1H, pyrimidine-H) and 10.43 (s, 1H, NH); Anal. Calcd. For $C_{15}H_8N_6O$: C, 62.50; H, 2.80; N, 29.15 Found: C, 62.32; H, 2.68; N, 28.94

Synthesis of 3-(cyano(2-oxoindolin-3-ylidene) methyl)-5-((5,5-dimethyl-3-oxocyclohex-1-en-1-yl)amino)-1H-pyrazole-4-carbonitrile 11.

To a mixture of 5-amino-3-(cyano(2-oxoindolin-3-ylidene)methyl)-1H-pyrazole-4-carbonitrile **5** (2.76g, 0.01mole) with 5,5-dimethylcyclohexane-1,3-dione (dimedone) **10** (1.4g, 0.01mole) in ethanol and triethylamine was heated under reflux for 4 hours. After cooling, the precipitate was collected by filtration and recrystallized from ethanol as brown crystals, Yield: (2.9g, 72.8%), m.p. = 240-242 °C; IR (KBr, cm^{-1}): 3330, 3298, 3209 (3NH), 2959(CH aliphatic), 1723 and 1692 (2 C=O); ^{1}HNMR (DMSO-d_6, ppm): 1.15 (s, 6H, CH_3), 2.07 (s, 2H, dimedone-CH_2), 2.4 (s, 2H, dimedone-CH_2), 5.5 (s, 1H, dimedone-CH) 6.88 (d, 1H, Ar-H), 7.28 (m, 2H, Ar-H), 8.56 (d, 1H, Ar-H), 9.12 10.5 and 12.4 (3br, 3H, 3NH); Anal. Calcd. For $C_{22}H_{18}N_6O_2$: C, 66.32; H, 4.55; N, 21.09; Found: C, 66.12; H, 4.45; N, 20.78**.**

Synthesis of 2-(cyano(2-oxoindolin-3-ylidene) methyl)-5,7-dimethylpyrazolo [1,5-a]pyrimidine-3-carbonitrile 12.

Method A: A mixture of 5-amino-3-(cyano(2-oxoindolin-3-ylidene)methyl)-1H-pyrazole-4-carbonitrile **5** (2.76g, 0.01mole) with acetyl acetone (1.03ml, 0.01mole) in acetic acid was heated under reflux for 6 hours. After cooling, the precipitate was collected by filtration Yield: (2.5g, 73.5%).

Method B: A mixture of isatin **1** (1.47 g, 0.01mole) with 2-(cyanomethyl)-5,7-dimethylpyrazolo[1,5-a]pyrimidine-3-carbonitrile **13** (2.11g, 0.01mole) in ethanol and some drops of piperidine was heated under reflux for 4 hours. After cooling, the precipitate was collected by filtration Yield: (2.8g, 82.3%).

Recrystallized from ethanol as deep brown crystals, m.p. over 300 °C; IR (KBr, cm^{-1}): 3398 (NH), 2945(CH aliphatic), 2228 (CN) and 1716 (C=O); ^{1}HNMR (DMSO-d_6, ppm): 2.52, 267 (2s, 6H, $2CH_3$), 6.95 (d, 1H, Ar-H), 7.32 (m, 2H, Ar-H), 8.52 (d, 1H, Ar-H), 10.4 (s, 1H, NH); Anal. Calcd. For $C_{19}H_{12}N_6O$: C, 67.05; H, 3.55; N, 24.69; Found: C, 66.94; H, 3.42; N, 24.53**.**

Synthesis of 3,3'-(hydrazine-1,2-diylidene) bis(indolin-2-one) 15.

Method A: a mixture of compound **3** (2.61g, 0.01mole) and hydrazine hydrate (excess) in ethanol was refluxed for 3 h. After cooling, the precipitate was filtered; Yield: (2.3g, 79.3%).

Method B: a mixture of compound **5** (2.76g, 0.01mole) and hydrazine hydrate (excess) in ethanol was refluxed for 3 h. After cooling, the precipitate was filtered; Yield: (2.1g, 72.4%).

Recrystallized from ethanol as brown crystals, m.p. over 300 °C [Lit. over 300 °C] [36]; IR (KBr, cm^{-1}): 3325 (2NH), 3065(CH aromatic), and 1750 (2 C=O); ^{1}HNMR (DMSO-d_6, ppm): 7.25(d, 2H, Ar-H), 7.34 (m, 4H, Ar-H), 7.75(d, 2H, Ar-H)and 10.6 (s, 2H, 2NH); Anal. Calcd. For $C_{16}H_{10}N_4O_2$: C, 66.20; H, 3.47; N, 19.30 Found: C, 66.09; H, 3.29; N, 19.18**.**

Biological Screening:

a) Agar well diffusion method:

The antimicrobial activities were carried out by well diffusion method [37]. Wheel diameter was 6 mm filled with 50 µl of the 10% (w/v) test samples. Chloramphenicol (5 mg/ml) was used as positive control for bacteria and nystatine (30000 Iu/ml) for fungi, while dimethylformamide (DMF) was used as negative control. Muller-Hinton agar (MHA) plates previously inoculated with 24 h old broth cultures of the bacterial isolates were used for antibacterial activity. The diameter of the inhibition zone around the disc, measured in millimeter, is used as positive bioactivity.

b) Test microorganisms:

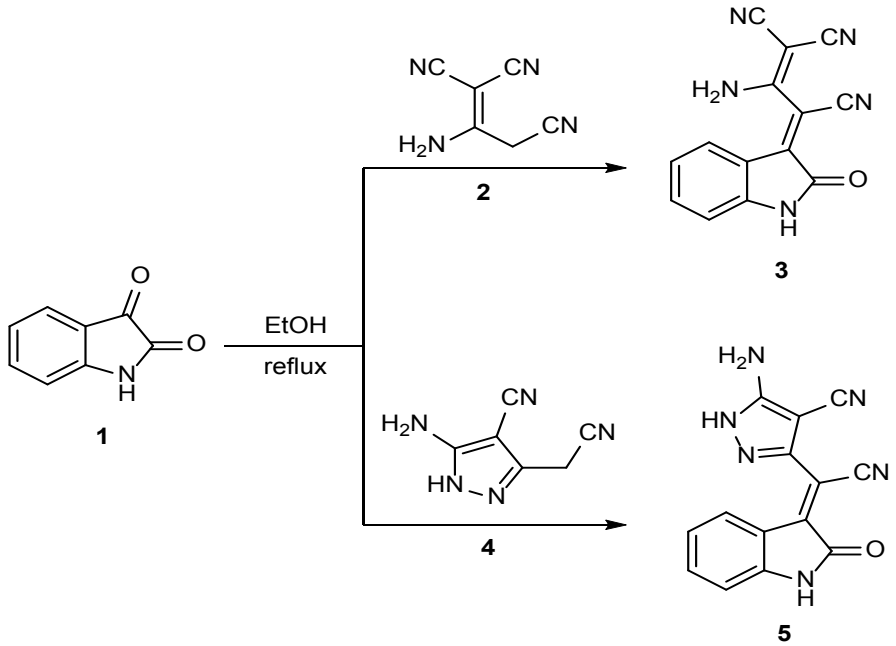
Five isolates of pathogenic bacteria (two Gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus,* and three Gram-negative bacteria, *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* were used as test organisms.

Results and Discussion

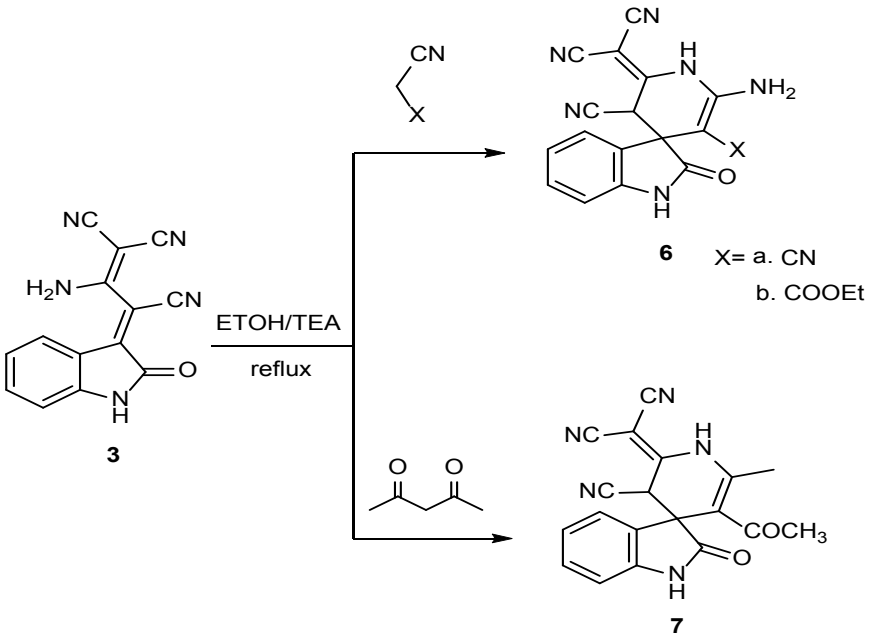
Chemistry:

Isatin **1** reacted with malononitrile dimmer **2** and 5-amino-3-(cyanomethyl)-1H-pyrazole-4-carbonitrile **4** in ethanol as solvent afforded 2-Amino-3-(2-oxoindolin-3-ylidene)prop-1-ene-1,1,3-tricarbonitrile 3 and 5-amino-3-(cyano(2-oxoindolin-3-ylidene)methyl)-1H-pyrazole-4-carbonitrile **5** respectively (Scheme 1). The structure of compounds **3** and **5** was determined by their elemental analysis and spectral data. Whereas, IR spectrum of compound **3** showed the appearance of absorption bands at 3325, 2215 cm^{-1} corresponding to amino and cyano groups, respectively, and IR spectrum of compound **5** showed the appearance of absorption bands at 3387, 3337, 3262 cm^{-1} for NH_2 and two NH and 2213 , 1707 cm^{-1} for cyano and carbonyl groups and ^{1}H NMR of compound **5** shows the appearance of a broad signal at 6.25 ppm corresponding to amino group and two singlet signals at 10.35 and 12.28 ppm for two NH groups.

The utility of arylidenes **3** and **5** as starting materials for synthesis of heterocyclic compounds were investigated. Thus, treating arylidene **3** with malononitrile, ethyl 2-cyanoacetate and acetyl acetone in ethanol and presence of triethylamine afforded 6'-amino-2'-(dicyanomethylene)-2-oxo-2',3'-dihydro-1'H-spiro[indoline-3,4'-pyridine]-3',5'-dicarbonitrile **6a**, 2'- (dicyanomethylene) -2,6' dioxospiro [indoline-3,4'-piperidine-3',5'-dicarbonitrile **6b** and 2-(5'-acetyl-3'-cyano-6'-methyl-2-oxo-1'H-spiro [indoline-3,4'-pyridin]-2'(3'H)-ylidene) malono-nitrile **7** respectively. (Scheme 2) mechanism of this reaction occurs by Michael addition follow by cyclization and aromatization. The structures of the isolated compounds were established by their elemental analyses and spectral data. The IR spectrum of compound **6a** showed three bands at 3395, 3320, 3295 $cm^{-1}$for NH_2 and two NH groups and the ^{1}HNMR spectrum showed the appearance of two singlet signals at 4.12 and 9.08 ppm for CH and NH group of the pyridine ring. Similarly, the, IR spectrum of compound **6b** showed the appearance of the band at 1685 cm^{-1} and 1734 cm^{-1} for two carbonyl groups and ^{1}HNMR spectrum showed the appearance of a triplet signal at 1.21 and a quartet signal at 4.32 ppm corresponding to the ethyl group. IR spectrum of compound **7** showed the appearance of two absorption bands at 1690, 1715 cm^{-1} for two carbonyl groups and ^{1}HNMR spectrum showed four singlet signals at 2.25, 3.02, 4.16 and 9.24 ppm corresponding to CH_3, CH_3CO, CH and NH groups of the pyridine ring respectively.



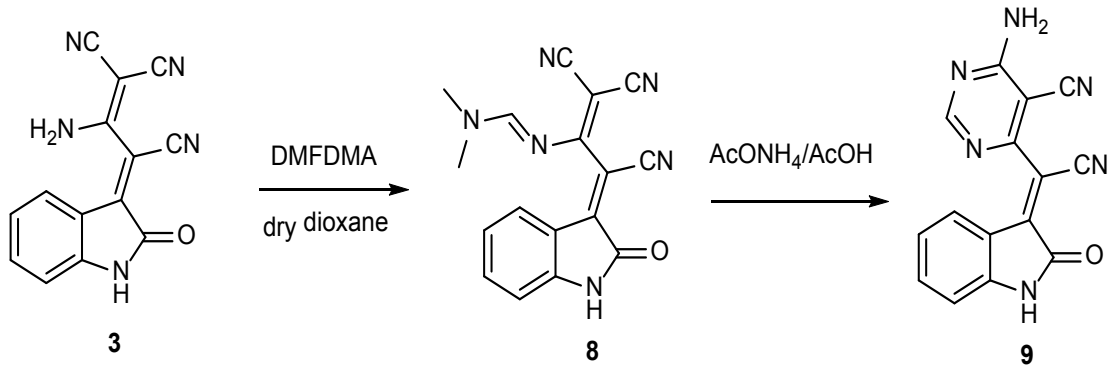
Scheme 1. Synthesis of arylidenes 3 and 5.



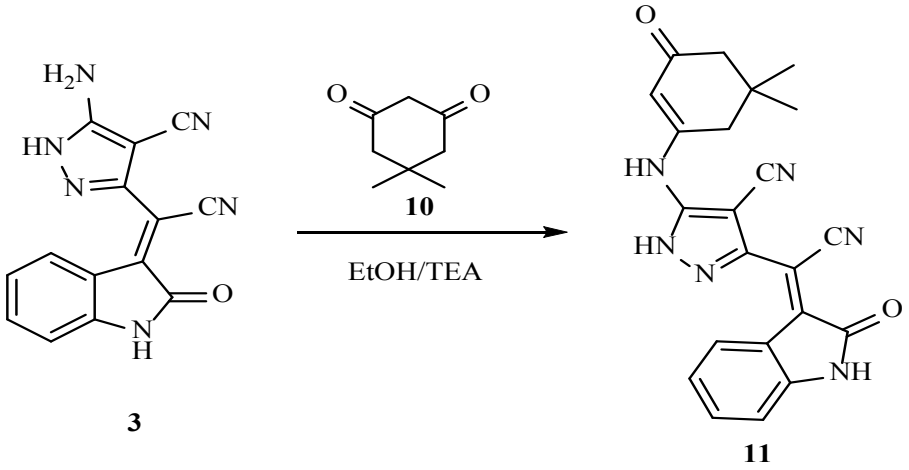
Scheme 2. Synthesis of 2-oxo-1'H-spiro indoline-3,4'-pyridine derivatives

On the other hand, reaction of arylidene **3** with dimethylformamide dimethyl acetal (DMFDMA) afforded N,N-dimethyl-N'-(1,1,3-tricyano-3-((Z)-2-oxoindolin-3-ylidene)prop-1-en-2 yl)formimid amide **8 (**Scheme 3). The structure of compound **8** is confirmed by its elemental analysis and spectral data; where, its IR spectrum showed the disappearance of bands due to amino group and appearance of the band at 2942 cm^{-1} for (CH-aliphatic) and its ^{1}HNMR spectrum showed the appearance of two singlet signals at 3.25 and 8.8 ppm corresponding to enamine moiety. The structure of compound **8** could be also confirmed by its heating it with ammonium acetate and acetic acid to afford 4-amino-6-(cyano(2-oxoindolin-3-ylidene) methyl) pyrimidine-5-carbonitrile **9 (**Scheme 3). The structure of **9** was supported by elemental analysis and spectral data**.** IR spectrum showed the appearance of the band at 3420 cm^{-1} for amino group and ^{1}HNMR spectrum showed the appearance of the singlet signal at 8.68 ppm for (CH-pyrimidine).

Similarly, treating arylidene **5** with 5,5-dimethylcyclohexane-1,3-dione (dimedone) **10** in ethanol and triethylamine afforded 3-(cyano(2-oxoindolin-3-ylidene)methyl)-5-((5,5-dimethyl-3-oxocyclohex-1-en-1-yl)amino)-1H-pyrazole-4-carbonitrile **11** (Scheme 4). The structure of compound **11** is confirmed by its elemental analysis and spectral data; where, its IR spectrum showed the appearance of bands at 2958 and 1692, 1723 cm^{-1} for C-H aliphatic and two carbonyl groups and ^{1}H NMR showed singlet signals at 1.15, 2.07, 2.4 and 5.5 ppm corresponding to dimedone protons and three singlet signals at 9.12, 10.5 and 12.4 ppm for three NH groups.



Scheme 3. Synthesis of 4-amino-6-(cyano(2-oxoindolin-3 ylidene)methyl) pyrimidine-5-carbonitrile 9.



Scheme 4. Synthesis of 3-(cyano(2-oxoindolin-3-ylidene)methyl)-5-((5,5-dimethyl-3-oxocyclohex-1-en-1-yl)amino)-1H-pyrazole-4-carbonitrile 11.

Also, arylidene **5** reacted with acetyl acetone in acetic acid to afford 2-(cyano(2-oxoindolin-3-ylidene)methyl)-5,7dimethylpyrazolo[1,5-a] pyrimidine-3-carbonitrile **12** (Scheme 5). The structure of compound **12** is confirmed by its elemental analysis and spectral data; where, IR spectrum showed disappearance of amino and one of two NH groups and appearance band at 2945 cm^{-1} which corresponding to C-H aliphatic and ^1H NMR showed appearance two singlet signals at 2.52, 267 ppm for two methyl groups.

Also, compound **12** can be prepared by refluxing of isatin **1** with 2-(cyanomethyl)-5,7-dimethylpyrazolo[1,5-a]pyrimidine-3-carbonitrile **13 (**which synthesized from reaction of 5-amino-3-(cyanomethyl)-1H-pyrazole-4-carbonitrile **4** with acetyl acetone in acetic acid) [38] in ethanol and piperidine (Scheme 5).

Finally, boiling of arylidenes **3** with hydrazine hydrate in ethanol failed to give the expected compound **14**. However the spectral data of the isolated product confirmed the formation of 3,3'-(hydrazine-1,2-diylidene)bis(indolin-2-one) **15** rather than the expected 3-amino-5-(cyano(2-oxoindolin-3-ylidene) methyl)-1H-pyrazole-4-carbonitrile **14** (Scheme 6). [39]. Whereas, the IR spectrum of **15** showed the lack of absorption bands due to amino and cyano groups and ^{1}HNMR spectrum shown signals at 7.25-7.75 ppm for aromatic protons and 10.6 ppm for NH group of isatin.

Similarly, arylidenes **5** reacted with hydrazine hydrate in ethanol afforded 3,3'-(hydrazine-1,2-diylidene)bis(indolin-2-one) **15** (Scheme 6)**.**

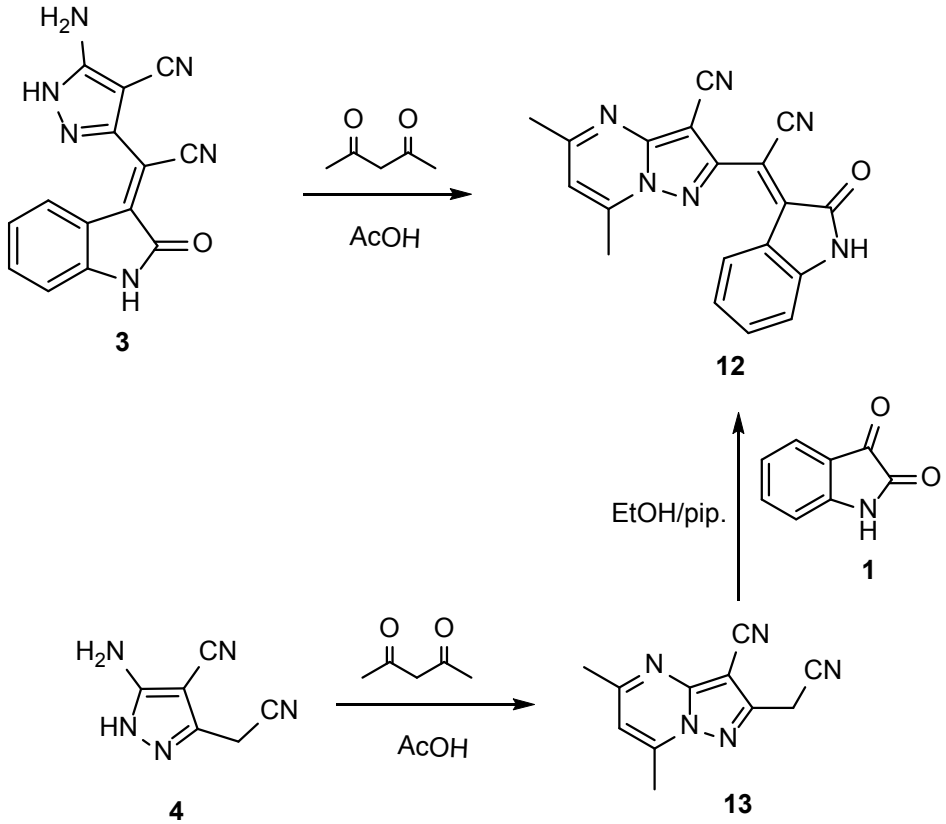
The formation of 3,3'-(hydrazine-1,2-diylidene)bis(indolin-2-one) **15** can be explained by the reaction pathway depicted in Scheme 7. The formation of **15** is assumed to proceed *via* the nucleophilic attack of nitrogen in hydrazine molecule on compound **3** or **5** followed by an intermolecular attack of the second nitrogen of hydrazine to another molecule of compound **3** or **5** to formed 3,3'-(hydrazine-1,2-diylidene) bis(indolin-2-one) **15**.

Antibacterial evaluation:

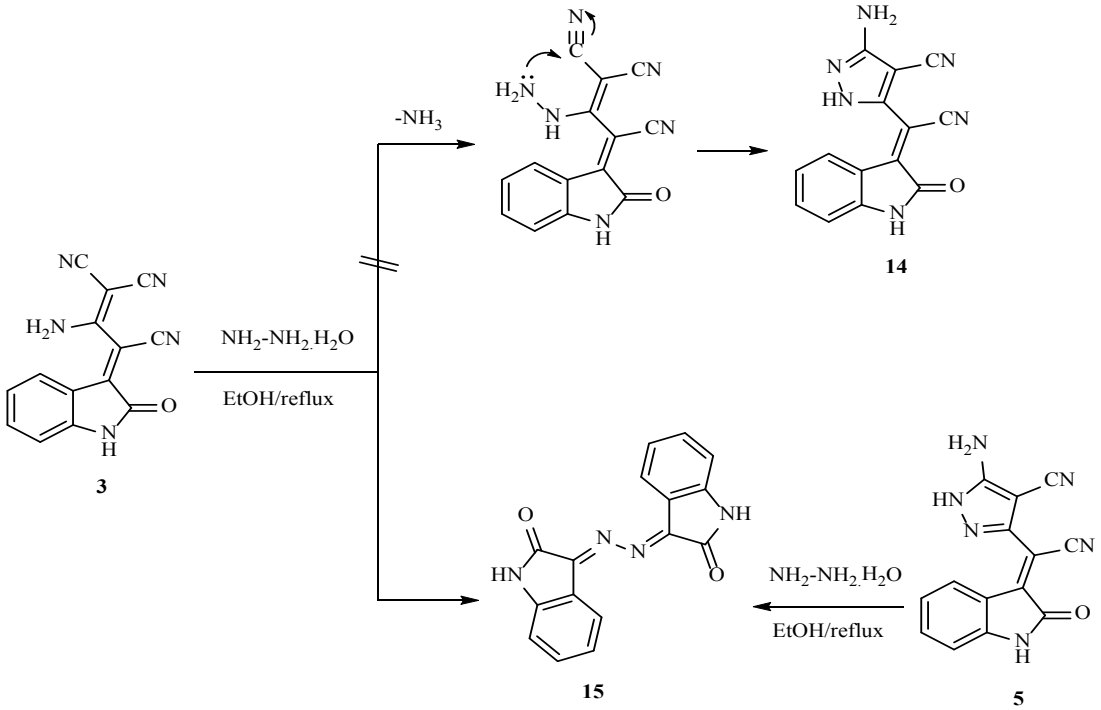
The prepared compounds were evaluated *in vitro* for their antibacterial activity against Gram-positive bacteria *(Staphylococcus aureus* and *Bacillus subtilis)* and Gram-negative bacteria *(Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeuroginosa)*.The obtained results were compared to the positive reference Chloramphenicol.

Antibacterial effects of testing chemicals:

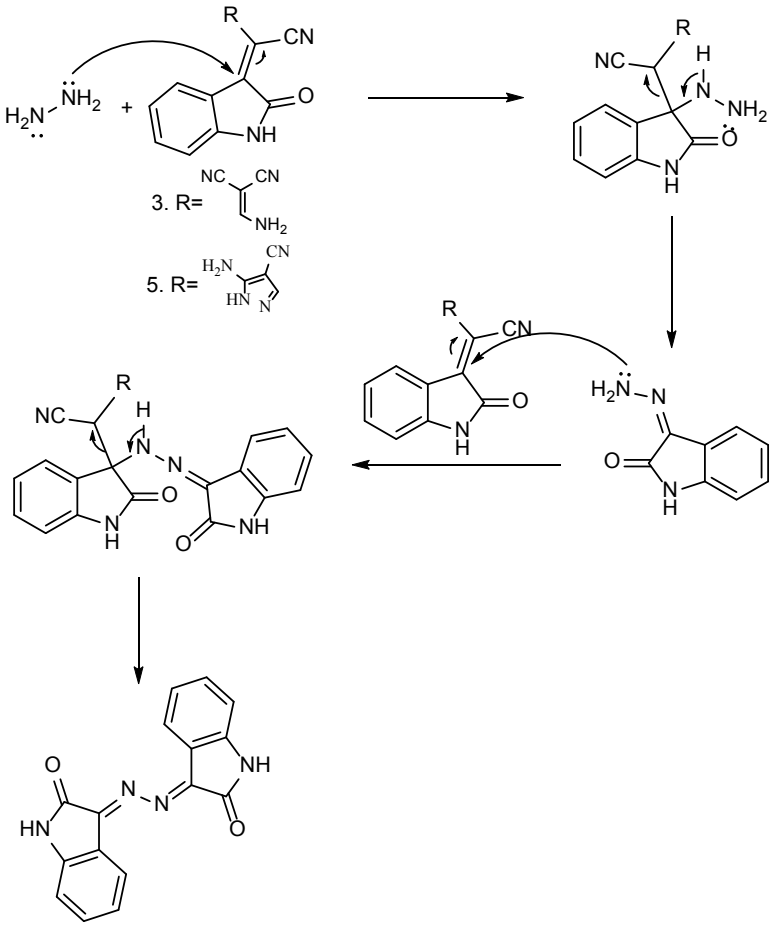
According to the results (Table 1), compounds **6a**, **6b** and **8** showed wide spectrum with higher antibacterial activity against all tested isolates, followed by the other compounds **3**, **5, 7, 11** and *15* exhibited activity against 3-4 bacterial species. Compound **8** was the highly active against all tested isolates followed by **6a** and **6b**, respectively. Compound **15** presented the narrow spectrum against **3** bacterial isolates. *Escherichia coli* were the most resistant bacteria isolate against the investigated compounds, while *Staphylococcus aureus* and *Pseudomonas aeruginosa* were more sensitive to the tested compounds.



Scheme 5. Synthesis of 2-(cyano(2-oxoindolin-3-ylidene)methyl)-5,7dimethylpyrazolo[1,5-a]pyrimidine-3-carbonitrile 12.



Scheme 6. Synthesis of 3,3'-(hydrazine-1,2-diylidene)bis(indolin-2-one) 15.



Scheme 7. The proposed mechanism for the synthesis of 3, 3'-hydrazino-bis-isatin 15

TABLE 1. The antibacterial activity of some synthesized compounds

Tested chemicals	Tested bacteria				
	Gram +ve		Gram -ve		
	B. subtilis	*S. aureus*	*E. coli*	*K. pneumonia*	*P. aeruginosa*
3	NI	17	8	7	18
5	17	NI	6	12	14
6a	16	14	9	14	16
6b	16	16	8	13	11
7	18	13	NI	10	15
8	23	15	10	18	16
11	20	15	NI	15	14
15	NI	17	7	NI	18
Chloramphenicol	21	12	15	18	13

NI = No Inhibition

Conclusion

Synthesis of arylidenes **3** and **5** from a reaction of isatin and active methylene compounds **2**, **4** and utility of them as starting materials for synthesis of Spiro-oxindole derivatives **6a**, **6b** and **7** and oxindole derivatives 8, 9, 11, 12 and 15. The antimicrobial activities of the synthesized compounds were evaluated.

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التوليف والتوصيف والتقييم المضاد للبكتيريا في المختبر لمشتقات الأوكسيدول الجديدة ومشتقات سبيرو أوكسيدول

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تخليق الأريليدين 3 و 5 من تفاعل مركبات الايزاتين مع مركبات تحتوى على مجموعة ميثيلين نشطة 2 و 4 وفائدتهما كمواد بدء لتخليق مشتقات سبيرواوكسيدول 6أ , 6ب , 7 وتخليق مشتقات اوكسيدول 8 و 9 و 11 و 12 و 15. ثم تقييم الأنشطة المضادة للميكروبات للمركبات المخلقة.