Journal of Enzyme Inhibition and Medicinal Chemistry, 2012; 27(3): 330–338

ISSN 1475-6366 print/ISSN 1475-6374 online
DOI: 10.3109/14756366.2011.588950

informa
healthcare

RESEARCH ARTICLE

Antiproliferative effects of metal complexes of new isatin hydrazones against HCT116, MCF7 and HELA tumour cell lines

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Abstract

New hydrazone ligands (HL) derived from 5-substituted isatins and 1-(4-(2-methoxybenzyl)-6-arylpyridazin-3-yl) hydrazines and its complexes with Co(II) and Cu(II) were synthesized. The new hydrazones and their complexes were characterized by means of elemental, spectral analyses and magnetic studies. Primary cytotoxicity evaluation of HL 5a and the new complexes showed that these complexes could act as anticancer agents since they reduced the growth of samples of human tumour cell lines ($HCT116_{(Colon)}$, $MCF7_{(Breast)}$ and $HELA_{(Cervix)}$) to ≤18.5 μg/mL for the new complexes.

Keywords: Hyrazone ligands, 5-substituted isatins, cytotoxicity, anticancer drugs

Introduction

Metals, in particular, transition metals offer potential advantages over the more common organic-based drugs, including a wide range of coordination numbers and geometries, accessible redox states, 'tune-ability' of the thermodynamics and kinetics of ligand substitution and a wide structural diversity. Medicinal inorganic chemistry is a thriving area of research, which was initially fuelled by the discovery of the metallopharmaceutical cisplatin about 40 years ago[1,2]. Regardless of the achievements of current platinum drugs, there are some major drawbacks: they are efficient only for a limited range of cancers; some tumours can have acquired or intrinsic resistance; and they often cause severe side effects, such as nausea, bone marrow suppression and kidney toxicity. Although ~10 other platinum compounds are currently in clinical trials, the cisplatin derivatives have not been able to address sufficiently many of the disadvantages associated with cisplatin. There is a need, therefore, for new approaches that are purposefully designed to circumvent these drawbacks. Recent progress in the field of cell biology has resulted in the discovery of receptors and growth factors that are up-regulated in cancer cells. These provide new targets for anticancer drug design.

The field of metal-based anticancer drug design can be divided into two different approaches: classical and non-classical chemotherapeutics[3]. Hydrazones, which may also be considered as Schiff bases or imines, are an important class of compounds in medicinal and pharmaceutical field; they show biological applications including antibacterial, antifungal[4–6]; it can act as ligands in interaction with various metal ions. Hydrazone ligands (HL) possessing potential donor sites that have coordination capability are biologically active may be due to the presence of an azomethine and uncoordinated α-nitrogen of the hydrazone chromosphere; this lead to more research on the synthesis of metal complexes of new hydrazones with significant biological activity[7–9]. One of the most important goals of pharmacological research is the search for new molecular structures, which exhibit effective antitumour activities. This has driven inorganic and organometallic chemists to look for new metal compounds with good activities, preferably against tumours that are responsible for high cancer mortality. In view of

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(Received 04 April 2011; revised 10 May 2011; accepted 11 May 2011)

these observations and as a part of our ongoing research programme for the synthesis of biologically active compounds[10–13], the present article includes synthesis, spectral characterization and preliminary evaluation of their antiproliferative activities of new hydrazones and its metal complexes.

Experimental

Chemistry

Chemicals and solvents

All the chemicals used were of reagent grade. 5-Substituted isatins were synthesized using Sandemyer methodology starting from the corresponding amines. 1-(4-(2-Methoxybenzyl)-6-arylpyridazin-3-yl)hydrazines were synthesized according to earlier reports[13]. Metal salts were of analytical reagent (E. Merk) as chloride salts. The organic solvents used were either spectroscopically pure or purified by the recommended methods.

Instrumental measurement

Elemental analyses (C, H, N and Cl) of the synthesized compounds were carried out by Microanalytical Center, Cairo University. The metal percentage was estimated using Inductively Coupled Argon Plasma (ICP) technique of the type 6500 Duo, Thermo Scientific, England. The 1000 mg/L multi-element and certified standard solution (Merck, Germany) were used as stock solution for instrument standardization. Microwave Digestion Lab station closed system, Ethos Pro, Milestone, Italy was used to digest the organic matter in aqua regia. The infrared spectra were measured on Jasco instrument (Model 6100, Japan) in the transmittance mode at a resolution of 4 cm^{-1} using potassium bromide Wafer technique. The mass spectra were measured on mass spectrophotometer HP Model GC-MS-QPL000EX (Shimadzu) at 70 eV. UV–vis spectra were measured on UV-1600 spectrophotometer using dimethylformamide (DMF) as solvent. The ^{1}H NMR were measured on Varian Genini-300 MHz spectrophotometer using dimethyl sulphoxide (DMSO) as solvent. The magnetic moment measurement was performed using Faraday method.

Synthesis of hydrazones (5a–5r)

A mixture of a 1-[4-(2-methoxybenzyl)-6-arylpyridazin-3-yl]hydrazines (**4a**–**4c**) (0.01 mol) and 5-substituted isatin (0.01 mol) was heated under reflux in absolute ethanol (20 mL) in presence of catalytic amount of glacial acetic acid for 3 h. During this period, the product was separated from the reaction mixture as a precipitate was collected and crystallized from ethanol to give the title compounds.

3-[2-(4-(2-Methoxybenzyl)-6-phenylpyridazin-3-yl) hydrazono]indolin-2-one (5a). Orange. Yield 70%, mp 208–210°C[14].

3-[2-(4-(2-Methoxybenzyl)-6-p-tolylpyridazin-3-yl)hydrazon] indolin-2-one (5b). Orange. Yield 75%, mp 260–262°C[14].

3-[2-(4-(2-Methoxybenzyl)-6-(4-chlorophenyl)pyridazin-3-yl) hydrazono]-indolin-2-one (5c) Orange. Yield 83%, mp 270–272°C. IR [cm^{-1}]: 3406 $NH_{(hydrazone)}$, 3155 $NH_{(oxindole)}$, 1680 $C{=}O_{(oxindole)}$, 1616, 1546 C=N. GC-MS (*m*/*z*): 469.75 (M^+). Calcd. for $C_{26}H_{20}ClN_5O_2$: C, 66.39; H, 4.25; N, 14.89; Cl, 7.54; Found: C, 66.35; H, 4.25; N, 14.64; Cl, 7.22.

3-[2-(4-(2-Methoxybenzyl)-6-phenylpyridazin-3-yl) hydrazon]-5-methylindolin-2-one (5d) Red. Yield 51.9%, mp 228°C. IR [cm^{-1}]: 3420 $NH_{(hydrazone)}$, 3180 $NH_{(oxindole)}$, 1686 $C{=}O_{(oxindole)}$, 1625, 1545 C=N. ^{1}H NMR [DMSO d6, 300 MHz] δ:11.14 (s, 1H, $NH_{(oxindole)}$), 10.37 (s, 1H, $NH_{(hydrazone)}$), 7.83–7.15 (m, 12H, 3Ar-H), 6.70 (s, 1H, CH), 4.34 (s, 2H, CH_2), 3.80 (s, 3H, OCH_3 of CH_3O-Ar), 2.56 (s, 3H, CH_3 of CH_3-Ar). GC-MS (*m*/*z*): 450 (M^+). Calcd. for $C_{27}H_{23}N_5O_2$: C, 72.14; H, 5.16; N, 15.58; Found: C, 72.25; H, 5.15; N, 15.32.

3-[2-(4-(2-Methoxybenzyl)-6-p-tolylpyridazin-3-yl) hydrazono]-5-methylindolin-2-one (5e) Red. Yield 76.7%, mp 248°C. IR [cm^{-1}]: 3398 $NH_{(hydrazone)}$, 3167 $NH_{(oxindole)}$, 1680 $C{=}O_{(oxindole)}$, 1618, 1584 C=N. GC-MS (*m*/*z*): 464 (M^++1). Calcd. for $C_{28}H_{25}N_5O_2$: C, 72.55; H, 5.44; N, 15.11; Found: C, 72.52; H, 5.45; N, 15.00.

3-[2-(4-(2-Methoxybenzyl)-6-(4-chlorophenyl)pyridazin-3-yl)hydrazono]-5-methylindolin-2-one (5f). Red. Yield 70%, mp 234–235°C. IR [cm^{-1}]: 3406 $NH_{(hydrazone)}$, 3146 $NH_{(oxindole}$), 1682 $C{=}O_{(oxindole)}$, 1621, 1582 C=N. GC-MS (*m*/*z*): 484 (M^+), 486 (M^++2). Calcd. for $C_{27}H_{22}ClN_5O_2$: C, 67.00; H, 4.58; N, 14.47; Cl, 7.33; Found: C, 67.15; H, 4.60; N, 14.32; Cl 7.30.

3-[2-(4-(2-Methoxybenzyl)-6-phenylpyridazin-3-yl) hydrazon]-5-chloroindolin-2-one (5g) Orange. Yield 60%, mp 240°C. IR [cm^{-1}]: 3370 $NH_{(hydrazone)}$, 3156 $NH_{(oxindole)}$, 1704 $C{=}O_{(oxindole)}$, 1618, 1589 C=N. GC-MS (*m*/*z*): 470 (M^+), 472 (M^++2). Calcd. for $C_{26}H_{20}ClN_5O_2$: C, 66.39; H, 4.25; N, 14.89; Cl, 7.54; Found: C, 66.69; H, 4.52; N, 14.90; Cl 7.55.

3-[2-(4-(2-Methoxybenzyl)-6-p-tolylpyridazin-3-yl) hydrazono]-5-chloroindolin-2-one (5h) Violet. Yield 76%, mp 268–270°C. IR [cm^{-1}]: 3413 $NH_{(hydrazone)}$, 3182 $NH_{(oxindole)}$, 1705 $C{=}O_{(oxindole)}$, 1624, 1566 C=N. ^{1}H NMR [DMSO d6, 300 MHz] δ:11.12 (s, 1H, $NH_{(oxindole)}$), 10.58 (s, 1H, $NH_{(hydrazone)}$), 7.98–7.13 (m, 11H, 3Ar-H), 6.82 (s, 1H, CH), 4.33 (s, 2H, CH_2), 3.38 (s, 3H, OCH_3 of CH_3O-Ar), 2.51 (s, 3H, CH_3 of CH_3-Ar). GC-MS (*m*/*z*): 484 (M^+). Calcd. for $C_{27}H_{22}ClN_5O_2$: C, 67.00; H, 4.58; N, 14.47; Cl, 7.33; Found: C, 66.90; H, 4.48; N, 14.40; Cl, 7.33.

3-[2-(4-(2-Methoxybenzyl)-6-(4-chlorophenyl)pyridazin-3-yl)hydrazono]-5-chloroindolin-2-one (5i) Violet. Yield 73%, mp 264–265°C. IR [cm^{-1}]: 3433 $NH_{(hydrazone)}$, 3145 $NH_{(oxindole)}$, 1689 $C{=}O_{(oxindole)}$, 1624, 1546 C=N. GC-MS

(*m/z*): 504 (M^+), 505 (M^++1). Calcd. for $C_{26}H_{19}Cl_2N_5O_2$: C, 61.91; H, 3.80; N, 13.89; Cl, 14.06; Found: C, 61.85; H, 3.68; N, 13.72; Cl 14.14.

3-[2-(4-(2-Methoxybenzyl)-6-phenylpyridazin-3-yl) hydrazono]-5-methoxyindolin-2-one (5j) Red. Yield 90%, mp 210–211°C. IR [cm^{-1}]: 3422 $NH_{(hydrazone)}$, 3152 $NH_{(oxindole)}$, 1680 $C{=}O_{(oxindole)}$, 1625, 1587 C=N. ^1H NMR [DMSO d6, 300 MHz] δ:10.97(s, 1H, $NH_{(oxindole)}$), 10.30 (s, 1H, $NH_{(hydrazone)}$) 8.00–6.85 (m, 12H, 3Ar-H), 6.77 (s, 1H, CH), 4.11 (s, 2H, CH_2), 3.38–3.16 (s, 6H, 2OCH_3 of 2CH_3O-Ar). GC-MS (*m/z*): 466 (M^+). Calcd. for $C_{27}H_{23}N_5O_3$: C, 69.66; H, 4.98; N, 15.05; Found: C, 69.67; H, 4.95; N, 14.92.

3-[2-(4-(2-Methoxybenzyl)-6-p-tolylpyridazin-3-yl) hydrazono]-5-methoxyindolin-2-one (5k) Brick red. Yield 94%, mp 220–222°C. IR [cm^{-1}]: 3362 $NH_{(hydrazone)}$, 3165 $NH_{(oxindole)}$, 1683 $C{=}O_{(oxindole)}$, 1627, 1586 C=N. GC-MS (*m/z*): 480 (M^+). Calcd. for $C_{28}H_{25}N_5O_3$: C, 70.13; H, 5.26; N, 14.61; Found: C, 69.58; H, 5.21; N, 14.57.

3-[2-(4-(2-Methoxybenzyl)-6-(4-chlorophenyl)pyridazin-3-yl)hydrazon]-5-methoxyindolin-2-one (5l) Brick red. Yield 94%, mp 250°C. IR [cm^{-1}]: 3415 $NH_{(hydrazone)}$, 3153 $NH_{(oxindole)}$, 1683 $C{=}O_{(oxindole)}$, 1625, 1590 C=N. GC-MS (*m/z*): 500 (M^+). Calcd. for $C_{27}H_{22}ClN_5O_3$: C, 64.86; H, 4.44; N, 14.01; Cl, 7.09; Found: C, 64.57; H, 4.33; N, 13.80; Cl, 7.09.

3-[2-(4-(2-Methoxybenzyl)-6-phenylpyridazin-3-yl) hydrazono]-5-nitroindolin-2-one (5m) Orange. Yield 74.44%, mp 280–282°C. IR [cm^{-1}]: 3403 $NH_{(hydrazone)}$, 3162 $NH_{(oxindole)}$, 1699 $C{=}O_{(oxindole)}$, 1624, 1585 C=N. GC-MS (*m/z*): 480 (M^+). Calcd. for $C_{26}H_{20}N_6O_4$: C, 64.99; H, 4.20; N, 17.50; Found: C, 64.80; H, 4.50; N, 17.25.

3-[2-(4-(2-Methoxybenzyl)-6-p-tolylpyridazin-3-yl) hydrazono]-5-nitroindolin-2-one (5n) Red. Yield 75.67%, mp 308–310°C. IR [cm^{-1}]: 3417 $NH_{(hydrazone)}$, 3104 $NH_{(oxindole)}$, 1677 $C{=}O_{(oxindole)}$, 1614, 1591 C=N. ^1H NMR [DMSO d6, 300 MHz] δ: 11.17 (s, 1H, $NH_{(oxindole)}$), 9.19 (s, 1H, $NH_{(hydrazone)}$), 7.96–7.02 (m, 11H, 3Ar-H), 6.92 (s, 1H, CH), 4.34 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3 of CH_3O-Ar), 2.49 (s, 3H, CH_3 of CH_3-Ar). GC-MS (*m/z*): 495 (M^+). Calcd. for $C_{27}H_{22}N_6O_4$: C, 65.57; H, 4.48; N, 17.00; Found: C, 65.64; H, 4.40; N, 16.77.

3-[2-(4-(2-Methoxybenzyl)-6-(4-chlorophenyl)pyridazin-3-yl)hydrazono]-5-nitroindolin-2-one (5o) Orange. Yield 58.92%, mp 304°C. IR [cm^{-1}]: 3410 $NH_{(hydrazone)}$, 3168 $NH_{(oxindole)}$, 1716 $C{=}O_{(oxindole)}$, 1617, 1593 C=N. GC-MS (*m/z*): 516 (M^++2). Calcd. for $C_{26}H_{19}ClN_6O_4$: C, 60.64; H, 3.72; N, 16.32; Cl, 6.88; Found: C, 60.45; H, 3.92; N, 16.05; Cl, 7.03.

3-[2-(4-(2-Methoxybenzyl)-6-phenylpyridazin-3-yl) hydrazono]-5-fluoroindolin-2-one (5p) Orange. Yield 52%, mp 250°C. IR [cm^{-1}]: 3399 $NH_{(hydrazone)}$, 3162 $NH_{(oxindole)}$, 1699 $C{=}O_{(oxindole)}$, 1624, 1585 C=N. GC-MS (*m/z*): 454 (M^+), 455 (M^++1), 456 (M^++2). Calcd. for $C_{26}H_{20}FN_5O_2$: C, 68.86; H, 4.45; N, 15.45; Found: C, 68.99; H, 4.55; N, 15.10.

3-[2-(4-(2-Methoxybenzyl)-6-p-tolylpyridazin-3-yl) hydrazono]-5-fluoroindolin-2-one (5q) Orange. Yield 88.23%, mp 300°C. IR [cm^{-1}]: 3398 $NH_{(hydrazone)}$, 3158 $NH_{(oxindole)}$, 1697 $C{=}O_{(oxindole)}$, 1624, 1542 C=N. GC-MS (*m/z*): 467 (M^+), 468 (M^++1). Calcd. for $C_{27}H_{22}FN_5O_2$: C, 69.36; H, 4.74; N, 14.98; Found: C, 69.31; H, 4.72; N, 14.88.

3-[2-(4-(2-Methoxybenzyl)-6-(4-chlorophenyl)pyridazin-3-yl)hydrazon]-5-fluoroindolin-2-one (5r) Orange. Yield 55.24%, mp 296–298°C. IR [cm^{-1}]: 3334 $NH_{(hydrazone)}$, 3156 $NH_{(oxindole)}$, 1698 $C{=}O_{(oxindole)}$, 1621, 1579 C=N. ^1H NMR [DMSO d6, 300 MHz] δ: 11.12 (s, 1H, $NH_{(oxindole)}$), 10.45 (s, 1H, $NH_{(hydrazone)}$), 8.06–7.02 (m, 11H, 3Ar-H), 6.84 (s, 1H, CH), 4.19 (s, 2H, CH_2), 3.82 (s, 3H, OCH_3 of CH_3O-Ar). GC-MS (*m/z*): 488 (M^+), 489 (M^++1), 490 (M^++2). Calcd. for $C_{26}H_{19}ClFN_5O_2$: C, 64.00; H, 3.93; N, 14.36; Cl, 7.27; Found: C, 64.11; H, 4.18; N, 14.02; Cl, 7.33.

Synthesis of Co(II) and Cu(II) complexes (6a–6k)

Warm ethanolic solutions of the ligand (20 mmol) and the metal chloride (10 mmol) were mixed. The reaction mixture was refluxed and stirred for 3 h. The solid product obtained after evaporation under reduced pressure was filtered and crystallized from ethanol to give the complexes (**6a**–**6k**).

Copper(II) complex (6a) Brick red. Yield 60%, mp 280–281°C. IR [cm^{-1}]: 3327 $NH_{(hydrazone)}$, 3178 $NH_{(oxindole)}$, 1645 $C{=}O_{(oxindole)}$, 1575, 1540 C=N, 604, 509 (M-N), 426 (M-O). λ_{max} [nm]: 483, 394, 265. μ_{eff}: 1.87 B.M. Calcd. for $C_{56}H_{50}CuN_{10}O_6$: C, 65.77; H, 4.93; N, 13.70; Cu, 6.21; Found: C, 66.40; H, 4.47; N, 13.44; Cu, 6.57.

Cobalt(II) complex (6b) Violet. Yield 50%, mp 282–284°C. IR [cm^{-1}]: 3401 $NH_{(hydrazone)}$, 3183 $NH_{(oxindole)}$, 1615 $C{=}O_{(oxindole)}$, 1567, 1524 C=N, 591, 545 (M-N), 462 (M-O). λ_{max} [nm]: 485.5, 392, 265. μ_{eff}: 5.18 B.M. Calcd. for $C_{56}H_{50}CoN_{10}O_4$: C, 68.21; H, 5.11; N, 14.21; Co, 5.98; Found: C, 68.09; H, 5.34; N, 13.55; Co, 5.40.

Copper(II) complex (6c) Golden brown. Yield 68%, mp 273°C. IR [cm^{-1}]: 3316 $NH_{(hydrazone)}$, 3165 $NH_{(oxindole)}$, 1645 $C{=}O_{(oxindole)}$, 1568, 1523 C=N, 577, 504 (M-N), 467 (M-O). λ_{max} [nm]: 477.5, 381, 264. μ_{eff}: 1.88 B.M. Calcd. for $C_{52}H_{42}CuN_{10}O_4$: C, 66.83; H, 4.53; N, 14.99; Cu, 6.80; Found: C, 66.78; H, 4.53; N, 14.66; Cu, 6.45.

Cobalt(II) complex (6d) Violet. Yield 56.9%, mp 164°C. IR [cm^{-1}]: 3324 $NH_{(hydrazone)}$, 3096 $NH_{(oxindole)}$, 1644 $C{=}O_{(oxindole)}$, 1565, 1524 C=N, 593, 549 (M-N), 425 (M-O). λ_{max} [nm]: 478.5, 389, 266.5. μ_{eff}: 5.44 B.M. Calcd. for $C_{52}H_{42}CoN_{10}O_4$: C, 67.16; H, 4.55; N, 15.07; Co, 6.34; Found, 66.89; H, 4.29; N, 14.76; Co, 6.33.

Copper(II) complex (6e) Golden brown. Yield 52.22%, mp 310°C. IR [cm^{-1}]: 3318 $NH_{(hydrazone)}$, 3155 $NH_{(oxindole)}$, 1676 $C=O_{(oxindole)}$, 1569, 1533 C=N, 586, 533 (M-N), 447 (M-O). λ_{max} [nm]: 482.5, 264. μ_{eff}: 2.22 B.M. Calcd. for $C_{52}H_{42}Cl_2CuN_{12}O_{10}$: C, 55.30; H, 3.75; N, 14.89; Cu, 5.63; Cl, 6.28; Found, 55.31; H, 3.91; N, 15.22; Cu, 6.13; Cl, 7.12.

Cobalt(II) complex (6f) Dark brown. Yield 30.20%, mp 260°C. IR [cm^{-1}]: 3316 $NH_{(hydrazone)}$, 3185 $NH_{(oxindole)}$, 1647 $C=O_{(oxindole)}$, 1566, 1527 C=N, 592, 507 (M-N), 456 (M-O). λ_{max} [nm]: 480, 414, 267. μ_{eff}: 3.82 B.M. Calcd. for $C_{52}H_{38}Cl_2CoF_2N_{10}O_4$: C, 60.35; H, 3.70; N, 13.54; Co, 5.70; Cl, 6.85; Found: C, 60.29; H, 3.80; N, 13.26; Co, 5.51; Cl, 6.89

Cobalt(II) complex (6g) Violet. Yield 34.23%, mp 258–260°C. IR [cm^{-1}]: 3421 $NH_{(hydrazone)}$, 3100 $NH_{(oxindole)}$, 1635 $C=O_{(oxindole)}$, 1569, 1492 C=N, 601, 551 (M-N), 443 (M-O). λ_{max} [nm]: 485.5, 417, 265. μ_{eff}: 4.82 B.M. Calcd. for $C_{52}H_{40}Cl_4CoN_{10}O_5$: C, 58.50; H, 3.59; N, 13.12; Co, 5.52; Cl, 13.28; Found: C, 58.30; H, 3.60; N, 12.96; Co, 6.16; Cl, 13.59.

Copper(II) complex (6h) Brown. Yield 47.05%, mp >350°C. IR [cm^{-1}]: 3336 $NH_{(hydrazone)}$, 3070 $NH_{(oxindole)}$, 1643 $C=O_{(oxindole)}$, 1569, 1500 C=N, 597, 509 (M-N), 455 (M-O). λ_{max} [nm]: 483, 381, 265. μ_{eff}: 2.48 B.M. Calcd. for $C_{54}H_{44}CuF_2N_{10}O_4$: N, 14.03; Cu, 6.36; Found: N, 13.86; Cu, 6.42.

Cobalt(II) complex (6i) Brick red. Yield 50.38%, mp 140°C. IR [cm^{-1}]: 3314 $NH_{(hydrazone)}$, 3166 $NH_{(oxindole)}$, 1647 $C=O_{(oxindole)}$, 1599, 1491 C=N, 577, 504 (M-N), 423 (M-O). λ_{max} [nm]: 485, 388, 267. μ_{eff}: 4.64 B.M. Calcd. for $C_{54}H_{44}CoF_2N_{10}O_4$: C, 65.25; H, 4.46; N, 14.09; Co, 5.82; Found: C, 64.95; H, 4.62; N, 14.12; Co, 5.78.

Copper(II)complex (6j) Brown. Yield 34%, mp 254°C IR [cm^{-1}]: 3321 $NH_{(hydrazone)}$, 3056 $NH_{(oxindole)}$, 1647 $C=O_{(oxindole)}$, 1569, 1531 C=N, 594, 513 (M-N), 470 (M-O). λ_{max} [nm]: 488, 381, 265. μ_{eff}: 2.28 B.M. Calcd. for $C_{56}H_{50}CuN_{10}O_4$: N, 14.14; Cu, 6.41; Found: N, 13.84; Cu, 6.25.

Cobalt(II) complex (6k) Violet. Yield 48.5%, mp 280–282°C. IR [cm^{-1}]: 3300 $NH_{(hydrazone)}$, 3177 $NH_{(oxindole)}$, 1627 $C=O_{(oxindole)}$, 1573, 1496 C=N, 613, 520 (M-N), 432 (M-O). λ_{max} [nm]: 485, 392, 266. μ_{eff}: 3.87 B.M. Calcd. for $C_{54}H_{46}CoN_{10}O_4$: C, 67.71; H, 4.84; N, 14.62; Co, 6.15; Found: C, 67.67; H, 4.54; N, 14.46; Co, 6.84.

Biological activity

The primary evaluation of *in vitro* cytotoxicity against human tumour cell of the complexes under investigation has been tested in National Cancer Institute (NCI), Cairo University using the method of Skehan and Storeng[15]. The study also involved the cytotoxicity evaluation of vinblastine sulphate[16] as antitumour agent reference 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 cell to the wall of the plate.
2. Different concentrations of the compound under test (0, 1, 2.5, 5 and 10 μg/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, cells were fixed, washed and stained with sulpho-rhodamine-B stain.
5. Excess stain was washed with acetic acid and attached stain was recovered with Tris–EDTA buffer.
6. Colour intensity was measured in an ELISA reader.
7. The relation between the surviving fraction and drug concentration is plotted to give the survival curve of each tumour cell line after the specified compound.

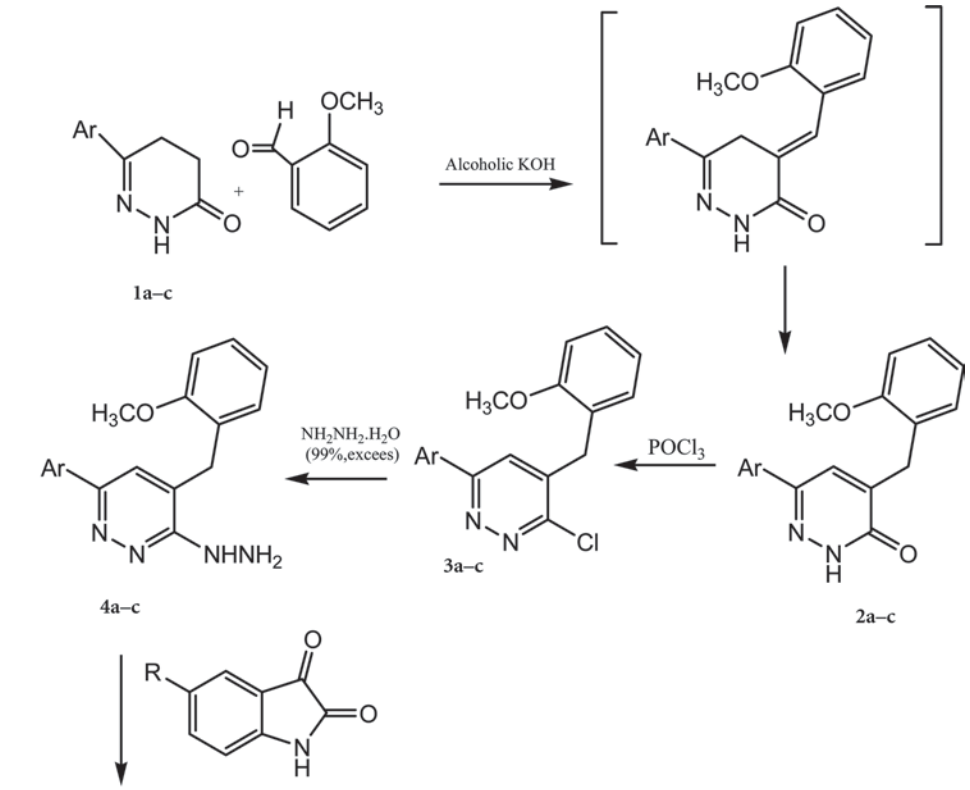
The results of the *in vitro* cytotoxicity activity on human tumour cell lines HCT116 (colon cell), MCF7 (breast cell) and HELA (cervix cell) were determined according to the dose values of the drug exposure required to reduce survival in the cell lines to 50%.

Results and discussion

Chemistry

The sequence of reactions followed in the synthesis of the target compounds is illustrated in Scheme 1. This article describes a facile synthesis of the new hydrazones (**5a**–**5r**) and its Co(II) and Cu(II) complexes (**6a**–**6k**). The hydrazine derivatives (**4a**–**4c**) required for the synthesis of the new hydrazone derivatives were obtained through our reported methods[10–14]. 6-Aryl-4-(2-methoxybenzyl) pyridazin-3-(2*H*)-ones (**2a**–**2c**) were obtained via reaction of 4-methoxybenzaldehyde with 6-aryl-4,5-dihydropyridazin-3(2*H*)-one (**1a**–**1c**) in alcoholic 5% KOH. Then reaction of (**2a**–**2c**) with phosphorus oxychloride yielded the corresponding 3-chloropyridazin derivatives (**3a**–**3c**), which on reaction with hydrazine hydrate afforded the corresponding hydrazines 1-[4-(2-methoxybenzyl)-6-arylpyridazin-3-yl]hydrazines (**4a**–**4c**). In the present study, hydrazine derivatives or its tautomeric structures (**4a**–**4c**) (Figure 1) were used as key starting materials for the synthesis of new hydrazones derivatives (**5a**–**5r**).

Condensation of hydrazine derivatives (**4a**–**4c**) with isatin and 5-substituted isatins afforded the corresponding hydrazones (**5a**–**5r**). The structure of these hydrazones were determined on the basis of elemental analysis and spectroscopic methods such as IR, ^{1}H NMR and mass fragmentation. Support for the structure was evidenced by the presence of prominent bands in the IR spectra for NH (3433–3334 cm^{-1} and 3182–3104 cm^{-1}), carbonyl (1716–1677 cm^{-1}) and C=N (1627–1542 cm^{-1}). The structures were further established by ^{1}H NMR spectral data. The signal showed at δ: 12.35–10.97 for $NH_{(oxindole)}$, 10.58–9.19 for $NH_{(hydrazone)}$, 8.06–6.86 for Ar-H, 6.92–6.70



No.	R	Ar
5a–c	H	C_6H_5,$C_6H_4CH_3$(p),C_6H_4Cl(p)
5d–f	CH_3	C_6H_5,$C_6H_4CH_3$(p),C_6H_4Cl(p)
5g–i	Cl	C_6H_5,$C_6H_4CH_3$(p),C_6H_4Cl(p)
5j–l	OCH_3	C_6H_5,$C_6H_4CH_3$(p),C_6H_4Cl(p)
5m–o	NO_2	C_6H_5,$C_6H_4CH_3$(p),C_6H_4Cl(p)
5p–r	F	C_6H_5,$C_6H_4CH_3$(p),C_6H_4Cl(p)

Scheme 1. Experimental protocol for the synthesis of hydrazones (**5a**–**5r**).

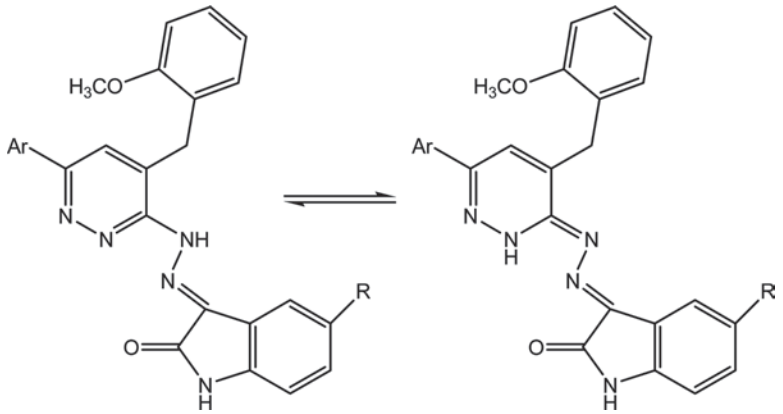


Figure 1. Tautomeric forms of new Hydrazones (**5a**–**5r**).

for $CH_{(hetero)}$, 4.34–4.11 for CH_2, 3.86–3.16 for OCH_3 of CH_3O-Ar and 2.83–2.49 for CH_3 of CH_3-Ar.

The hydrazones (**5a**–**5r**) reacted with the metal salt of Co(II) and Cu(II) to give the corresponding Co(II), Cu(II) complexes (**6a**–**6k**). The complexes were designed in the aim of exploring their anticancer activity. The structure of metal complexes (**6a**–**6k**) was confirmed by elemental analysis and spectral studies. The elemental analyses showed 1:2 (metal:ligand) stoichiometry for all complexes (Figure 2). The analytical data of ligands and complexes are given in the "Experimental" section, which showed that they are agreed well with the general formula $[M(HL)_2\ yH_2O]$ (where M = Co(II), Cu(II) and y = 0–1).

IR spectral studies and mode of coordination

The important infrared frequencies of hydrazones and metal complexes are reported in the "Experimental" section. In the IR spectra of the complexes (**6a**–**6k**), the band located at 1716–1680 cm^{-1} in all ligands attributed to carbonyl group in oxindole moiety moved to the lower frequency side by values up to 84 cm^{-1}, also the band due to (C=N) of azomethine moiety at 1627–1618 cm^{-1} lowered by value up to 51 cm^{-1} because of coordination of these sites to the metal. New bands in the 613–577 cm^{-1}, 551–504 cm^{-1} and 470–425 cm^{-1} regions are assigned to stretching frequencies of (M-N), (M-N) and (M-O) bonds, respectively. Thus the IR spectral results provide strong evidence for the complexation of hydrazones with metal ions in tridentate mode.

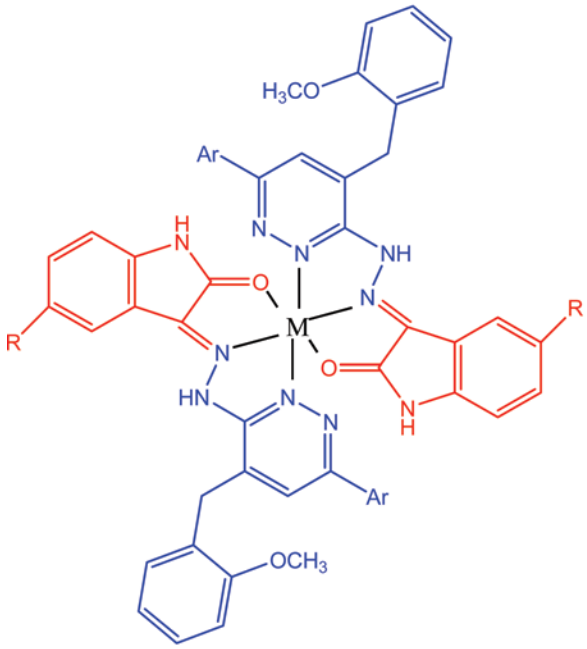
Electronic spectra and magnetic moment studies

The Co(II) complexes exhibit two bands at 485–478 and 392–388 nm. The bands can be assigned to $^4T_{1g}(F)$–$^4A_{2g}$ and $^4T_{1g}(F)$–$^4T_{1g}(P)$, which are in accordance with Co(II) high-spin octahedral geometry. The magnetic susceptibility measurement for the solid Co(II) complexes (3.82–5.44 B.M.) are also indicative of three unpaired electrons per Co(II) ion suggesting consistency with their octahedral environment.

The Cu(II) complexes exhibit two bands at 488–477 and 394–381 nm may be attributed to $^2B_{1g}$–$^2A_{1g}$ and $^2B_{1g}$–^2E_g transitions, respectively. Also, the magnetic susceptibility measurements (1.87–2.48 B.M.) for the copper (II) complex are indicative of octahedral geometry[17].

In vitro cytotoxicity evaluation "antiproliferative studies"

All the newly synthesized copper and cobalt complexes (**6a**–**6k**) and HL (**5a**) are chosen as prototypes were screened against a panel of three human cancer cell lines HCT116 (colon cell), MCF7 (breast cell) and HELA (cervix cell). Primary anticancer assay was performed in accordance with the protocol of the National Cancer Institute, Cairo University, Egypt by the method described in the "Experimental" section. The cytotoxicity of the complexes comparable with that obtained with widely used anticancer drug vinblastine sulphate[16]. The study also involved the examination of the cytotoxicity of the ligand (**5a**) in order to study the impact of metal complexation. The cobalt complexes (**6b** and **6d**) and copper complex (**6c**) are the



Comp.No	R	Ar	M
6a	OCH_3	C_6H_4-$CH_{3(p)}$	Cu
6b	CH_3	C_6H_4-$CH_{3(p)}$	Co
6c	H	C_6H_5	Cu
6d	H	C_6H_5	Co
6e	NO_2	C_6H_4-$Cl_{(p)}$	Cu
6f	F	C_6H_4-$Cl_{(p)}$	Co
6g	Cl	C_6H_4-$Cl_{(p)}$	Co
6h	F	C_6H_4-$CH_{3(p)}$	Cu
6i	F	C_6H_4-$CH_{3(p)}$	Co
6j	CH_3	C_6H_4-$CH_{3(p)}$	Cu
6k	H	C_6H_4-$CH_{3(p)}$	Co

Figure 2. The proposed structure of complexes (**6a**–**6k**).

most active on the colon tumour cell line (HCT116). In addition as with HCT116 cell line copper complex (**6c**) and cobalt complex (**6d**) showed the highest activity towards the breast tumour cell line (MCF7) together with the cobalt complex (**6i**). Metal complexes (**6e**–**6h**) are the most active on cervix tumour cell line (HELA). All data of the antiproliferative activity of metal complexes **6a**–**6k** and HL **5a** virus vinblastin sulphate (in means of IC_{50} values) were recorded in (Figures 3–5) and Table 1.

Conclusion

In the present study, a series of new hydrazones and its metal complexes were designed, synthesized, characterized and screened for anticancer activity against a panel of three human cancer cell lines. The new HL were coordinated to the metal ion resulting octahedral mononuclear complexes; they coordinated essentially through the carbonyl oxygen of the oxoindole moiety, the nitrogen atom of the azomethine group and the nitrogen of the pyridazine fragments. The hydrazone acts as a monobasic tridentate ligand. The two series of metal complexes possessed considerable cytotoxicity activity using colon, breast and cervix human cancer cell lines. The study involved the cytotoxicity of the reference vinblastine sulphate according to this arrangement the cobalt complexes (**6b** and **6d**) were more potent than the remaining complexes on the colon human cancer cell line, for the

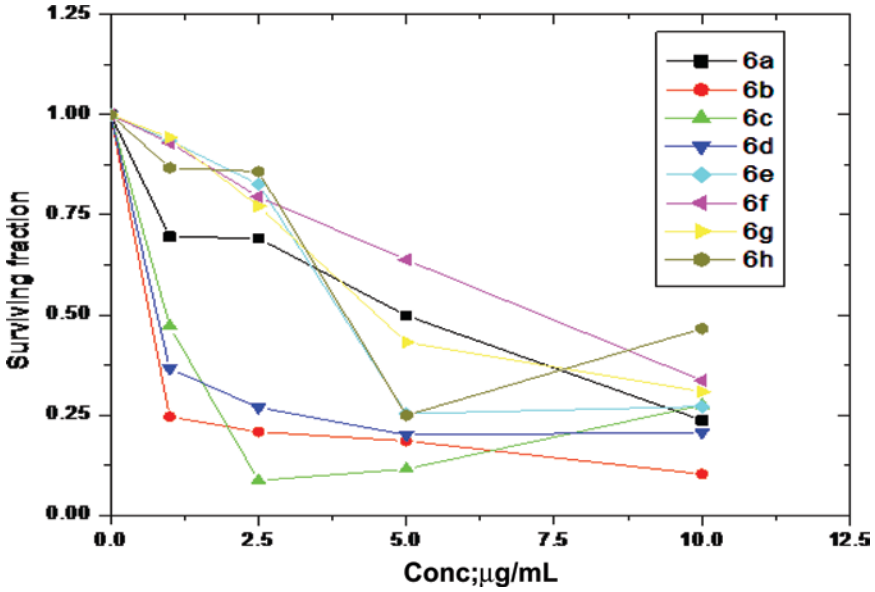


Figure 3. Cell viability dose–response curve of tested compounds against HCT116 cells.

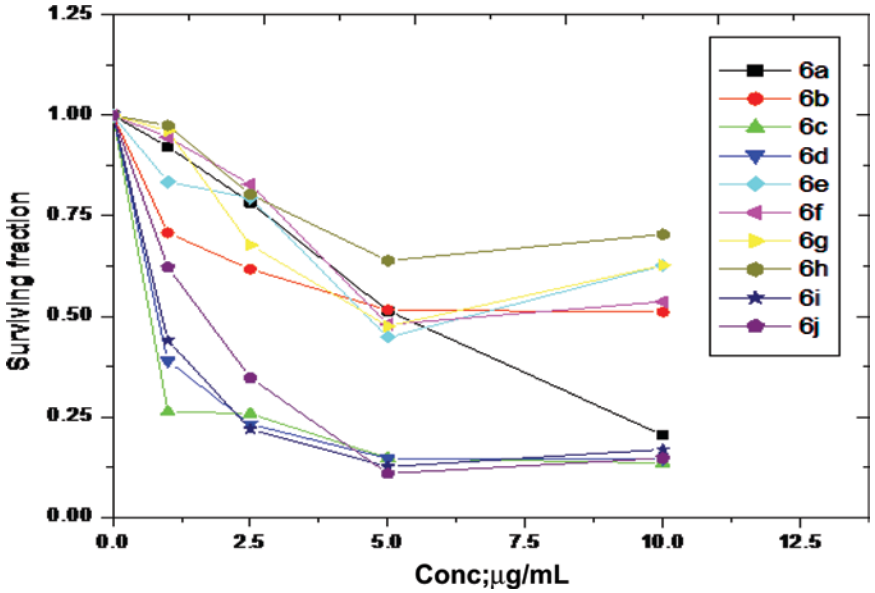


Figure 4. Cell viability dose–response curve of tested compounds against MCF7 cells.

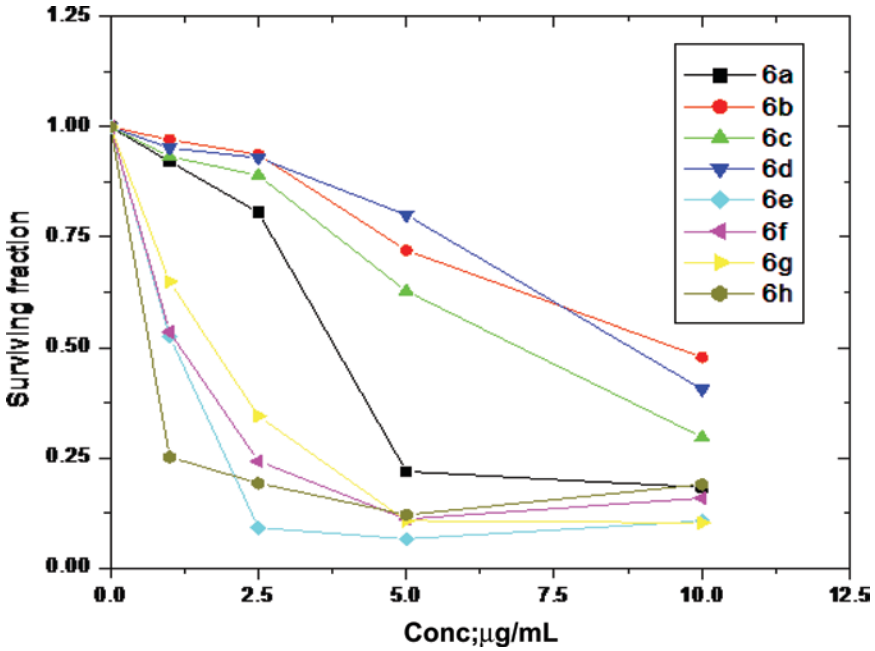


Figure 5. Cell viability dose–response curve of tested compounds against HELA cells.

Table 1. The antiproliferative activity of metal complexes **6a**–**6k** and ligand **5a** virus vinblastin sulphate (in means of IC_{50} values).

Compound	HCT116 (Colon)	MCF7 (Breast)	HELA (Cervix)
5a	Negative	77.8 µg/mL	14.5 µg/mL
6a	5.03	5.23	3.56
6b	$IC_{50}=0.54 IC_{10}=9.6$	9.53	9.33
6c	0.94	0.54	6.88
6d	0.69	0.74	8.82
6e	3.95	4.62	1.03
6f	7.25	4.85	1.14
6g	4.52	4.68	1.71
6h	4.02	Negative	0.656
6i	8.77	0.896	12.9
6j	13	1.66	8.77
6k	14.4	18.5	14.6
Vinblastine sulphate	9.8	11.6	10.9

breast human cancer cell line the two complexes (**6b** and **6c**) with unsubstituted aromatic rings in their structure showed the most potent cytotoxicity effect and finally the two copper complexes (**6e** and **6h**) were the most potent complexes against the cervix human cancer cell line. In conclusion, the cytotoxicity of the metal complexes on the human cancer cells exhibited improved anticancer activity as compared with the hydrazino ligand (**5a**) on the selected human cancer cell lines. Compound (**5a**) showed no cytotoxicity against colon cell line, very weak cytotoxicity against breast and less cytotoxicity against cervix cell line comparable with the metal complexes (**6a**–**6k**).We can conclude that coordination reduces the polarity of the metal ion mainly because of partial sharing of its positive charge with donor group within the whole chelate ring system. The process of chelation thus increases the lipophilic nature of the central metal atom, which in turn is increasing the activity of the complexes. Besides many other factors such as solubility, dipole moment, conductivity influenced by metal ion may be reasons for remarkable activities of these complexes. Also, it was observed that some moieties such as azomethine linkage or heteroaromatic introduced into such compounds exhibit extensive biological activities that may be responsible for the increase in hydrophobic character and liposolubility of the molecules in crossing the cell membrane human tumour cell and enhance biological utilization ratio and activity of complexes.

Declaration of interest

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

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