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Synthesis and antitumor activity of indolylpyrimidines: Marine natural product meridianin D analogues

Mohamed A. A. Radwan[a,*] and Mahmoud El-Sherbiny[b]

[a]*Applied Organic Chemistry Department, National Research Centre, Dokki, Giza, Egypt*
[b]*Medicinal Chemistry Department, National Research Centre, Dokki, Giza, Egypt*

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Abstract—Marine indole alkaloid meridianin D analogues have been synthesized starting from the appropriate 3-cyanoacetyl indole. A facile two-step conversion of 3-cyanoacetyl indole to the corresponding cyano meridianin D analogue by treatment with dimethylformamide-dimethylacetal and further cyclization of the resulting enaminonitrile with aminoguanidine is described. Then, alkaline hydrolysis of cyano meridianin D afforded the carboxylic acid analogue. The treatment of acid with 75% H_2SO_4 afforded the desired 6-debromomeridianin D. Simply treatment of cyano meridianin D analogue with hydrazine hydrate afforded the amidrazone analogue. The biological evaluation indicated that cyano analogue showed good cytotoxic activity with IC_{50} values of 0.85 and 2.65 μg (against MCF7 and HeLa, respectively), but acid and amidrazone analogues showed high cytotoxicity with IC_{50} values of 0.75 and 0.25 μg, respectively (against MCF7).

1. Introduction

Marine organisms represent a very promising source of unique bioactive molecules and antitumor secondary metabolites. Various biologically active indole alkaloids have been produced from the marine invertebrates over the past few years.[1–4] Along with these, 3-substituted indoles represented a promising structural class of marine alkaloids based upon their high degree of biological activity. The substitution at 3-position of the indole ring connecting an extra heterocyclic ring: imidazole (topsentins[4,5] and nortopsentins[6]); dihydroimidazole (discodermindole[7]); oxazole (martefrgin,[8] amazol[9]); oxadiazine (alboinon[10]); maliemide (didemidines[11]); and piperazine (dragmacidon[3]) takes place. Recently, five novel indole alkaloids, meridianins A–E (Fig. 1), have been isolated from the *tunicate Splidium meridiaum*.[12] They show cytotoxicity toward murine tumor cell lines[13] and show potent inhibition against several protein kinases[14] and are therefore interesting synthetic targets. This forms the basis for the aim of the synthesis of the marine indole alkaloids.

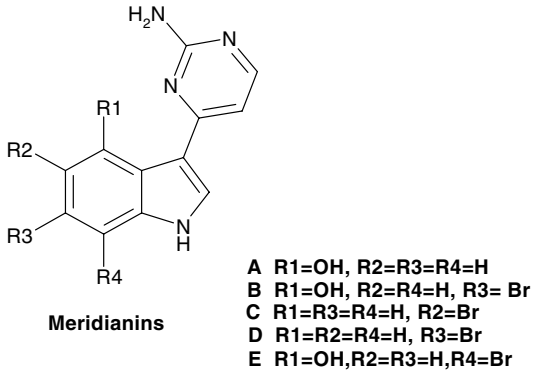

Figure 1. Structure of meridianins A–E.

2. Chemistry

The natural meridianins have previously been synthesized by two different strategies: either the construction of the 2-aminopyrimidine ring from a β-dicarbonylic substituted indole precursor,[15,16] or by a cross-coupling reaction between the indole and pyrimidine moieties.[17] This synthetic methodology has also been applied for the synthesis of natural Variolin B.[18] Recently, Stanovnik et al. reported the synthesis of both condensed indolylpyrimidones as meridianin analogues[19] and polycyclic meridianin analogues with uracil structural unit.[20]

Keywords: Alkaloids; Indole; Enaminonitrile; Heterocycles; Meridianines; Cyclizations; Antitumor.
* Corresponding author. Tel.: +20 2 7201588; fax: +20 2 3370931; e-mail: m1radwan@yahoo.com

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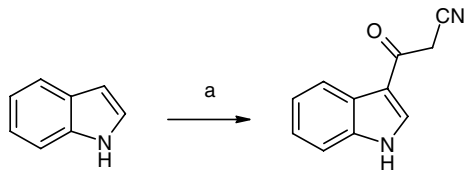


Scheme 1. Reagents and condition: (a) cyanoactic acid, Ac_2O, Δ.

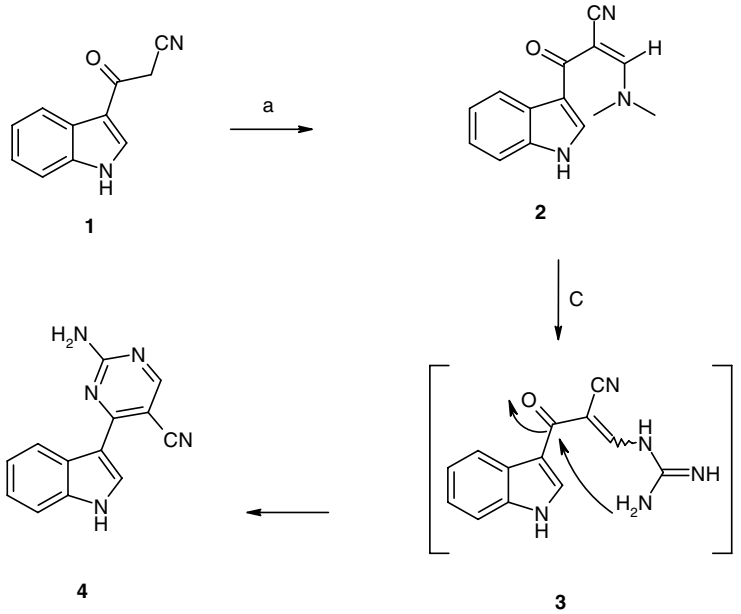
Here a facile synthesis of indolylpyrimidines was achieved via the cyanoacetyl side chain at the indole 3-position using C2 moiety for the construction of the 2-aminopyrimidine ring, through the cyclization of (2*Z*)-3-dimethylamino-2-(1*H*-indole-3-carbonyl)acrylonitrile **2** with aminoguanidine as *N*,*N*-1,3-dinucleophile.

The starting compound 3-cyanoacetyl indole **1** was previously prepared by Kreher and Wagner[21] and recently by Bergman et al.[22] via a new facile approach starting from indole and cyanoacetic acid (Scheme 1). A novel (2*Z*)-3-dimethylamino-2-(1*H*-indole-3-carbonyl)acrylonitrile **2** was prepared from 3-cyanoacetyl indole **1** by treatment with dimethylformamide-dimethylacetal (DMF-DMA) in dry toluene at 120 °C for 15 min, the enaminonitrile **2** was obtained in a good yield (84.5%). The structure of compound **2** was established on the basis of its elemental analysis and spectral data. For example, its ^{1}H NMR spectrum revealed a singlet signal at δ 8.30 characteristic of the olefinic proton and two singlet signal at δ 3.25 and 3.30 as a result of magnetically non-equivalent $N(CH_3)_2$ group.

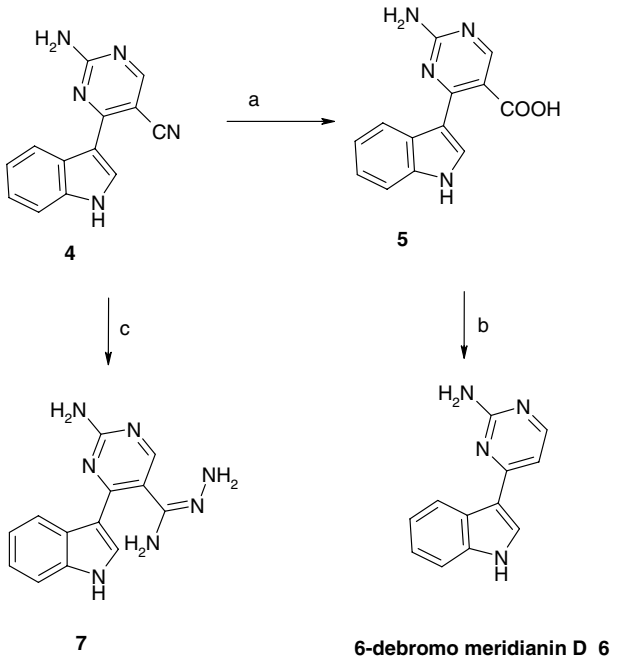
Pyrimido annelation took place by further cyclization of the enaminonitrile **2** with guanidine hydrochloride in absolute ethanol in the presence of anhydrous potassium carbonate under refluxing temperature for 12 h to give 2-amino-4-(1*H*-indolyl-3-)-pyrimidine-5-carbonitrile **4** in 71.4% yield through the nonisolable intermediate **3** (Scheme 2). This indolylpyrimidine derivative **4** can be considered as 5′-cyano-6-debromomeridianin D. The structure of compound **4** was established on the basis of its elemental analysis and spectral data. For example, its IR spectrum showed the presence of absorption peak at 2222 cm^{-1} due to a cyano group and its ^{1}H NMR spectrum revealed a singlet signal at δ 8.53 characteristic of the pyrimidine proton (H-6′), which was confirmed by ^{13}C NMR and HSQC experiments at δ 163.11 (see Section 5).

Alkaline hydrolysis of compound **4** with 20% NaOH solution in ethanol under refluxing temperature for 6 h afforded the carboxylic acid analogue **5**. The structure of compound **5** was established on the basis of its elemental analysis and spectral data. For example, its IR spectrum showed the presence of absorption peaks at 3125 and 1690 cm^{-1} due to the OH and carbonyl groups, respectively, and its ^{1}H NMR spectrum revealed a singlet signal at δ 8.25 characteristic of the pyrimidine proton (H-6′). Decarboxylation of acid **5** with 75% H_2SO_4 under refluxing temperature for 6 h afforded the previously prepared 6-debromomeridianin D **6**, but in a higher yield.[17] In addition, simply treatment of cyano meridianin D analogue with hydrazine hydrate in absolute ethanol under refluxing temperature for 3 h afforded the desired amidrazone analogue **7** (Scheme 3). The structure of compound **7** was established on the basis of its elemental analysis and spectral data. For example, its IR spectrum showed the presence of absorption peaks at 3425, 3345, 3285 cm^{-1} due to 3 NH_2 groups and its ^{1}H NMR spectrum revealed a singlet signal at δ 8.45 characteristic of the pyrimidine proton (H-6′).

The structures of the novel compounds were determined by spectroscopic methods (IR, ^{1}H and ^{13}C NMR, HSQC, NOE and mass spectrometry) and by elemental analyses for C, H, and N. Compound **2** was character-



Scheme 2. Reagents and conditions: (a) DMF-DMA, toluene, 120 °C, 15 min; (b) $H_2N(C{=}NH)NH_2{\cdot}HCl$, K_2CO_3, ethanol, reflux, 12 h.



Scheme 3. Reagents and conditions: (a) 20% NaOH, ethanol 220°C, 6 h; (b) 75% H_2SO_4, reflux, 6 h; (c) hydrazine hydrate, ethanol, reflux, 3 h.

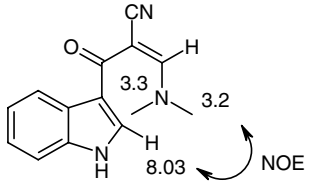


Figure 2. NOE experiment of (*Z*)-isomer **2**.

ized as pure (*Z*)-olefin, of which the configuration around the olefinic double bond was determined by NOE experiment (Fig. 2).

3. Conclusions

In summary, simple, cost-effective, and convenient method for the synthesis of four meridianin D analogues starting from easily prepared 3-cyanoacetyl indole was demonstrated. In this approach cyanoacetyl group at the heteroaromatic 3-position is used as a precursor to construct the appropriately substituted pyrimidines. In this method, we do not need to protect the *N*-indole compared to the previous methods.[16] Further application of (2*Z*)-3-dimethylamino-2-(1*H*-indole-3-carbonyl)acrylonitrile **2** in the synthesis of a variety of other meridianin analogues and 3-indole heterocyclic compounds is currently underway. The cytotoxic activity in vitro and structure–activity relationship (SAR) of the newly synthesized compounds were provided.

4. Biological evaluation

The synthesized meridianin D analogues were evaluated in National Cancer Institute (Egypt) for its cytotoxic activity in in vitro disease-oriented antitumor screening using sulforhodamine B (SRB) assay including 4 human tumor cell lines representing different cancer types.[24] And also tested for its cytotoxicity against Ehrlich Ascites Carcinoma.[23] The cyano meridianin D **4** analogue exhibited a good cytotoxic activity against breast carcinoma cell line (MCF7) and cervix cell line (HeLa) with the IC_{50} values of 0.85 and 2.65 μg, respectively, but carboxylic acid analogue **5** and amidrazone analogue **7** showed high cytotoxicity with IC_{50} values of 0.75 and 0.25 μg, respectively (against MCF7).

The natural product, meridianin D, showed weak antitumor activities against a variety of tumor cell lines, and its analogue 6-debromomeridianin D **6** did not show any antitumor activity at all.[13]

The tested compounds were proven to have no cytotoxic activities against liver carcinoma and brain tumor cell lines. And it showed no effect on the viability of the Ehrlish Ascites Carcinoma at concentrations 25, 50, and 100 μg/mL.

In summary, we have synthesized cyano **4**, carboxylic acid **5**, and amidrazone **7** meridianin D analogues that showed good cytotoxic effects in the growth of different human cell lines in vitro, especially, cyano analogue **4** against breast and cervix carcinoma cell lines. And carboxylic acid **5** and amidrazone **7** analogues showed high antitumor activity against breast carcinoma cell line. In comparison,[13] these meridianin D analogues were considered to be more potent than the natural meridianin D which was isolated from the *tunicate Splidium meridiaum*.[12]

5. Experimental

5.1. Chemistry

5.1.1. General methods. Melting points were determined on a Gallenkamp apparatus and are uncorrected. IR spectra were recorded on Shimadzu FT-IR 8101 PC infrared spectrophotometer. ^{1}H NMR spectra were recorded on a Jeol EX-270 MHz spectrometer using DMSO-d_6 as solvent and TMS as the internal standard, ^{13}C NMR and 2D NMR spectra were recorded on a varian Mercury VX 300 NMR using DMSO-d_6 as solvent and TMS as an internal standard. Mass spectra were recorded on a Finnigan mat. SSQ-7000 GC-MS spectrometer. Microanalyses were performed at the Microanalytical Center of Cairo University.

5.1.2. 3-(1*H*-Indol-3-yl)-3-oxo-propionitrile 1. Prepared according to the literature procedure;[20] colorless prisms; yield (91%); mp 240–241 °C. IR: 3214 (NH), 2251 (CN), 1633 (C=O), 1580 (C=C) cm^{-1}. ^{1}H NMR (270 MHz, DMSO-d_6): δ 4.48 (s, 2H), 7.22–7.24 (m, 2H, H-5, H-6), 7.49–7.51 (m, 1H, H-7), 8.13–8.15 (m, 1H, H-4), 8.37 (br s, 1H, H-2), 12.16 (br s, 1H, NH). ^{13}C NMR: δ 29.4 (t), 112.4 (d), 114.4 (s), 116.4 (s), 121.0 (d),

122.3 (d), 123.3 (d), 125.1 (s), 135.4 (d), 136.6 (s), 182.8 (s).

5.1.3. (2*Z*)-3-Dimethylamino-2-(1*H*-indole-3-carbonyl)acrylonitrile 2. To a solution of 3-(1*H*-indol-3-yl)-3-oxo-propionitrile **1** (1.18 g, 6.4 mmol) in dry toluene (10 mL) was added a solution of dimethylformamide-dimethylacetal (1.2 g, 10 mmol) in the same solvent (5 mL). The resultant solution was heated at 120 °C for 15 min. After cooling, the mixture was filtered to leave enaminonitrile **2** as a yellow solid (1.35 g, 84.5%). Recrystallization from EtOH gave analytically pure material; mp 160–163 °C (yellow prisms). ^{1}H NMR (270 MHz, DMSO-d_6) 3.20 (br s, 3H, NCH_3), 3.30 (br s, 3H, NCH_3), 7.15–7.19 (m, 2H, H-5, H-6), 7.45–7.48 (m, 1H, H-7), 8.03 (br s, 1H, H-2), 8.13–8.15 (m, 1H, H-4), 8.30 (br s, 1H, H-β), 11.80 (br s, 1H, NH). MS: *m*/*z* (%) (EI positive) 238, M^+-1, (57), 222 (53), 210 (30), 195 (24), 184 (69), 159 (43), 144 (100), 130 (29), 116 (61), 89 (65), 77 (38), 62 (55). Anal. Calcd for $C_{14}H_{13}N_3O$: C, 70.28; H, 5.48; N, 17.56; O, 6.69. Found: C, 70.18; H, 5.41; N, 1767; O, 6.74.

5.1.4. 5′-Cyano-6-debromomeridianin D 4. A mixture of enaminonitrile **2** (2.0 g, 8.36 mmol), guanidine hydrochloride (1.15 g, 12.0 mmol), anhydrous K_2CO_3 (2.0 g, 15.0 mmol), and absolute ethanol (20 mL) was heated at reflux temperature for 12 h. After cooling, the mixture was filtered off to leave 5′-cyano-6-debromomeridianin D **4** as a yellow solid (1.4 g, 71.4%). Recrystallization from EtOH gave analytically pure material; mp 258–259 °C (yellow prisms).

IR (KBr) ν_{max} 3408 (NH_2), 3329 (NH_2), 3174 (NH), 2222 (CN), 1658, 1569, 1494, 1436, 1335, 1241, 1129, 808, 741, 684 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6): δ 7.17–7.22 (m, 2H, H-5, H-6), 7.47–7.49 (dd, 1H, H-7), 7.55–7.65 (d, 2H, NH_2), 8.45 (s, 1H, H-2), 8.53 (s, 1H, H-6′), 8.70–8.75 (dd, 1H, H-4), 11.93 (br s, 1H, NH). ^{13}C NMR (300 MHz, DMSO-d_6): δ 89.31 (C-5′), 111.57 (C-7), 111.99 (C-3), 119.76 (CN), 121.04 (C-3a), 122.75 (C-6), 123.22 (C-5), 125.72 (C-4), 130.01 (C-2), 136.35 (C-7a), 163.11 (C-6′), 163.16 (C-4′), 163.27 (C-2′); EIMS *m*/*z* (%) 235 (M+, 100), 208 (11), 194 (37), 180 (14), 166 (15), 139 (18), 117 (19), 97 (9), 77 (10), 61 (12). Anal. Calcd for $C_{13}H_9N_5$: C, 66.37; H, 3.86; N, 29.77. Found: C, 66.18; H, 3.56; N, 29.83.

5.1.5. 2-Amino-4-(1*H*-indol-3-yl)-pyrimidine-5-carboxylic acid (5), carboxylic acid analogue. A mixture of compound **4** (0.3 g, 1.28 mmol), aqueous NaOH solution (5 mL, 20%), and ethanol (15 mL) was heated at refluxing temperature of 220 °C for 6 h. The reaction mixture was filtered off while hot and the filtrate left to cool and acidified with 6 N HCl. The solid precipitate was filtered, washed with water, and air-dried to form **5** as an orange solid (0.27 g, 83.1%). Recrystallization from ethanol gave analytically pure material; mp 157–159 °C.

IR (KBr) ν_{max} 3401 (NH_2), 3331(NH_2), 3125 (OH), 1690, 1570, 1494, 1446, 1256, 745, 688 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6): δ 7.15–7.20 (m, 2H, H-5, H-6), 7.42–7.45 (dd, 1H, H-7), 7.45–7.50 (d, 2H, NH_2), 8.25 (s, 1H, H-6′), 8.41 (s, 1H, H-2), 8.50–8.55 (dd, 1H, H-4), 11.91 (br s, 1H, NH), 11.97 (br s, 1H, OH). ^{13}C NMR (300 MHz, DMSO-d_6): δ 111.14 (C-7), 112.13 (C-3), 112.44 (C-5′), 120.85 (C-3a), 122.56 (C-6), 123.56 (C-5), 126.24 (C-4), 129.32 (C-2), 136.17 (C-7a), 161.23 (C-6′), 163.12 (C-4′), 163.34 (C-2′), 167.57 (COOH); EIMS *m*/*z* (%) 254 (M+, 100), 237 (91), 209 (54). Anal. Calcd for $C_{13}H_{10}N_4O_2$: C, 61.41; H, 3.96; N, 22.04, O, 12.59. Found: C, 61.12; H, 3.71; N, 21.89; O, 12.67.

5.1.6. 6-Debromomeridianin D (6). To a 75% aqueous solution of sulfuric acid (5 mL), compound **5** (0.25 g, 0.98 mmol) was added gradually and the mixture was heated at 90 °C for 2 h and at 120 °C for further 4 h. The obtained mixture was cooled, poured into 20% aqueous solution of sodium hydroxide (5 mL), and extracted with hexane–ethyl acetate. The extract was washed with water, concentrated, and purified by silica gel column chromatography (eluent, hexane/ethyl acetate, 4:6) to give 6-debromomeridianin D. Yield: 0.18 g (87%); mp 263–265 °C (lit. 262–264 °C).[17]

IR (KBr) ν_{max} 3409 (NH_2), 3329 (NH_2), 3172 (NH), 1659, 1569, 1454, 1416, 1241, 1129, 808, 741, 684 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6): δ 6.4 (br s, 2H, NH_2), 7.03 (d, 1H, H-5′), 7.15 (m, 2H, H-5, H-6), 7.44–7.46 (d, 1H, H-7), 8.11 (d, 1H, H-6′), 8.19 (s, 1H, H-2), 8.58–8.61 (d, 1H, H-4), 11.65 (br s, 1H, NH). ^{13}C NMR (300 MHz, DMSO-d_6): δ 105.2 (C-5′), 111.71 (C-7), 113.70 (C-3), 120.21 (C-3a), 121.85 (C-6), 122.32 (C-5), 125.30 (C-4), 128.10 (C-2), 136.90 (C-7a), 156.91 (C-6′), 162.62 (C-4′), 163.40 (C-2′); EIMS *m*/*z* (%) 210 (M+, 100), 209 (36), 169 (49), 155 (4), 140 (10), 114 (8). Anal. Calcd for $C_{12}H_{10}N_4$: C, 68.56; H, 4.79; N, 26.65. Found: C, 68.72; H, 4.76; N, 26.47.

5.1.7. 2-Amino-4-(1*H*-indol-3-yl)pyrimidine-5-carbohydrazonamide (7), amidrazone analogue. To a solution of compound **4** (0.2 g, 0.85 mmol) in 5 mL ethanol, hydrazine hydrate (99%) was added. The mixture was refluxed for 3 h and the solvent was removed under reduced pressure. The precipitate was crystallized from ethanol as a white solid. Yield: 0.18 g (79%); mp 267–269 °C; IR (KBr) ν_{max} 3389 (NH_2), 3296 (NH_2), 3172 (NH), 1619, 1559 (C=N groups) cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6): δ 4.85 (br s, 2H, NH_2), 5.65 (br s, 2H, NH_2), 7.22 (m, 2H, H-5, H-6), 7.43 (br s, 2H, NH_2), 7.47 (d, 1H, H-7), 8.31 (s, 1H, H-2), 8.45 (d, 1H, H-6′), 8.66 (d, 1H, H-4), 11.76 (br s, 1H, NH); EIMS *m*/*z* (%) 267 (M+, 100), 251 (76), 209 (34). Anal. Calcd for $C_{13}H_{13}N_7$: C, 58.42; H, 4.90; N, 36.68. Found: C, 58.14; H, 4.89; N, 36.43.

5.2. Materials and methods

5.2.1. Human tumor cell lines

a– MCF7 (Breast carcinoma cell line)
b– HeLa (Cervix carcinoma cell line)
c– U251 (Brain tumor cell line)
d– HEPG2 (Liver carcinoma cell line) (Fig. 3).

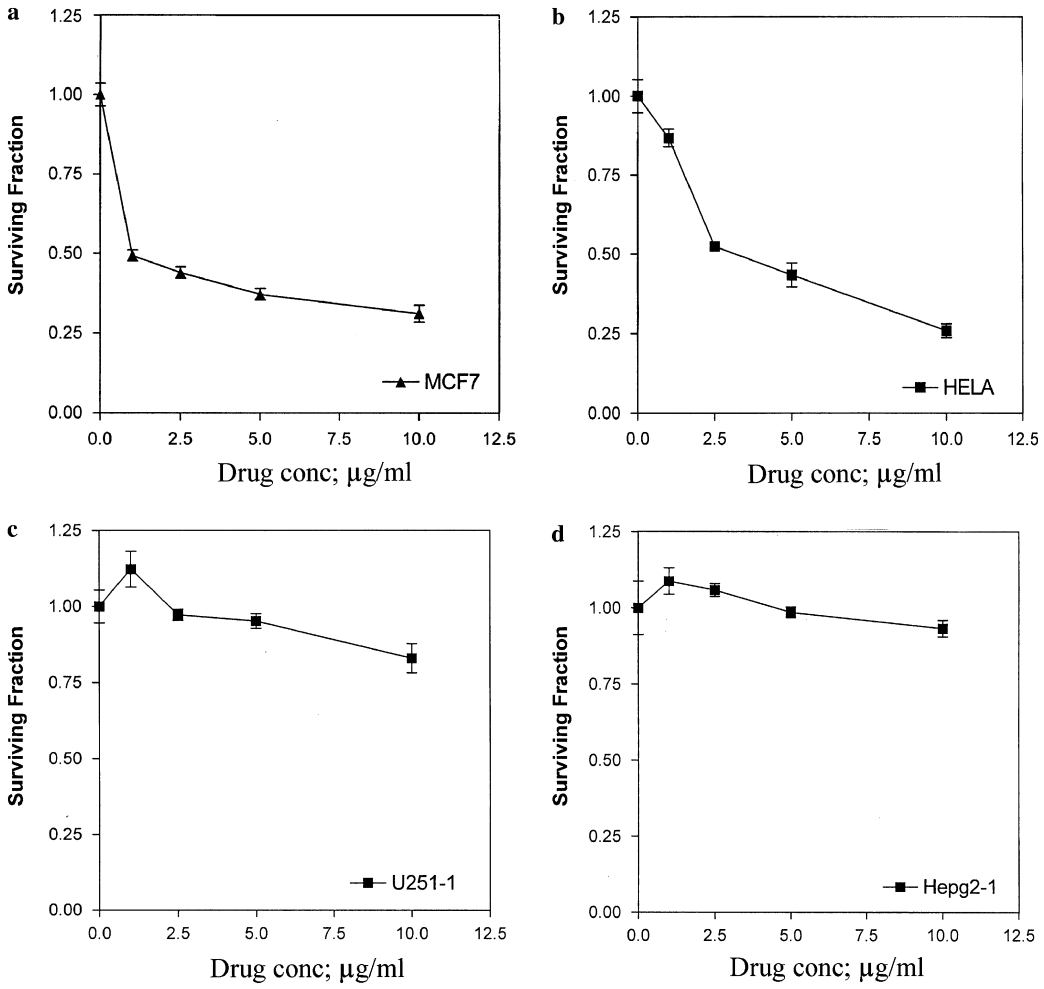


Figure 3. Cytotoxic activities of the compound **4** (at concentrations 1, 2.5, 5, and 10 µg/mL) against: (a) breast carcinoma cell line; (b) cervix carcinoma cell line; (c) brain tumor cell line; (d) liver carcinoma cell line.

5.2.2. Ehrlich ascites carcinoma (EAC). Maintained in the laboratory by weekly interperitoneal transplantation in female Swiss albino mice.

5.2.3. Animals. Female Swiss albino mice from the animal house of National Cancer Institute (Egypt), weighing 18–22 g, were used. Animals were maintained on standard pellet diet and water.

5.2.4. Antitumor activity of the Ehrlich Ascites Carcinoma (EAC). A set of sterile test tubes were used where 2.5×105 tumor cells per ml were suspended in phosphate-buffered saline. Then 25, 50, and 100 µg/mL from tested compound were added to the suspension, kept at 37 °C for 2 h. Trypan blue dye exclusion test was then carried out to calculate the percentage of nonviable cells.[23]

5.2.5. Measurement of potential cytotoxicity by SRB assay. Potential cytotoxicity of the compounds was tested using the method of Skehan et al.[24]

- Cells were plated in 96-multiwell plate (104 cells/well) for 24 h before treatment with the compounds to allow attachment of cell to the wall of the plate.
- Different concentrations of the compounds under test (0, 1, 2.5, 5, and 10 µg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose.
- Monolayer cells were incubated with the compound for 48 h at 37 °C and in atmosphere of 5% CO_2.
- After 48 h, cells were fixed, washed, and stained with sulforhodamine B stain.
- Excess stain was washed out with acetic acid and attached stain was recovered with EDTA buffer.
- Color intensity was measured in an ELISA reader.
- The relation between surviving fraction and tested compound concentrations is plotted to get the survival curve of each tumor cell line after the cytotoxicity of the specified compound and IC_{50} (dose of the tested compound which reduces survival to 50%) were evaluated.

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