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Synthesis of new Schiff bases bearing 1,2,4-triazole, thiazolidine and chloroazetidine moieties and their pharmacological evaluation

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ABSTRACT

New compounds based on oxindole moiety were synthesized via the reaction of 5-substitued isatins **1a**–**e** with different nucleophiles such as benzidine, 3,3′-dimethoxybenzidine **2a,b** and 2,6-diaminopyridine **3** to afford three different classes of bis-Schiff bases **4a**–**e**, **5a**–**e** and **6a**–**e**, respectively. The structures of the new compounds were elucidated on the basis of their FTIR, ^{1}H NMR, ^{13}C NMR, GC/MS spectral data and elemental analysis. The *in vitro* antimicrobial activity of the new compounds was evaluated using a broth dilution technique in terms of minimal inhibitory concentration (MIC) against four bacterial and two fungal pathogens and anticancer activities against HELA cervix. The revealed data showed that compound **9d** has excellent activity against Gram + ve and Gram –ve bacteria, and compounds **11b** presented promising anticancer activity against HELA cervix.

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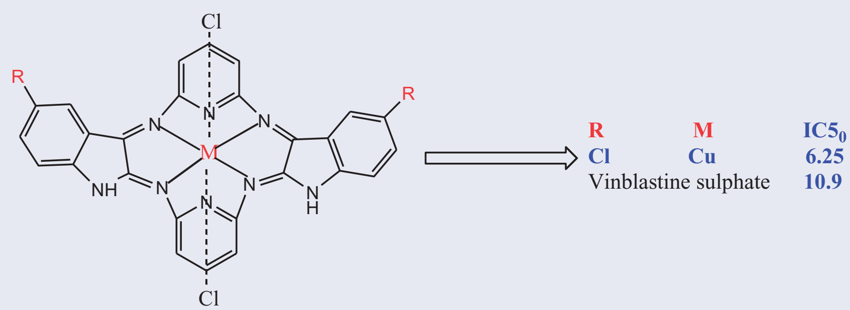

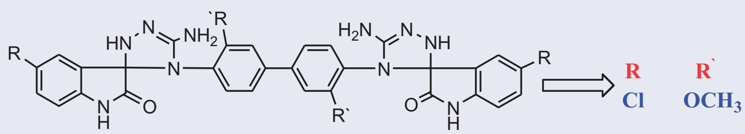

The compound shows the highest activity against Gram +Ve and -Ve bacteria

Introduction

The oxo-derivatives of indole particularly isatins and related compounds are important class of compounds due to their biological effects, including antifungal, antiviral, anticancer and antiproliferative activities[1,2]. These compounds are of great interest in oncology, microbiology and immunology[2]. Hence a significant rising research interest in the design of different oxindoles and related compounds as drugs is currently observed in the field of medicinal chemistry[3]. Schiff-bases and spiro-thiadiazoline derivatives of isatins have shown remarkable biological activities[4–6]. Also oxindoles were reported earlier due to their marked cytotoxicity[7–10].

The compounds carrying azomethine functional group –C=N– which are known as Schiff bases have gained importance in medicinal and pharmaceutical fields due to the most versatile organic synthetic intermediates and also showing a broad range of biological activities, such as antituberculosis[11,12], anticancer[13], analgesic and anti-inflammatory[14], anticonvulsant[15,16], antibacterial and antifungal activities[17,18].

Schiff bases are good intermediates for the synthesis of many heterocyclic ring systems like thiazolidinones[19] and azetidinones[20] etc.

Schiff bases are used as substrates in the preparation of a number of industrial and biologically active compounds via ring closure, cycloaddition and replacement reactions[21]. Moreover, Schiff bases derived from various heterocycles have been reported to possess cytotoxic[22], anticonvulsant[23], antiproliferative[24], antimicrobial[25] and anticancer activities[26].

Schiff bases are reported to possess antimicrobial activities. Heterocycles bearing nitrogen, sulfur and thiazole moieties constitute the core structure of a number of biologically interesting

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compounds[27]. Schiff base complexes derived from heterocyclic compounds have found increased interest in the context of bioinorganic chemistry[28–31].

The chemistry of 1,2,4-triazole and its fused heterocyclic derivatives has received considerable attention owing to their synthetic and effective biological importance. 1,2,4-triazole moieties have been incorporated into a variety of therapeutically interesting drug candidates including antiviral (ribavarin), anti-migraine (rizatriptan), antifungal (fluconazole) and antianxiety compounds (alprazolam).The pharmacological importance of heterocycles derived from 1,2,4-triazole paved the way towards active research in a triazole chemistry[32].

In view of these reports and in continuation of our research on synthesis of biologically active molecules[33–41], we hereby report the synthesis of some new Schiff bases bearing triazole, thiazolidine and chloroazetidine moieties and evaluation of their biological activities as antimicrobial and anticancer agents.

Materials and methods

Chemistry

General remarks

All melting points are uncorrected and were determined on a Gallenkamp Instrument (London, UK). IR and NMR recorded on Perkin-Elmer-1430 infrared spectrophotometer (Waltham, MA) using the potassium bromide wafer or the Nujol mull technique for metal complexes and ^{1}H NMR, ^{13}C NMR spectra were measured in DMSO-d_6 measured on a Varian Genini-300, 500 MHz spectrophotometer (Varian, Palo Alto, CA) and chemical shifts δ are in ppm. The mass spectra were measured on a HP GC MS-QPL000EX (Shimadzu, Tokyo, Japan) mass spectrophotometer at 70 ev. Microanalyses were carried out using a Perkin Elmer 2400 CHN elemental analyzer (Waltham, MA). The metal percentage was estimated using inductively coupled argon plasma (ICP) technique on a 6500 Duo apparatus, Thermo Scientific (Mahwah, NJ).

A 1000 mg/L multi-element and certified standard solution (Merck, Darmstadt, Germany) was used as the stock solution for instrument standardization. A microwave Digestion Lab Station closed system, Ethos Pro; Milestone, Italy was used to digest the organic matter in aqua regia. UV-Vis spectra were measured on UV-1600 spectrophotometer. The solid reflectance spectra were measured on a Shimadzu 3101 pc spectrophotometer. Magnetic susceptibilities of the metal complexes were measured at room temperature using a magnetic susceptibility Sherwood Scientific apparatus (Cambridge, UK). The molar
conductance values of the metal chelates were calculated using a conductivity meter ORION model 150 with a 0.6 cell constant.

In this study, Explorer Automated Microwave Synthesis Work station (CEM) was used for the synthesis of the compounds.

Four bacterial strains and two fungal strains from the Basic Science Department, Faculty of Applied Medical Science, October 6th University were employed for minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) microbial counts.

The primary evaluation of the *in vitro* cytotoxicity of the compounds under investigation against human tumor cells was tested at the National Cancer Institute (NCI), Cairo University, Egypt.

General procedure for the synthesis of bis N-*[(1,3-dihydro)-5-substituted-indol-2-one]benzidine derivatives 4a–e and bis* N-*[(1,3-dihydro)-5-substituted-indol-2-one] 3,3′-dimethoxybenzidine derivatives 5a–e*

Using thermal conditions 5-Substituted isatins **1a**–**e** (0.02 mol) and each of the benzidine/3,3′-dimethoxybenzidine **2a,b** (0.01 mol) were dissolved in warm ethanol (20 ml) containing glacial acetic acid (0.45 ml). The reaction mixture was refluxed for 3 h. The major part of product precipitated while hot. The solid formed on cooling was filtered, washed with hot ethanol and dried under vacuum to give **4a**–**e** and **5a**–**e**, respectively.

Using microwave irradiation A mixture (0.02 mol) of 5-substituted isatins **1a**–**e** and benzidine/3,3′-dimethoxybenzidine **2a,b** (0.01 mol) in the minimum quantity of ethanol (5 ml required to form a slurry) was irradiated with microwave radiation under controlled conditions [power: 150 watt, temperature: 78 °C]. On cooling, pure crystals were separated (TLC).

Bis N-*[(1,3-dihydro)-2H-indol-2-one]benzidine 4a*

Orange crystals, yield 50%; mp >350 °C; IR: 3174 for $NH_{(oxindole)}$, 3116 3001 for $CH_{(aromatic)}$, 1743 for $C=O_{(oxindole)}$ and 1612 for C=N cm^{-1}. ^{1}H NMR (DMSO d6, 300 MHz) δ: 10.97 (s, 2H, $2NH_{(oxindole)}$, $D_2O_{exchangeable}$), 7.90–6.60 (m, 16H, 4Ar–H) ppm. Anal. $C_{28}H_{18}N_4O_2$ (442.47): Calcd: N, 12.66. Found: N, 12.36. MS: *m/z* 442 (M^+).

Bis N-*[(1,3-dihydro)-5-methylindol-2-one]benzidine 4b*

Red crystals, yield 82%; mp >350 °C; IR: 3240 (broad) for $NH_{(oxindole)}$, 3111 3047 for $CH_{(aromatic)}$, 1751 for $C=O_{(oxindole)}$ and 1612 for C=N cm^{-1}. ^{1}H NMR (DMSO d6, 300 MHz) δ: 10.84 (s, 2H, $NH_{(oxindole)}$, $D_2O_{exchangeable}$), 7.69–6.42 (m,14H, 4Ar–H), 2.50 (s, 6H, $2CH_3$ of CH_3–Ar) ppm. Anal. $C_{30}H_{22}N_4O_2$ (470.52): Calcd: C, 76.58; H, 4.71; N, 11.91. Found: C, 76.28; H, 4.62; N, 12.16. MS: *m/z* 471 (M^+).

Bis N-*[(1,3-dihydro)-5-chloroindol-2-one]benzidine 4c*

Red crystals, yield 79%; mp >350 °C; IR: 3236 (broad) for $NH_{(oxindole)}$, 3109, 2989 for $CH_{(aromatic)}$, 1747 for $C=O_{(oxindole)}$ and 1608 for C=N cm^{-1}. Anal. $C_{28}H_{16}Cl_2N_4O_2$ (511.36): Calcd: C, 65.77; H, 3.15; N, 10.96; Cl, 13.87. Found: C, 65.65; H, 3.45; N, 11.10; Cl, 13.96. MS: *m/z* 511 (M^+).

Bis N-*[(1,3-dihydro)-5-nitroindol-2-one]benzidine 4d*

Violet crystals, yield 58%; mp 316 °C; IR: 3255 (broad) for $NH_{(oxindole)}$, 3039 for $CH_{(aromatic)}$, 1751 for $C=O_{(oxindole)}$ and 1620 for C=N cm^{-1}. ^{1}H NMR (DMSO d6, 300 MHz) δ: 11.67(s, 2H, $NH_{(oxindole)}$, $D_2O_{exchangeable}$), 8.37–6.66 (m, 14H, 4Ar–H). Anal. $C_{28}H_{16}N_6O_6$ (532.46): Calcd: N, 15.78. Found: N, 15.76. MS: *m/z* 532 (M^+).

Bis N-*[(1,3-dihydro)-5-fluoroindol-2-one]benzidine 4e*

Brick red crystals, yield 59%; mp >350 °C; IR: 3298 for $NH_{(oxindole)}$, 3035, 2997 for $CH_{(aromatic)}$, 1747 for $C=O_{(oxindole)}$ and 1620 for C=N cm^{-1}. Anal. $C_{28}H_{16}F_2N_4O_2$ (478.45): Calcd: C, 70.29; H, 3.37; N, 11.71. Found: C, 70.15; H, 3.60; N, 11.41. MS: *m/z* 479 (M^+), 480 (M^++1), 481 (M^++2).

Bis* N-*[(1,3-dihydro)-2H-indol-2-one] 3,3′-dimethoxybenzidine 5a
Orange crystals, yield 54.90%; mp 342–344 °C; IR: 3174 for $NH_{(oxindole)}$, 3000 for $CH_{(aromatic)}$, 2935 for $CH_{(aliphatic)}$, 1747 for $C=O_{(oxindole)}$ and 1608 for C=N cm^{-1}. ^1H NMR (DMSO d6, 300 MHz) δ: 10.95 (s, 2H, $2NH_{(oxindole)}$, $D_2O_{exchangeable}$), 7.49–6.66 (m, 14H, 4Ar–H), 3.84–3.76 (s, 6H, $2OCH_3$) ppm. Anal. $C_{30}H_{22}N_4O_4$ (502.16): Calcd: C, 71.70; H, 4.41; N, 11.51. Found: C, 72.00; H, 4.32; N, 10.86. MS: *m/z* 502 (M^+), 503 (M^++1).

Bis* N-*[(1,3-dihydro)-5-methylindol-2-one] 3,3′-dimethoxybenzidine 5b
Red crystals, yield 50%; mp 335–336 °C; IR: 3186 (broad) for $NH_{(oxindole)}$, 3000 for $CH_{(aromatic)}$, 2923 2835 for $CH_{(aliphatic)}$, 1732 for $C=O_{(oxindole)}$ and 1616 for C=N cm^{-1}. ^1H NMR (DMSO d6, 300 MHz) δ: 10.84 (s, 2H, $2NH_{(oxindole)}$, $D_2O_{exchangeable}$), 7.52–6.52 (m, 12H, 4Ar–H), 3.88–3.84 (s, 6H, $2OCH_3$), 2.50 (s, 6H, $2CH_3$ of CH_3–Ar) ppm. Anal. $C_{32}H_{26}N_4O_4$ (530.57): Calcd: C, 72.44; H, 4.94. Found: C, 71.47; H4.70. MS: *m/z* 531 (M^+).

Bis* N-*[(1,3-dihydro)-5-chloroindol-2-one] 3, 3′-dimethoxybenzidine 5c
Brick red crystals, yield 37.50%; mp 260 °C; IR: 3182 (broad) for $NH_{(oxindole)}$, 3082 3008 for $CH_{(aromatic)}$, 2966 for $CH_{(aliphatic)}$, 1728 for $C=O_{(oxindole)}$ and 1608 for C=N cm^{-1}. ^1H NMR (DMSO d6, 300 MHz) δ: 11.10 (s, 2H, $2NH_{(oxindole)}$, $D_2O_{exchangeable}$), 7.54–6.60 (m, 12H, 4Ar–H), 3.90–3.84 (s, 6H, $2OCH_3$) ppm. Anal. $C_{30}H_{20}Cl_2N_4O_4$ (571.41): Calcd: N, 9.81; Cl, 12.41. Found: N, 9.87; Cl, 12.40. MS: *m/z* 571 (M^+), 572 (M^++1), 573 (M^++2).

Bis* N-*[(1,3-dihydro)-5-nitroindol-2-one] 3,3′-dimethoxybenzidine 5d
Brick red crystals, yield 82.35%; mp 172–174 °C; IR: 3183 (broad) for $NH_{(oxindole)}$, 3115, 3014 for $CH_{(aromatic)}$, 2925, 2865 for $CH_{(aliphatic)}$, 1725 for $C=O_{(oxindole)}$ and 1618 for C=N cm^{-1}. Anal. $C_{30}H_{20}N_6O_8$ (592.52): Calcd: C, 60.81; H, 3.40; N, 14.18. Found: C, 60.80; H, 3.70; N, 13.87. MS: *m/z* 593 (M^+).

Bis* N-*[(1,3-dihydro)-5-fluoroindol-2-one] 3,3′-dimethoxybenzidine 5e
Violet crystals, yield 63.70%; mp 338–340 °C; IR: 3182, 3136 for $NH_{(oxindole)}$, 3077, 3011 for $CH_{(aromatic)}$, 2940 for $CH_{(aliphatic)}$, 1725 for $C=O_{(oxindole)}$ and 1621 for C=N cm^{-1}. Anal. $C_{30}H_{20}FN_4O_4$ (538.50): Calcd: C, 66.91; H, 3.74; N, 10.40. Found: C, 66.66; H, 4.00; N, 10.06. MS: *m/z* 538 (M^+), 539 (M^++1), 540 (M^++2).

General procedure for synthesis of bis* N-*[(1,3-dihydro)-5-substituted-indol-2-one]pyridine-2,6-diamine derivatives 6a–e
Using thermal conditions Pyridine-2,6-diamine **3** (0.004 mol, 0.436 gm) was added to a stirring solution of 5-substituted isatins **1a–e** (0.008 mol) in ethanol (20 ml). The resulting mixture was refluxed at 60 °C and stirred for 3 h. The reaction mixture was cooled to room temperature and the resulting precipitate was filtered and washed with cold methanol. The solid was purified by column chromatography (20% ethyl acetate/80% petroleum ether (60–80 °C) to give pure title product.

Using microwave irradiation A mixture of pyridine-2,6-diamine **3** (0.004 mol, 0.436 g) and 5-substituted isatins **1a–e** (0.008 mol) in the minimum quantity of ethanol (5 ml, required to form a slurry) was irradiated with microwave radiation under controlled condition. On cooling, pure crystals were separated (TLC).

Bis* N-*[(1,3-dihydro)-2H-indol-2-one]pyridine-2,6-diamine 6a
Orange crystals, yield 86.9%; mp >350 °C; IR: 3166 for $NH_{(oxindole)}$, 3022 for $CH_{(aromatic)}$, 1720 for $C=O_{(oxindole)}$ and 1620 for C=N cm^{-1}. ^1H NMR (DMSO d6, 300 MHz) δ: 11.00 (s, 2H, $NH_{(oxindole)}$, $D_2O_{exchangeable}$), 7.54–6.5 (m, 11H, 3Ar–H) ppm. Anal. $C_{21}H_{13}N_5O_2$ (367.36): Calcd: C, 68.66; H, 3.57; N, 19.06. Found: C, 68.25; H, 3.32; N, 18.86. MS: *m/z* 368 (M^++1).

Bis* N-*[(1,3-dihydro)-5-methylindol-2-one]pyridine-2,6-diamine 6b
Buff crystals, yield 48.20%; mp 238–240 °C; IR: 3197 for $NH_{(oxindole)}$, 3039 for $CH_{(aromatic)}$, 1712 for $C=O_{(oxindole)}$ and 1627 for C=N cm^{-1}. ^1H NMR (DMSO d6, 300 MHz) δ: 10.07 (s, 2H, $NH_{(oxindole)}$, $D_2O_{exchangeable}$), 6.92–5.84 (m, 9H, 3Ar–H), 2.50 (s, 6H, $2CH_3$ of CH_3–Ar) ppm. Anal. $C_{23}H_{17}N_5O_2$ (395.41): Calcd: N, 17.71. Found: N, 17.46. MS: *m/z* 397 (M^++2).

Bis* N-*[(1,3-dihydro)-5-chloroindol-2-one]pyridine-2,6-diamine 6c
Orange crystals, yield 46.23%; mp 260 °C; IR: 3190 for $NH_{(oxindole)}$, 3050 for $CH_{(aromatic)}$, 1724 for $C=O_{(oxindole)}$ and 1616 for C=N cm^{-1}. ^1H NMR (DMSO d6, 300 MHz) δ: 11.09 (s, 2H, $NH_{(oxindole)}$, $D_2O_{exchangeable}$), 7.58–6.87 (m, 9H, 3Ar–H) ppm. Anal. $C_{21}H_{11}Cl_2N_5O_2$ (436.25): Calcd: N, 16.05; Cl, 16.25. Found: N, 16.38; Cl, 16.55. MS: *m/z* 437 (M^++1), 440 (M^++4).

Bis* N-*[(1,3-dihydro)-5-nitroindol-2-one]pyridine-2,6-diamine 6d
Green crystals, yield 95.23%; mp 212 °C; IR: 3200 for $NH_{(oxindole)}$, 3097 for $CH_{(aromatic)}$, 1735 for $C=O_{(oxindole)}$ and 1620 for C=N cm^{-1}. Anal. $C_{21}H_{11}N_7O_6$ (457.36): Calcd: C, 55.15; H, 2.42; N, 21.44. Found: C, 54.89; H, 2.70. N, 21.87. MS: *m/z* 459 (M^++2).

Bis* N-*[(1,3-dihydro)-5-fluoroindol-2-one]pyridine-2,6-diamine 6e
Orange crystals, yield 56.70%; mp >350 °C; IR: 3201 for $NH_{(oxindole)}$, 3085 for $CH_{(aromatic)}$, 1720 for $C=O_{(oxindole)}$ and 1627 for C=N cm^{-1}. Anal. $C_{21}H_{11}FN_5O_2$ (403.34): Calcd: C, 62.53; H, 2.75; N, 17.36. Found: C, 62.66; H, 3.00; N, 17.06. MS: *m/z* 404 (M^++1).

General procedures for synthesis of bis spiro[(5-methylindoline-3,2-(4H)thiazolidine)-2,4′(1H)-dione]1,1′-biphenyl 7a and bis spiro [(indoline-3,2-(4H)thiazolidine)-2,4′(1H)-dione]3,3′-dimethoxybenzidine 7b
[Method A] A solution of Schiff bases **4b, 5a** (0.001 mol) and mercaptoacetic acid (0.002 mol) in dry dioxane (50 ml) in presence of anhydrous $ZnCl_2$ was refluxed for 6–10 h. After completion of the reaction excess of solvent was removed through distillation, and sticky solid obtained was poured onto crushed ice, then filtered, dried and recrystallized from ethanol to give **7a,b**.

[Method B] A mixture of Schiff bases **4b, 5a** (0.001 mol) and mercaptoacetic acid (0.002 mol) was taken in DMF in a round-bottom flask fitted with a Dean Stark apparatus. The mixture was refluxed for 6 h with removal of water azeotropically. A sticky solid was formed on evaporating of solvent and was treated with a solution of sodium bicarbonate to remove excess of acid. The solid formed was filtered, washed with water, dried and recrystallized from ethanol to give **7a,b**.

Bis spiro[(5-methylindoline-3,2-(4H)thiazolidine)-2,4′(1H)-dione]1,1′-biphenyl 7a
Orange crystals, yield 53%; mp 187–179 °C; IR: 3291(broad) for $NH_{(oxindole)}$, 3071, 3030 for $CH_{(aromatic)}$, 2915, 2828 for $CH_{(aliphatic)}$, 1700 for $C=O_{(oxindole)}$, 1679 for $C=O_{(thiazolidine)}$, 1246 for C–N and 638 for C–S–C cm^{-1}. ^1H NMR (DMSO d6,300 MHz) δ: 10.44 (s, 2H,

$NH_{(oxindole)}$), 8.27–6.47 (m,12H, 4Ar-H), 5.04 (s, 4H, $2CH_2$ of $_{thiazolidinone}$), 2.46 (s, 6H, $2CH_3$ of CH_3–Ar) ppm, ^{13}C NMR (DMSO, 500 MHz) δ: 167.53, 167.26, 138.52, 137.15, 135.21, 135.11, 127.79, 127.02, 126.57, 126.26, 125.87 125.73, 120.86, 120.31, 119.33, 119.11, 118.87, 118.69, 118.44, 117.84, 113.63, 48.05, 46.78, 28.96, 28.16. Anal. $C_{34}H_{26}N_4O_4S_2$ (618.12): Calcd: C, 66.00; H, 4.24; N, 9.06; S, 10.36. Found: C, 66.29; H, 4.25; N, 9.31; S, 10.45. MS: *m/z* 618 (M^+).

Bis spiro[(indoline-3,2-(4H)thiazolidine)-2,4′(1H)-dione]3,3′-dimethoxybenzidine 7b

Yellow crystals, yield 58%; mp 218–220 °C; IR: 3247 for $NH_{(oxindole)}$, 3069, 3000 for $CH_{(aromatic)}$, 2927, 2873 for $CH_{(aliphatic)}$, 1681 for $C=O_{(oxindole)}$, 1658 for $C=O_{(thiazolidine)}$, 1257 for C–N and 675 for C–S–C cm^{-1}. ^1H NMR (DMSO d6, 300 MHz) δ: 9.65 (s, 2H, $NH_{(oxindole)}$, $D_2O_{exchangeable}$), 8.29–7.19 (m, 12H, 4Ar–H), 4.28–3.96 (s, 4H, $2CH_2$ of $_{thiazolidinone}$), 3.85–3.82 (s, 6H, $2OCH_3$) ppm. Anal. $C_{34}H_{26}N_4O_6S_2$ (650.13): Calcd: S, 9.86. Found: S, 9.60. MS: *m/z* 650 651(M^++1) and 652 (M^++2).

General procedures for synthesis of bis spiro[(5-methylindoline-3,2-(2H)-3-chloro-azetidine)-2,4′(1H)-dione]1,1′-biphenyl 8a and bis spiro[(indoline-3,2-(2H)-3-chloro-azetidine)-2,4′(1H)-dione]-3,3′-dimethoxybenzidine 8b

To a solution of Schiff bases **4b, 5a** (0.001 mol) in DMF (30 ml), chloroacetyl chloride (0.002 mol) and triethylamine (0.002 mol) were added at 0–5 °C with constant stirring. The reaction mixture was refluxed on water bath for 7 h, and then excess of solvent was distilled off. The sticky solid was cooled, poured on ice water then filtered, further recrystallized from ethanol to give the title products.

Bis spiro [(5-methylindoline-3,2-(2H)-3-chloroazetidine)-2,4′(1H)-dione] 1,1′-biphenyl 8a

Brown crystals, yield 52%; mp 148–150 °C; IR: 3267(broad) for $NH_{(oxindole)}$, 3103, 3071 for $CH_{(aromatic)}$, 2885 for $CH_{(aliphatic)}$, 1777 for $C=O_{(azetidine)}$, 1739 for $C=O_{(oxindole)}$, 1204 for C–N and 756 for C–Cl cm^{-1}. ^1H NMR (DMSO d6, 300 MHz) δ: 11.19 (s, 2H, $2NH_{(oxindole)}$), 7.91–7.04 (m,16H, 4Ar-H), 5.83 (s, 2H, 2CH-Cl) ppm, ^{13}C NMR (DMSO, 500 MHz) δ: 171.71, 169.16, 165.318, 163.36 163.06, 162.26, 160.86, 142.36, 135.55, 128.02, 127.90, 127.27, 126.64, 126.47, 122.52, 121.07, 120.54, 119.64, 116.95, 112.30, 111.27, 66.94, 63.83, 62.35. Anal. $C_{32}H_{20}N_4O_4Cl_2$ (594.09): Calcd: C, 64.55; H, 3.39; N, 9.41; Cl, 11.91. Found: C, 64.76; H, 5.22; N, 9.31; Cl, 12.01. MS: *m/z* 595 (M^++1).

Bis spiro [(indoline-3,2-(2H)-3-chloroazetidine)-2,4′(1H)-dione]-3,3′-dimethoxybenzidine 8b

Olive crystals, yield 67%; mp 196–198 °C; IR: 3217(broad) for $NH_{(oxindole)}$, 3100, 3078 for $CH_{(aromatic)}$, 2939, 2839 for $CH_{(aliphatic)}$, 1778 for $C=O_{(azetidine)}$, 1739 for $C=O_{(oxindole)}$, 1207 for C–N and 756 for C–Cl cm^{-1}. ^1H NMR (DMSO d6, 300 MHz) δ: 10.90,10.81 (s, 2H, $2NH_{(oxindole)}$, $D_2O_{exchangeable}$), 8.18–6.75 (m,16H, 4Ar–H), 5.71–5.59 (s, 2H, 2CH–Cl), 3.94–3.89 (s, 6H, $2OCH_3$) ppm, ^{13}C NMR (DMSO, 500 MHz) δ: 173.35, 172.22, 166.99, 162.03, 161.57, 149.76, 148.92, 143.34, 142.76, 138.45, 138.25, 131.58, 130.53, 125.46, 123.91, 122.87, 122.53, 121.77, 120.45, 119.33, 118.79, 69.696, 64.14, 62.67, 55.85, 54.712. Anal. $C_{34}H_{24}N_4O_6Cl_2$ (654.11): Calcd: N, 8.55; Cl, 10.82. Found: N, 8.26; Cl, 10.55. MS: *m/z* 655 (M^++1).

General procedures for synthesis of bis spiro[(3H)indole-3,3′-(3H)1,2,4-triazole-2–(1H)-one] 1,1′-biphenyl 9a and bis spiro[(5-substituted)indole-3,3′-(3H)1,2,4-triazole-2-(1H)-one]-3,3′-dimethoxybenzidines 9b–e

A mixture of Schiff bases **5a**–**d** (0.01 mol) and thiosemicarbazide (0.02 mol) in a little amount of ethanol made as slurry was irradiated under MW. The solid product obtained after cooling was filtered and recrystallized from ethanol to give spiro compounds **9a**–**e**.

Bis spiro[(3H)indole-3,3′-(3H)-1,2,4-triazol-2-(1H)-one] 1,1′-biphenyl 9a

Yellow crystals, yield 57%; mp 278–280 °C; IR: 3399 for NH_2, 3328 for $NH_{(triazole)}$, 3195 for $NH_{(oxindole)}$, 3030 for $CH_{(aromatic)}$, 1694 for $C=O_{(oxindole)}$, 1616 for C=N and 1263 for C–N cm^{-1}. ^1H NMR (DMSO d6, 300 MHz) δ: 12.44 (s,2H,$NH_{(oxindole)}$ $D_2O_{exchangeable}$), 11.21 (s, 2H, $NH_{(triazole)}$, $D_2O_{exchangeable}$), 9.00, 8.62 (s, 4H, $2NH_2$, $D_2O_{exchangeable}$), 7.61–6.86 (m, 14H, 4Ar–H) ppm, ^{13}C NMR (DMSO, 500 MHz) δ: 179.19 178.63, 163.15, 158.65, 142.88 142.83, 132.58, 132.17, 131.40, 123.47, 122.31, 121.48, 120.47, 112.06, 111.02, 100.01, 72.60. Anal. $C_{30}H_{24}N_{10}O_2$ (556.21): Calcd: N, 25.17. Found: N, 25.82. MS: *m/z* 556 (M^+).

Bis spiro [(3H) indole-3, 3′-(3H)-1,2,4-triazol-2–(1H)-one]-3,3′-dimethoxybenzidine 9b

Yellow crystals, yield 58.27%; mp 260–2 °C; IR: 3340 for NH_2, 3260 for $NH_{(triazole)}$, 3166 for $NH_{(oxindole)}$, 3062 3012 for $CH_{(aromatic)}$, 2885 for $CH_{(aliphatic)}$, 1674 for $C=O_{(oxindole)}$, 1624 for C=N and 1276 for C–N cm^{-1}. ^1H NMR (DMSO d6, 300 MHz) δ: 12.46 (s, 2H, $NH_{(oxindole)}$ $D_2O_{exchangeable}$), 11.15 (s, 2H, $NH_{(triazole)}$, $D_2O_{exchangeable}$), 9.00,8.63 (s, 4H, $2NH_2$, $D_2O_{exchangeable}$), 7.61–6.87 (m, 14H, 4Ar–H), 3.79 (s, 6H, $2OCH_3$) ppm, ^{13}C NMR (DMSO, 500 MHz) δ: 179.19, 163.15 142.89 142.84, 141.79, 132.58 132.17, 131.40, 126.98, 123.46, 122.33, 121.49, 120.49, 112.08, 111.04, 83.15 (*spiro* C), 32.26 . Anal. $C_{32}H_{28}N_{10}O_4$ (616.63): Calcd: C, 62.33; H, 4.58; N, 22.71. Found: C, 62.00; H, 4.68; N, 23.12. MS: *m/z* 616 (M^+).

Bis spiro [5-methylindole-3,3′-(3H)-1,2,4-triazol-2-(1H)-one]-3,3′-dimethoxybenzidine 9c

Violet crystals, yield 66.60%; mp 286–8 °C; IR: 3417 for NH_2, 3255 for $NH_{(triazole)}$, 3166 for $NH_{(oxindole)}$, 3066 for $CH_{(aromatic)}$, 2931 2888 for $CH_{(aliphatic)}$, 1689 for $C=O_{(oxindole)}$, 1612 for C=N and 1261 for C–N cm^{-1}. Anal. $C_{34}H_{32}N_{10}O_4$ (644.26): Calcd: N, 21.73. Found: N, 21.90. MS: *m/z* 644 (M^+), 645 (M^++1), 647 (M^++3).

Bis spiro [5-chloroindole-3,3′3H)-1,2,4-triazol-2–(1H)-one]-3,3′-dimethoxybenzidine 9d

Yellow crystals, yield 77.30%; mp 305–8 °C; IR: 3413 for NH_2, 3267 for $NH_{(triazole)}$, 3166 for $NH_{(oxindole)}$, 3062 for $CH_{(aromatic)}$, 2875 for $CH_{(aliphatic)}$, 1681 for $C=O_{(oxindole)}$, 1612 for C=N and 1253 for C–N cm^{-1}. ^1H NMR (DMSO d6, 300 MHz) δ: 12.29 (s, 2H, $2NH_{(oxindole)}$, $D_2O_{exchangeable}$), 11.26 (s, 2H, 2NH $_{(triazole)}$, $D_2O_{exchangeable}$), 9.08, 8.78 (s, 4H, $2NH_2$, $D_2O_{exchangeable}$), 7.75–6.92 (m, 14H, 4Ar–H), 3.31 (s, 6H, $2OCH_3$) ppm. Anal. $C_{32}H_{26}Cl_2N_{10}O_4$ (684.15): Calcd: N, 20.43; Cl, 10.34. Found: N, 20.23; Cl, 10.48. MS: *m/z* 685 (M^++1).

Bis spiro [5-nitroindole-3,3′-(3H)-1, 2, 4-triazol-2-(1H)-one]-3,3′-dimethoxybenzidine 9e

Green crystals, yield 63.75%; mp 320–22 °C; IR: 3371 for NH_2, 3247 for $NH_{(triazole)}$, 3193 for $NH_{(oxindole)}$, 3066, 3008 for $CH_{(aromatic)}$, 2812 for $CH_{(aliphatic)}$, 1697 for $C=O_{(oxindole)}$, 1616 for C=N and 1257 for C–N cm^{-1}. Anal. $C_{32}H_{26}N_{12}O_8$ (706.62): Calcd: C, 54.39; H, 3.71; N, 23.79. Found: C, 54.66; H, 4.00; N, 23.99. MS: *m/z* 706(M^+).

General procedures for synthesis of bis N-[(1-morpholinomethyl) indolin-2-one]pyridine 2,6-diamines 10a,c and bis N-[(1-piperidinomethyl)indolin-2-one]pyridine-2,6-diamines 10b,d

A slurry consisting of the Schiff bases **6a,b** (0.001 mol), absolute ethanol (2 ml) and 37% formalin (0.3 ml). To this slurry secondary amine (0.002 mol) was added drop wise, with cooling and shaking. The reaction mixture was irradiated for an appropriate time until the completion of the reaction, as the reactants disappeared (TLC). On cooling, crystals separated out were recrystallized from ethanol to give **10a**–**d**.

Bis N-[(1-morpholinomethyl) indolin-2-one] pyridine 2,6-diamine 10a

Orange crystals, yield 55%; mp 216–8 °C; IR: 3039 for CH $_{(aromatic)}$, 2947, 2893, 2854, 2831 for $CH_{(aliphatic)}$, 1735 for $C=O_{(oxindole)}$ and 1612 for C=N cm^{-1}. ^1H NMR (DMSO d6, 300 MHz) δ: 7.69–7.12 (m, 11H, 3Ar–H),4.39 (s, 4H, $2NCH_2N$), 3.56–3.54 (t, 8H, $2CH_2–O–CH_2$), 2.57–2.50 (t, 8H, $2CH_2–N–CH_2$) ppm, ^{13}C NMR (DMSO, 500 MHz) δ: 183.66, 159.48, 151.91, 139.25, 138.58, 138.39, 125.55, 124.65, 124.37, 123.94, 123.19, 118.084, 112.04, 111.93, 67.63, 67.39, 66.54, 66.31, 62.32, 51.29, 50.96, 50.36, 50.00. Anal. $C_{31}H_{31}N_7O_4$ (565.62): Calcd: C, 65.83; H, 5.52; N, 17.33. Found: C, 66.03; H, 5.59; N, 17.54. MS: *m/z* 565 (M^+).

Bis N-[(1-piperidinomethyl) indolin-2-one] pyridine-2,6-diamine 10b

Orange crystals, yield 53%; mp 300 °C; IR: 3055 for $CH_{(aromatic)}$, 2935, 2854, 2804 for $CH_{(aliphatic)}$, 1716 for $C=O_{(oxindole)}$ and 1612 for C=N cm^{-1}. ^1H NMR (DMSO d_6, 300 MHz) δ: 7.13–6.68 (m, 11H, 3Ar–H),4.37 (s, 4H, $2NCH_2N$), 2.249 (t, 8H, $2CH_2–N–CH_2$) and 1.369 (m, 12H, $6CH_2$) ppm. Anal. $C_{33}H_{35}N_7O_2$ (561.68): Calcd: C, 70.57; H, 6.28; N, 17.46. Found: C, 70.23; H, 5.98; N, 17.87. MS: *m/z* 562 (M^+).

Bis N-[(1-morpholinomethyl)-5-methylindolin-2-one] pyridine-2,6-diamine 10c

Orange crystals, yield 53.5%; mp 118–120 °C; 3088 for $CH_{(aromatic)}$, 2923, 2839, 2804 for $CH_{(aliphatic)}$, 1712 for $C=O_{(oxindole)}$ and 1589 for C=N cm^{-1}. ^1H NMR (DMSO d6, 300 MHz) δ: 7.44–7.13 (m, 9H, 3Ar–H),4.33 (s, 4H, $2NCH_2N$), 3.51–3.49 (t, 8H, $2CH_2–O–CH_2$), 2.51–2.49 (t, 8H, $2CH_2–N–CH_2$), 2.25 (s, 6H, $2CH_3$ of CH_3–Ar) ppm. Anal. $C_{33}H_{35}N_7O_4$ (593.68): Calcd: N, 16.52. Found: N, 16.89. MS: *m/z* 594(M^+).

Bis N-[(1-piperidinomethyl)-5-methylindolin-2-one] pyridine-2,6-diamine 10d

Orange crystals, yield 51%; mp 150–151 °C; 3028 for $CH_{(aromatic)}$, 2931, 2854, 2804 for $CH_{(aliphatic)}$, 1720 for $C=O_{(oxindole)}$ and 1600 for C=N cm^{-1}. Anal. $C_{35}H_{39}N_7O_2$ (589.73): Calcd: N, 16.63. Found: N, 16.23. MS: *m/z* 590 (M^++1), 592 (M^++2), 593 (M^++3).

General procedures for the synthesis of mononuclear Cu(II), Co(II) and Ni(II) macrocyclic complexes of bis-N-[(1,3-dihydro)-5-substitutedindol-2-one] pyridine-2,6-diamine 11a–f

To a stirring methanolic solution of 2,6-diamine pyridine **3** (0.01 mol), metal chloride (0.005 mol) dissolved in a minimum quantity of methanol was added. The resulting solution was refluxed for 30 min. Then 5-substituted isatins **1b,c** (0.01 mol) dissolved in methanol (20 ml) was added to the refluxing mixture and complete reflux for 10 h. The reaction mixture was concentrated, the crystals obtained were filtered, washed with methanol, ether and dried in vacuum.

Synthesis of mononuclear Cu (II) macrocyclic complex of bis N-[(1,3-dihydro)-5-methyl-indol-2-one] pyridine-2,6-diamine 11a

Brown crystals, yield 57.80%; mp >300 °C; IR: 3201 for NH, 1612 for C=N and 505 for M–N cm^{-1}. UV λ_{max}: 767, 737, 657, 516, 480, 393, 242 nm. μ eff: 1.8 B.M. Anal. $C_{28}H_{20}CuCl_2N_8$ (601.05): Calcd: N, 18.58; Cu, 10.54; Cl, 11.76. Found: N, 18.90; Cu, 10.41; Cl, 11.72.

Synthesis of mononuclear Cu (II) macrocyclic complex of bis N-[(1,3-dihydro)-5-chloro-indol-2-one] pyridine-2, 6-diamine 11b

Violet crystals yield 56.89%; mp >300 °C; IR: 3213 for NH, 1612 for C=N and 525 for M–N cm^{-1}. UV λ_{max}: 752, 736, 706, 675, 658, 515, 506, 472, 393, 370, 333, 314, 234 nm. μ eff: 2.1 B.M. Anal. $C_{26}H_{14}CuCl_4N_8$ (640.94): Calcd: N, 17.41; Cu, 9.87; Cl, 22.03. Found: N, 16.70; Cu, 9.45; Cl, 21.98.

Synthesis of mononuclear Co (II) macrocyclic complex of bis N-[(1,3-dihydro)-5-methyl-indol-2-one] pyridine-2, 6-diamine 11c

Brown crystals, yield 67.90%; mp 320–22 °C; 3186 for NH, 1620 for C=N and 516 for M–N cm^{-1}. UV λ_{max}: 769, 734, 701, 656, 616, 510, 472, 433, 400, 369, 313, 254 nm. μ eff: 3.9 B.M. Anal. $C_{28}H_{20}CoCl_2N_8$ (597.05): Calcd: Co, 9.85; Cl, 11.85. Found: Co, 9.73; Cl, 11.84.

Synthesis of mononuclear Co (II) macrocyclic complex of bis N-[(1,3-dihydro)-5-chloro-indol-2-one] pyridine-2, 6-diamine 11d

Olive crystals, yield 60.10%; mp >300 °C; 3209 for NH, 1616 for C=N and 555 for M–N cm^{-1}. UV λ_{max}: 769, 735, 697, 664, 514, 473, 438, 396, 370, 314, 243 nm. μ eff: 3.5 B.M. Anal. $C_{26}H_{14}CoCl_4N_8$ (636.94): Calcd: N, 17.53; Co, 9.22; Cl, 22.19. Found: N, 17.27; Co, 9.00; Cl, 21.99.

Synthesis of mononuclear Ni (II) macrocyclic complex of bis N-[(1,3-dihydro)-5-methyl-indol-2-one] pyridine-2,6-diamine 11e

Olive crystals, yield 45.70%; mp >300 °C; 3136 for NH, 1620 for C=N and 505 for M–N cm^{-1}. UV λ_{max}: 768, 737, 698, 659, 519, 498, 439, 411, 381, 320, 219 nm. μ eff: 2.6 B.M. Anal. $C_{28}H_{20}NiCl_2N_8$ (596.05): Calcd: Ni, 9.81; Cl, 11.85. Found: Ni, 9.67; Cl, 11.97.

Synthesis of mononuclear Ni (II) macrocyclic complex of bis N-[(1,3-dihydro)-5-chloro-indol-2-one] pyridine-2, 6-diamine 11f

Brown crystals, yield 55.30%; mp 278–280 °C; 3213 for NH, 1616 for C=N and 520 for M–N cm^{-1}. UV λ_{max}: 767, 738, 696, 674, 616, 520, 472, 432, 384, 326, 266, 212 nm. μ eff: 2.74 B.M. Anal. $C_{26}H_{14}NiCl_4N_8$ (635.94): Calcd: N, 17.54; Ni, 9.19; Cl, 22.19. Found: N, 17.21; Ni, 10.00; Cl, 22.21.

Pharmacological evaluation

In vitro *antimicrobial measurement*

The compounds were tested for their *in vitro* antimicrobial activity by the broth-dilution technique in terms of minimum inhibitory concentrations (MIC). Experimentally for potent Drugs[42]. The antimicrobial activities of the compounds in this study were evaluated against six pathogenic microbial species: Gram + ve bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis*, Gram –ve bacteria *Escherichia coli* and *Klebsiella pneumonia* and fungi *Aspergillus fumigatu*, and *Candida albicans*. Reference drugs used were sulfamethoxazole as an antibacterial standard and fluconazole as an antifungal standard.

In vitro *cytotoxicity evaluation*

The primary evaluation of *in vitro* cytotoxicity of the selected new compounds against human tumor cells was carried out at the NCI (Cairo University, Cairo, Egypt) using the method of Skehan and Storeng[43]. The cytotoxicity evaluation also involved the use of vinblastine sulfate or doxorubicin[44] as antitumor drug reference standards. The procedure used was as follows:

1. Cells were plated in 96-multiwell plate (105 cells/well) for 24 h. before treatment with the compound to allow attachment of the cell to the wall of the plate.
2. Different concentrations of the compound under test (0, 1, 2.5, 5 and 10 mg/mL) were added to the cell monolayer triplicate walls that were prepared for each individual dose.
3. Monolayer cells were incubated with the compound for 48 h. at 37 °C and in atmosphere of 5% CO_2.
4. After 48 h, the cells were fixed, washed and stained with sulfo-Rhodamine-B.
5. Excess stain was washed away with acetic acid and attached stain was recovered with Tris–EDTA buffer.
6. Color intensity was measured with an ELISA reader.
7. The relationship between the surviving fraction and drug concentration is plotted to give the survival curve of cancer breast cell line.

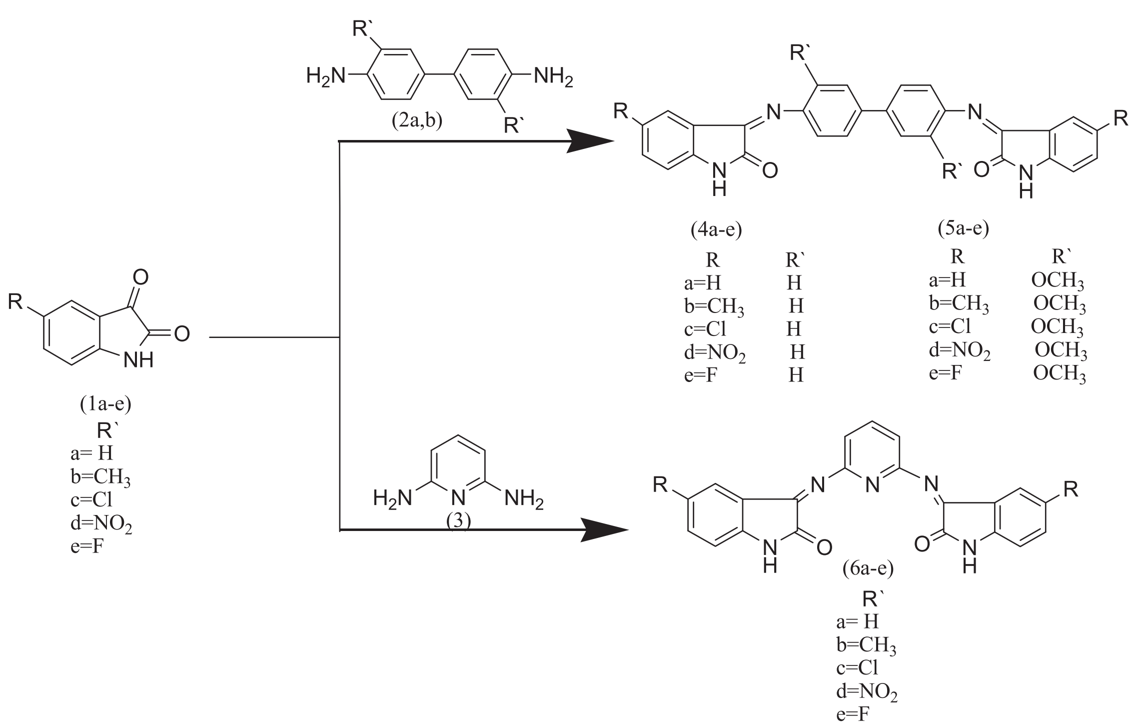
The results of the *in vitro* cytotoxicity activity on human tumor cell line HELA (cervix) were determined according to the dose values of the drug exposure required to reduce survival in the cell lines to 50%.

Results and discussion

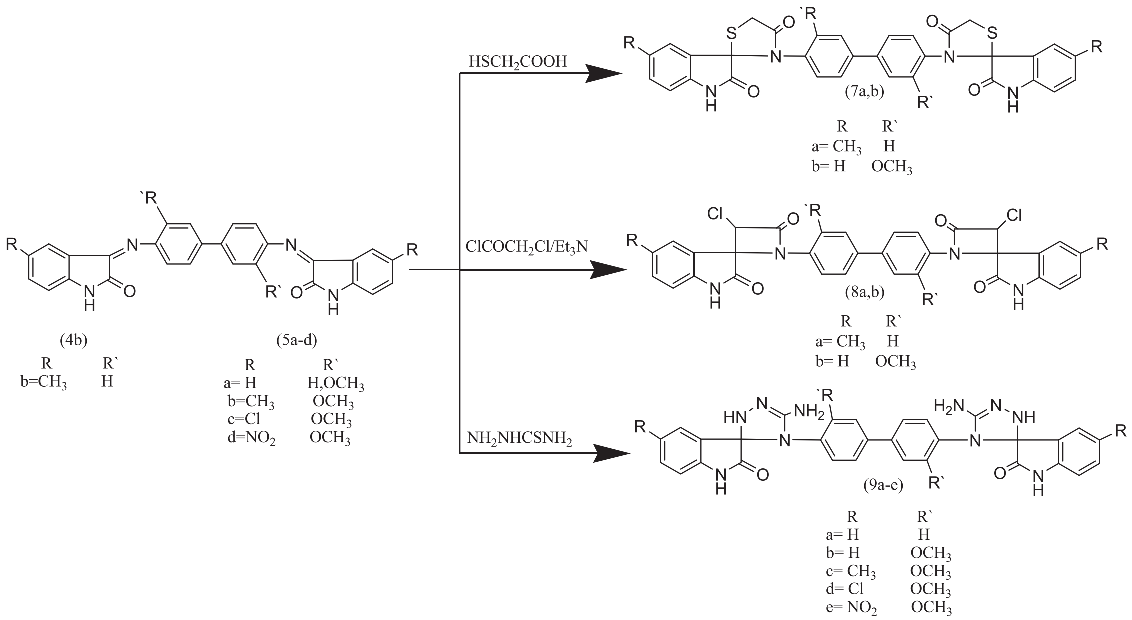
Chemistry

This study involve the synthesis of three different classes of bis-Schiff bases derived from three different diamines namely benzidine (4,4′-diamino-1,1′-biphenyl), 3,3′-dimethoxy-benzidine and 2,6-diaminopyridine. Thus condensation of (2 mol) of 5-substituted isatins **1a**–**e** with (1 mol) of diamines such as benzidine, 3,3′-dimethoxybenzidine **2a,b** and 2,6-diamino-pyridine **3** in ethanol at ambient temperature gave the desired bis-Schiff bases named by bis *N*-[(1,3-dihydro)-2H-indol-2-one] 4,4′-diamino-1,1′-biphenyl derivatives **4a**–**e**, bis *N*-[(1,3-dihydro)-2H-indol-2-one] 3,3′-dimethoxybenzidine derivatives **5a**–**e** and bis *N*-[(1,3-dihydro)-2H-indol-2-one]pyridine 2,6-diamine derivatives **6a**–**e**, respectively, as illustrated in Scheme 1. The structure of these compounds was confirmed by elemental analysis, FTIR, ^{1}H NMR and MS. ^{1}H NMR spectrum of compound **4a** showed bands at δ: 10.97 (s, 2H, $2NH_{(oxindole)}$, $D_2O_{exchangeable}$), 7.90–6.60 (m, 16H, 4Ar–H) ppm.

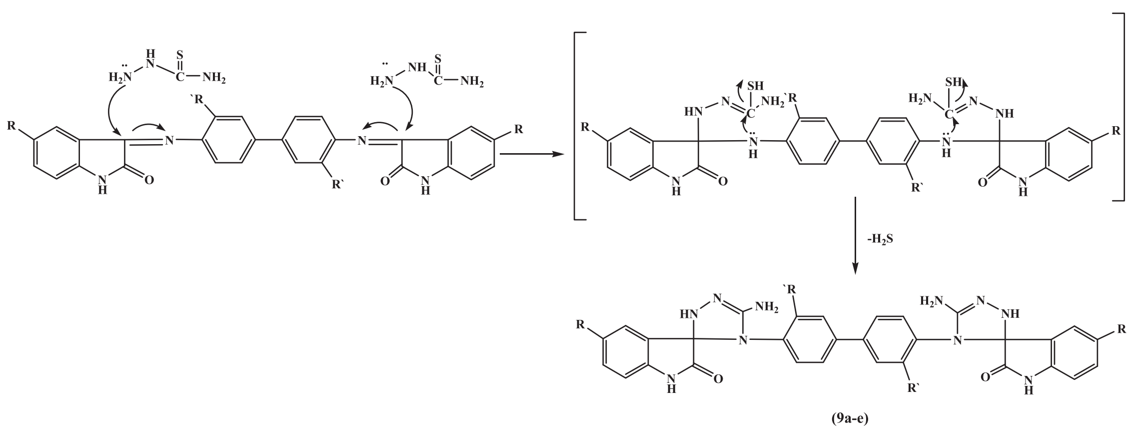
This study was extended to prepare new heterocycles such as bis spirothiazolidin-4-one derivatives **7a,b,** bis spiroazetidinone derivatives **8a,b** and bis spiro1,2,4-triazole derivatives **9a**–**e**, respectively by the reaction of bis-Schiff bases **4b, 5a**–**d** with mercaptoacetic acid in the presence of anhydrous $ZnCl_2$, chloroacetyl chloride in presence of triethylamine at temperature 80 °C and thiosemicarbazide as illustrated in Scheme 2. The structure of these compounds was confirmed by FTIR, ^{1}H NMR ^{13}C NMR, and MS spectra and elemental analysis. The ^{1}H NMR spectrum of bis



Scheme 1. Synthesis of the target compounds **4a**–**e**, **5a**–**e** and **6a**–**e**.



Scheme 2. Synthesis of the target compounds **7a,b**, **8a,b** and **9a–e**.



Scheme 3. Mechanism for the synthesis of target compounds **9a–e**.

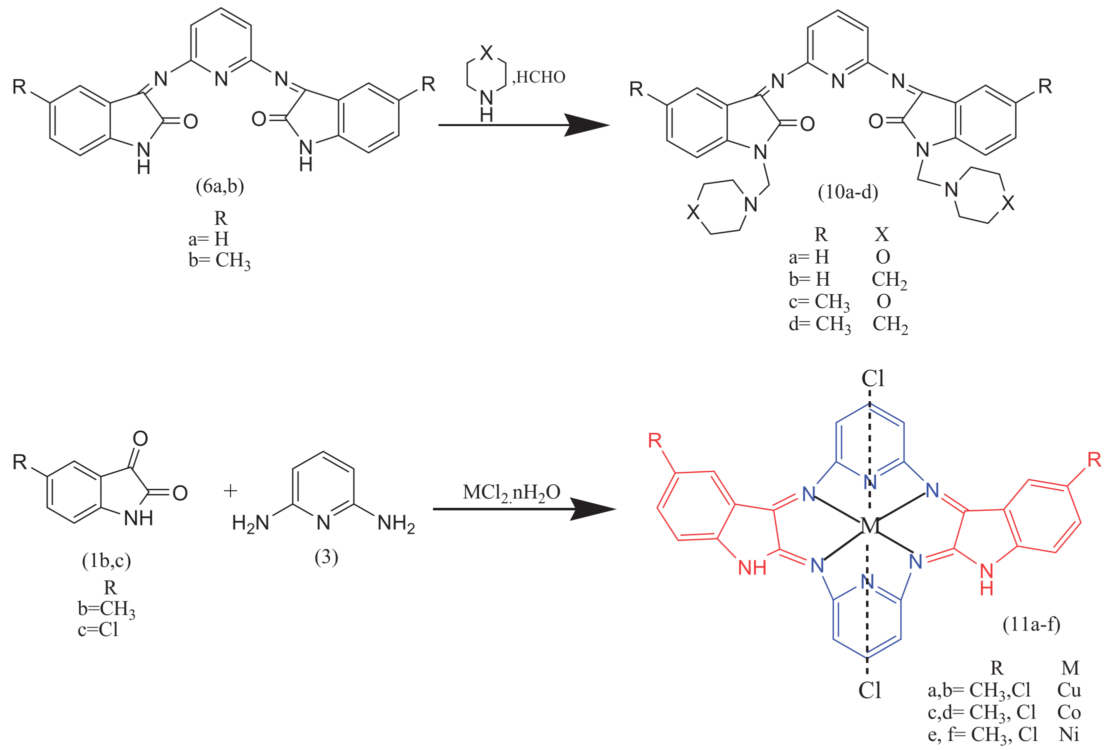
spiro[(3H)indole-3,3′3H)-1,2,4-triazol-2-(1H)-one]-3,3′-dimethoxybenzidine **9b** showed bands at δ: 12.46 (s, 2H, $NH_{(oxindole),}$ $D_2O_{exchangeable}$), 11.15 (s, 2H, $NH_{(triazole),}$ $D_2O_{exchangeable}$), 9.00,8.63 (s, 4H, $2NH_2$, $D_2O_{exchangeable}$),7.61–6.87 (m, 14H, 4Ar–H),3.79 (s, 6H, $2OCH_3$) ppm. The ^{13}C NMR spectrum of bis spiro[(3H)indole-3,3′-(3H)-1,2,4-triazol-2-(1H)-one]-3,3′-dimethoxybenzidine **9b** showed bands at δ: 179.19, 163.15, 142.89 142.84, 141.79, 132.58 132.17, 131.40, 126.98, 123.46, 122.33, 121.49, 120.49, 112.08, 111.04, 83.15(*spiro* C), 32.26.

The reaction of bis-Schiff bases **6a,b** with formaldehyde in the presence of a secondary amines such as piperidine and morpholine yielded the *N*-Mannich bases **10a–d**, respectively, as illustrated in Scheme 4. The structure of these compounds was established from FTIR, ^1H NMR, ^{13}C NMR and MS spectra and elemental analysis. The ^1H NMR spectrum of bis *N*-[(1-morpholinomethyl) indolin-2-one] pyridine 2,6-diamine **10a** showed bands at δ: 7.69–7.12 (m, 11H, 3Ar–H),4.39 (s, 4H, $2NCH_2N$), 3.56–3.54 (t, 8H, $2CH_2$–O–CH_2), 2.57–2.50 (t, 8H, $2CH_2$–N–CH_2) ppm. ^{13}C NMR spectrum of bis *N*-[(1-morpholinomethyl)indolin-2-one]pyridine 2,6-diamine **10a** showed bands at δ: 183.66, 159.48, 151.91, 139.25, 138.58, 138.39, 125.55, 124.65, 124.37, 123.94, 123.19, 118.084, 112.04, 111.93, 67.63, 67.39, 66.54, 66.31, 62.32, 51.29, 50.96, 50.36, 50.00.

The complexes **11a–f** were prepared by condensation reaction of 2,6-diamine pyridine with 5-substituted isatins **1b,c** in the presence of $MCl_2.nH_2O$ salts (where M = Cu, Co or Ni).The structure of metal complexes **11a–f** was confirmed by elemental analysis and spectral studies. The elemental analysis showed a ratio of 2:2:1 [isatins:DAP:MCl_2] as shown in Scheme 4. The theoretical values were in a good agreement with the found values. The presence of chlorine confirmed from element analysis and the low molar conductance values (15–82.30 S cm^2mol^{-1}) for the complexes **11a–f** supports the non-electrolytic nature of the metal complexes.

IR spectral studies and mode of coordination of complexes 11a–f

In the spectrum of 2,6-diaminopyridine a pair of medium intensity bands present at 3375–3400 cm^{-1} corresponding to (NH_2) but these are absent in the infrared spectra of all the complexes. Further, no strong absorption band was observed at



Scheme 4. Synthesis of the target compounds **10a–d** and **11a–f**.

1750–1700 cm^{-1} indicating the absence of (C = O) group of 5-substituted isatins. This indicates that the condensation of carbonyl groups of 5-substituted isatins and amino groups of pyridine – 2, 6-diamino might have taken place. These results provide strong evidence for the formation of macrocyclic frame. A strong absorption band in the region 1620–1612 cm^{-1} may be attributed to the (C=N) group. The lower values of (C=N) may be explained on the basis of drift of lone pair density of azomethine nitrogen towards the metal atom. The presence of band in the region 3213–3136 cm^{-1} in the isatin complexes may be assigned due to (N–H) stretching. New bands in the 555–505 cm^{-1} regions are assigned to stretching frequencies of (M–N) bonds. The unchanged pyridine ring vibrations in the complexes indicate non-coordination of the pyridine nitrogen atom. Moreover, the coordination through pyridine nitrogen is also ruled out on the basis that it will result in the formation of four membered heterocyclic rings, which are sterically unstable. Thus, in the presence of metal salts, a quadridentate macrocycle is formed which coordinates through suitably placed azomethine nitrogen while pyridine nitrogens do not take part in the coordination.

Electronic spectra and magnetic moment studies 11a–f

The magnetic susceptibility measurement for the solid Cu (II) complexes (1.8–2.1 B.M) is indicative of octahedral environment. The diffuse reflectance spectrum of the copper complexes **11a,b** showed two band groups at 393–314 and 242–234 nm, these bands can be attributed to π–π^* and n–π^* transition states within the hydrazone ligand. Bands at 767–472 nm can be attributed to d–d transition states and ligand to metal charge transfer.

The magnetic susceptibility measurement for the solid Co (II) complexes (3.50–3.90 B.M) is indicative of three unpaired electrons per Co (II) ion suggesting consistency with their octahedral environment. The diffuse reflectance spectrum of the cobalt complexes **11c,d** showed two band groups at 396–313 and 254–243 nm, these bands can be attributed to π–π^* and n–π^* transition states within the hydrazone ligand. Bands at 769–400 nm can be attributed to d–d transition states and ligand to metal charge transfer.

The magnetic susceptibility measurement for the solid Ni (II) complexe (2.60–2.74 B.M) is indicative of octahedral environment. The diffuse reflectance spectrum of the nickel complexes **11e,f** showed two band groups at 411–320 and 266–212 nm, these bands can be attributed to π–π^* and n–π^* transition states within the hydrazone ligand. Bands at 768–432 nm can be attributed to d–d transition states and ligand to metal charge transfer.

Table 1. Inhibition zone diameter in (mm) as a criterion of antibacterial and antifungal activities of the new synthesized compounds.

	Microorganism inhibition zone diameter(mm)					
	Gram + ve bacteria		Gram − ve bacteria		Fungi	
Compounds	*S. aureus*	*S. epidermidis*	*E. coli*	*K. pneumonia*	*A. fumigatu*	*C. albicans*
4a	16	16	19	15	10	13
4b	16	15	18	14	11	12
5a	17	15	17	14	10	11
5c	17	14	16	14	12	13
6a	16	18	19	15	13	15
7a	26	26	25	25	14	20
7b	25	30	30	27	17	21
8a	26	26	27	25	14	16
8b	26	28	25	23	15	15
9a	32	33	34	30	21	25
9d	34	35	36	32	23	25
10a	30	27	31	27	14	18
10b	30	29	30	29	16	19
10c	31	28	29	30	14	18
Sulfamethoxazole	36	36	39	33	–	–
Fluconazole	–	–	–	–	26	28

Pharmacological evaluation

In vitro antimicrobial measurement

Most of the synthesized compounds were tested for their *in vitro* antimicrobial activity by the broth-dilution technique in terms of

Table 2. MIC in µg/ml of the new synthesized compounds.

	Microorganism minimum inhibitory concentration					
	Gram + ve bacteria		Gram − ve bacteria		Fungi	
Compounds	*S. aureus*	*S. epidermidis*	*E. coli*	*K. pneumonia*	*A. flavus*	*C. albicans*
4a	24	24	24	48	48	48
4b	24	48	48	24	48	48
5a	24	48	48	48	48	48
5c	48	48	48	48	48	48
6a	48	24	24	48	48	24
7a	12	12	12	12	12	12
7b	6	6	6	6	12	6
8a	12	12	12	24	24	24
8b	12	6	12	24	24	12
9a	3	3	6	6	6	6
9d	3	3	3	3	6	6
10a	6	6	6	12	12	12
10b	12	12	12	12	24	24
10c	6	6	6	12	12	12
Sulfamethoxazole	3	3	3	3	–	–
Fluconazole	–	–	–	–	3	3

MIC: Minimum Inhibitory Concentration.

Table 3. MBC and MFC in µg/ml of the new synthesized compounds.

	Microorganism minimum bactericidal and fungicidal concentration					
	Gram + ve bacteria		Gram − ve bacteria		Fungi	
Compounds	*S. aureus*	*S. epidermidis*	*E. coli*	*K. pneumonia*	*A. flavus*	*C. albicans*
7a	24	24	24	24	48	48
7b	24	24	24	24	24	12
8a	48	48	24	48	48	96
8b	48	48	24	48	96	48
9a	12	12	24	24	12	12
9d	6	6	6	12	12	12
10a	12	12	24	24	48	48
10b	24	24	24	24	96	96
10c	24	24	12	48	24	24
Sulfamethoxazole	6	6	6	6	–	–
Fluconazole	–	–	–	–	6	6

MBC: Minimum Bactericidal Concentration; MFC: Minimum Fungicidal Concentration.

MIC, MBC and minimum fungicidal concentration (MFC) [Experimentally, for potent drugs the MBC and MFC is usually 2 to 4 x the MIC for the same isolate.] The antimicrobial activity of the compounds against six pathogenic microbial species are present in Tables 1–3. The study also included the activity of reference compounds Sulfamethoxazole as antibacterial agent and Fluconazole as antifungal agent. From the data obtained, the following conclusion can be drawn:

1. Schiff bases and bis-Schiff bases based on oxindole moiety have low antimicrobial activity compared to synthesized heterocyclic compounds obtained by formation of thiazolodine, azetidinone, 1,2,4 triazole and Mannich base derivatives.
2. Bis spiro thiazolodine **7b** with 3,3′-dimethoxybenzidine fragment according to MICs count gave the highest antimicrobial activity of the thiazolodine derivatives.
3. All the azetidinone derivatives nearly gave the same activities.
4. Bis spiro 1,2,4 triazole derivatives showed remarkable activity and gave relative values of the reference drugs and may serve as useful lead compounds in search for potent antimicrobial agent.
5. Mannich bases with morpholine moiety **10a** showed more activity compared to those with piperidine moiety **10b**.

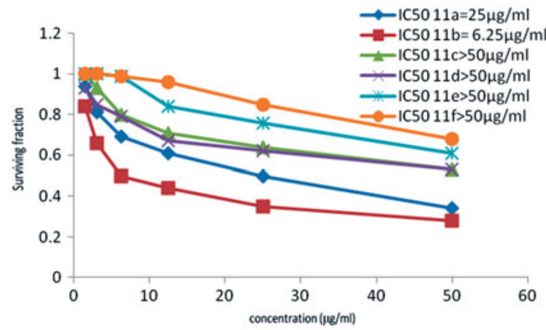


Figure 1. The cytotoxicity data of the activity of compounds **11a–f** against cervix (HELA) tumor cell line compared to Vinblastine sulfate **IC50:10.9.**

In vitro cytotoxicity evaluation of mononuclear Cu(II), Co(II) and Ni(II) macrocyclic complexes 11a–f

The activities of three different macrocyclic metal complexes **11a**–**f** were performed against the cervix human cancer cell line (HELA). Unexpected low values of activity were obtained with the three series of macrocyclic complexes although the copper complex **11b** gave remarkable activity comparable to the reference drug, also the activity obtained with the copper complex **11a** may be considered as moderate one Figure 1.

Conclusion

In this study, we report a convenient route for the synthesis of some novel heterocycles incorporating oxindole moiety in order to investigate their antimicrobial, antifungal activity. The *in vitro* evaluation of their antimicrobial against several pathogenic bacterial and fungal strains revealed that compound **9d** showed the highest activity against Gram + ve and Gram –ve bacteria. The activities of three different macrocyclic metal complexes **11a**–**f** were performed against the cervix human cancer cell line (HELA) .Unexpected low values of activity were obtained with the three series of macrocyclic complexes although the copper complex **11b** gave remarkable activity comparable to the reference drug, also the activity obtained with the copper complex **11a** may be considered as moderate one.

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Disclosure statement

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the article.

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