Synthesis and evaluation of antitumour activities of novel fused tri- and tetracyclic uracil derivatives

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Simple one-pot syntheses of indenopyrrolopyrimidines and indolopyrrolopyrimidines were achieved *via* the cyclocondensation of 6-aminouracils and, respectively, ninhydrin and isatin in the presence of catalytic amounts of glacial acetic acid. Similarly, 5,6-diaminouracil derivatives were used as starting materials for the synthesis of indenopteridines and indolopteridines *via* their reaction with ninhydrin and isatin, respectively. The synthesised compounds were evaluated for antitumour activity against a human hepatocellular carcinoma cell line (HepG2), some showing antitumour activity comparable with 5-fluorouracil and imatinib.

Keywords: 6-aminouracils, 5,6-diaminouracil, indenopyrrolopyrimidines, indolopyrrolopyrimidines, indenopteridines, indolopteridines

In recent years, pyrimidine derivatives have gained a lot of attention due to their various biological activities including antifungal, antibacterial, antitumour, antitubercular and herbicidal properties.[1–3] Kidder and Dewey have noted that the biological activity of pyrimidine differs with different substitutions.[4] Pyrrolopyrimidines such as imatinib are significant anticancer drugs which act as protein kinase inhibitors.[5] On the other hand, many alkaloids[6] and drugs[7,8] contain isatin as a main active ingredient. Researches indicate that different derivatives of isatin have a number of bioactivities including antibacterial,[9] antifungal,[10] antiviral,[11] anti-HIV,[12] anti-mycobacterial, anticancer[13] anti-inflammatory[14] and anticonvulsant properties.[15] Indane derivatives also possess a wide array of biological activities including antimicrobial[16,17] and anti-inflammatory action,[18–20] and antagonistic inhibition.[21–23]

Our plan was to investigate the (1:1) reaction of 6-amino- and 5,6-diaminouracils with ninhydrin and isatin. Previous studies of similar reactions in which 2 equiv. of 6-aminouracils were reacted with 1 equiv. of isatin[24,25] or with 1 equiv. of ninhydrin[24] in refluxing EtOH (plus added *p*-toluenesulfonic acid)[25] yielded pentacyclic spiro-pyrimidoquinolines[25] or pentacyclic spiro-pyridopyrimidines.[24] In the event, our procedure produced different tetracyclic heterocycles.

Here we report the synthesis of several examples of indenopyrrolopyrimidines, indolopyrrolo-pyrimidines, indenopteridines and indolopteridines by the reaction of 6-amino- and 5,6-diaminouracils with ninhydrin and isatin. The new compounds were evaluated as a possible antitumour agent.

Results and discussion

The nitrosation of 6-aminouracils **1**[26–34] yielded 5-nitroso derivatives **2** which upon reduction with $(NH_4)_2S$ afforded 5,6-diaminouracils **3–e**.[30–34] 6-Aminouracils and 5,6-diaminouracils used as direct starting materials for the synthesis of our target compounds.

Reaction of ninhydrin with sulfur-, oxygen-, nitrogen- and carbon-based nucleophiles takes place very quickly at C-2 due to the strong activity of this carbon centre which is located between two 1,3-carbonyl groups, [30] whereas isatin, which has two adjacent 1,2-carbonyl groups situated at the C-2 and C-3 positions, reacts with nucleophiles at the more reactive C-3 position.[35,36] Reaction at these reactive centres with 6-aminouracils **1** and 5,6-diaminouracils **3** yielded indenopyrrolopyrimidines **4–6**, indolopyrrolopyrimidines **7–9**, indenopteridines **10–14** and indolopteridines **18–20**, respectively.

Dihydroxyindenopyrrolopyrimidine derivatives **4–6** were synthesised by refluxing ninhydrin and 6-aminouracils **1** in the presence of acetic acid for 10–15 min. On the other hand, dihydroxyindolopyrrolopyrimidines **7–9** were obtained by refluxing isatin and 6-aminouracils **1** in the presence of acetic acid for 2–2.5 h as shown in Scheme 1.

The formation of **4–6** and **7–9** (Scheme 2) occurred *via* the initial attack of the nucleophilic C-5 of the uracil moiety, *via* its enol form, at the C-2 position of ninhydrin or at the C-3 position of isatin to yield non-isolable tricyclic intermediates. The final tetracyclic products **4–6** and **7–9** were formed *via* intramolecular cyclisation of the tricyclic intermediates by the attack of the 6-amino group of uracil, respectively, at C-1 of the carbonyl group of ninhydrin and at C-2 of the carbonyl group of isatin.

The structures of dihydroxyindenopyrrolopyrimidines **4–6** and dihydroxyindolo-pyrrolpyrimidines **7–9** were assigned partly on the basis of the position of the hydroxyl groups, NH absorptions and the disappearance of NH_2 stretching band in the IR spectra. Further support was given by their ^{1}H NMR spectra, which showed signals characteristic of two OH groups in **4–6** at δ 6.89–6.56 ppm and δ 6.08–5.82 ppm and in **7–9** at δ 11.11–10.89 and 10.73–10.66 ppm. The phenyl group in **4–6** showed signals at δ 7.97–6.53 ppm and in **7–9** at δ 7.56–6.87. The disappearance of both NH_2 signals at δ 5.00–6.00 ppm at positions C-5 and C-6 further confirmed the formation of the new compounds. Compounds **7–9** showed signals at δ 11.11–10.89 and 10.73–10.66 ppm for the NH groups.

Refluxing ninhydrin with 5,6-diaminouracil **3** in the presence of acetic acid for 10–15 min led to the formation of indenopteridines **10–14** through the formation of the intermediate Schiff's base. This reaction was faster than that with isatin (reaction time 2–3 h). This can be explained by an increase in the positive charge on carbon atom at position 2 in ninhydrin due to elimination of H_2O in acidic medium which facilitates the nucleophilic attack of more basic amino groups at this position. On the other hand, refluxing isatin with 5,6-diaminouracils **3** in ethanol in the presence of TEA involves the formation of Schiff's bases **15–17**. On the basis of the resonating structures of diaminouracils **3**, the $-NH_2$ group at position C-5 is expected to be more basic, attacking at the carbon atom at position 3 of isatin moiety. Refluxing diaminouracils **3** with isatin in acetic acid afforded the indolopteridines **18** and **19** *via* the formation of Schiff's base intermediates followed by cyclodehydration (Scheme 4). Refluxing compound **15** in acetic acid for 2h afforded compound **20** in good yield as shown in Scheme 3.

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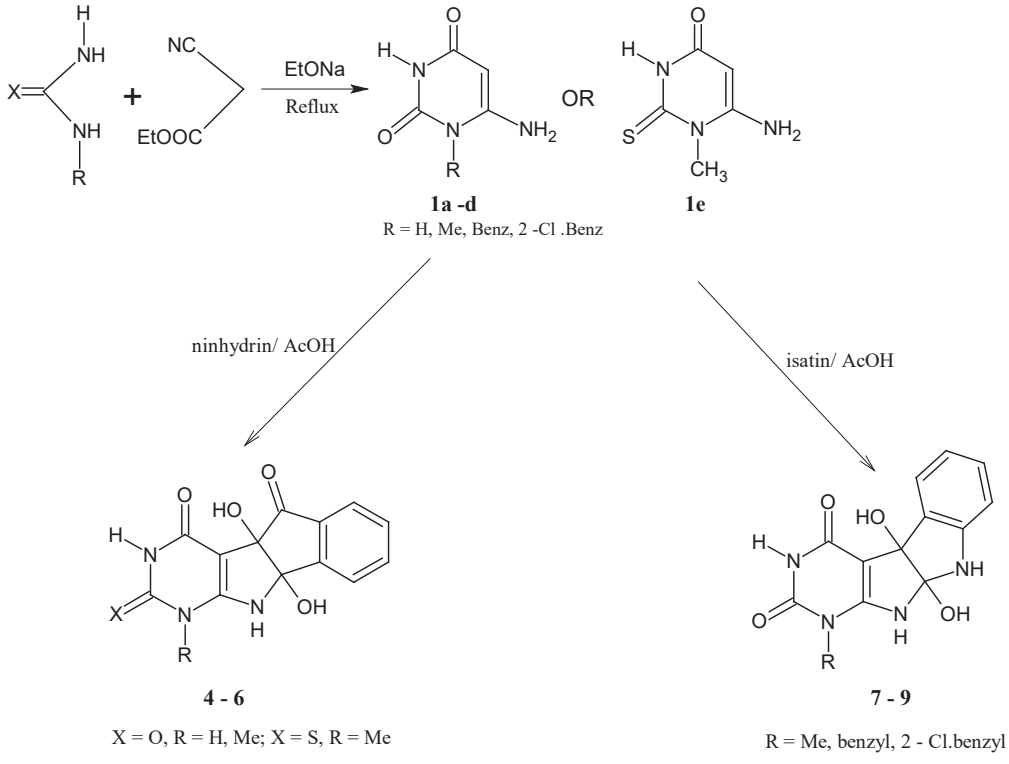


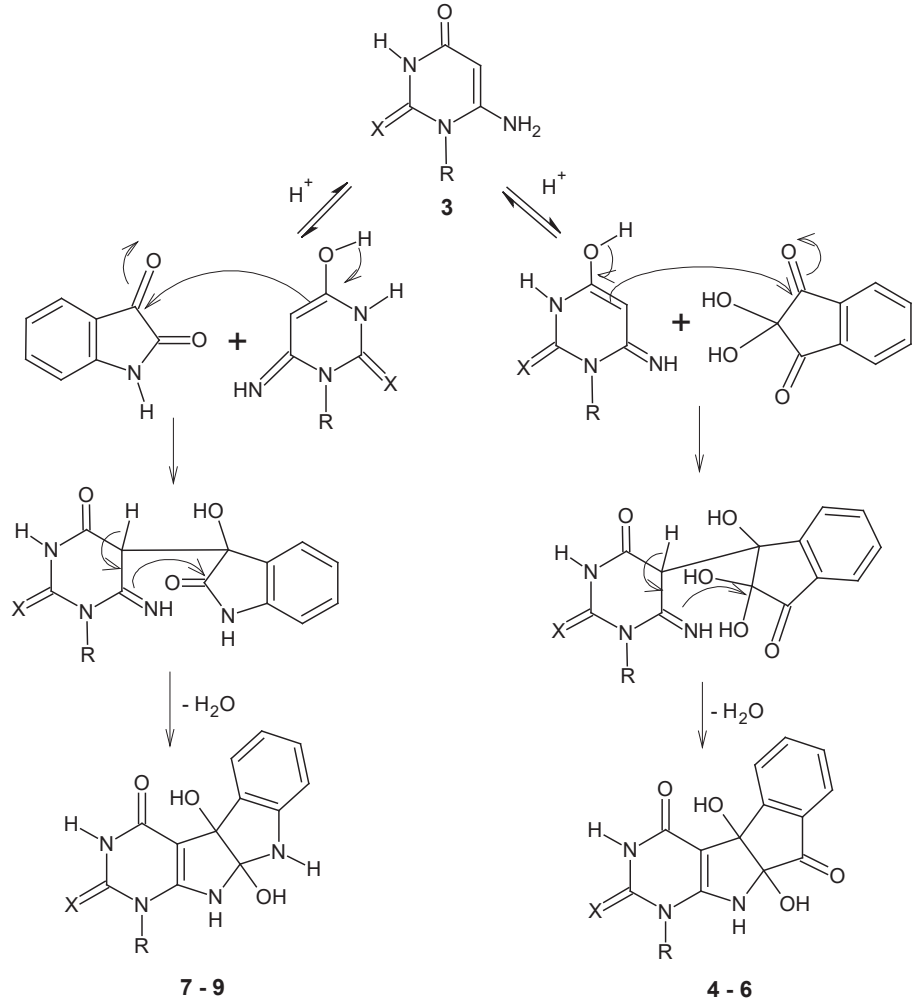
1a -d
R = H, Me, Benz, 2 -Cl .Benz

1e

4 - 6
X = O, R = H, Me; X = S, R = Me

7 - 9
R = Me, benzyl, 2 - Cl.benzyl

Scheme 1

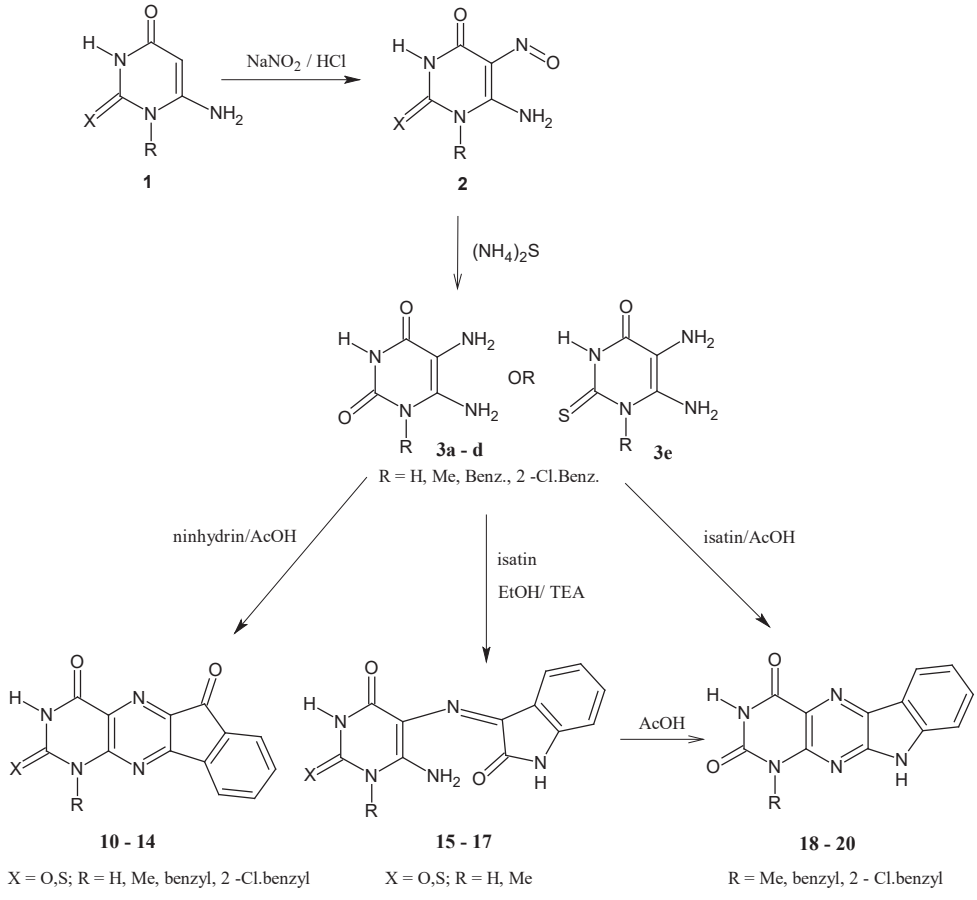


3

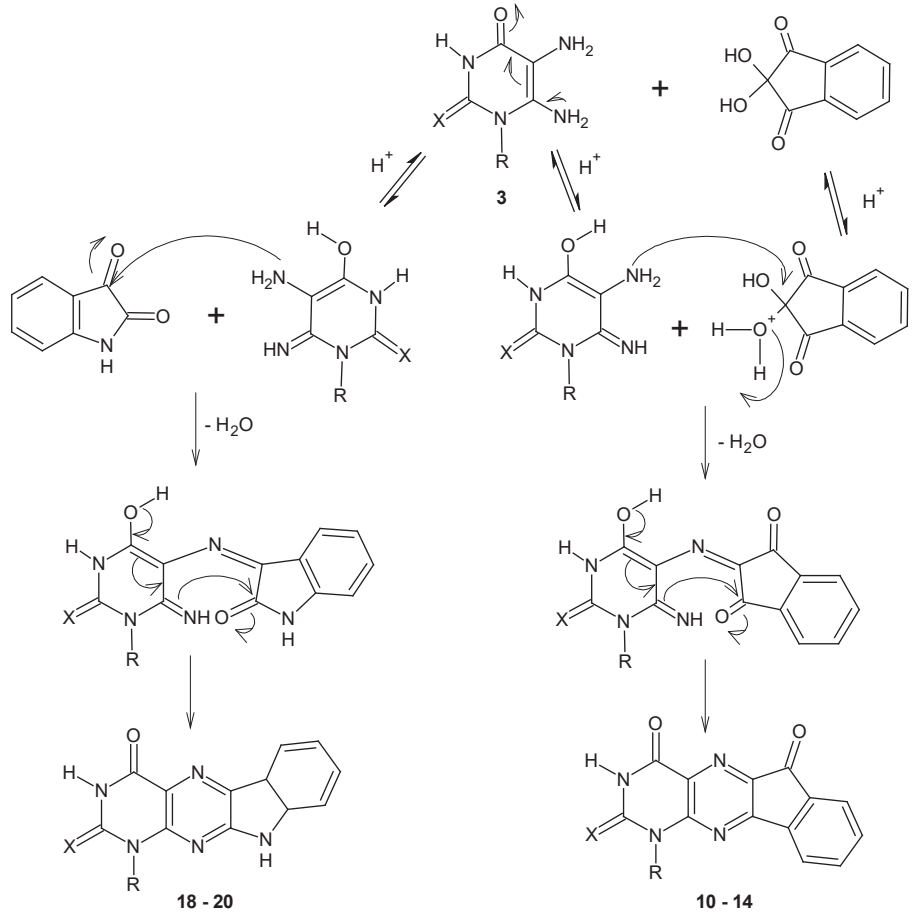
7 - 9

4 - 6

Scheme 2



Scheme 3



Scheme 4

Table 1 Growth inhibition (%) of human hepatocellular carcinoma cell line (HepG2) by decreasing concentration (0.8 to 0.0162 μM) of compounds **4–6, 9, 10, 13, 14, 17–19** (Schemes 1 and 3)

Compounds	Growth Inhibition /% Compound concentration / μM								*P* value
	0.062	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	
4	0	0	1.68	9.53	18.44	32.58	53.41	68.35	**< 0.001**[a]
5	0	0.86	2.13	8.52	14.07	22.85	39.18	57.3	**< 0.001**
6	3.62	12.85	20.58	32.79	45.16	61.84	75.03	84.57	**< 0.001**
9	6.26	11.84	20.48	29.57	40.35	56.18	68.05	79.64	**< 0. 001**
10	1.15	3.41	7.57	16.33	31.09	44.68	54.86	63.03	**< 0.001**
13	3.96	10.68	25.39	35.11	50.48	65.62	78.27	89.51	**< 0.001**
14	0	0	0	0	0.98	2.82	10.59	26.64	**< 0.001**[a]
17	0	1.28	5.82	13.54	21.88	34.97	62.59	71.81	**< 0.001**
18	0	0	1.21	3.92	10.25	28.46	43.83	59.18	**< 0.001**[a]
19	4.17	7.63	18.41	26.82	49.53	60.22	79.36	87.15	**< 0.001**

Significant value: $P \leq 0.01$, [a]Chi-square test conducted among inhibition rates of active concentrations only.

The structures of indenopteridines **10–14** were assigned on the basis of the position of NH and carbonyl absorptions and the disappearance of NH_2 stretching in the IR spectra. Further support was given by their ^{1}H NMR spectra, which showed signals at δ 8.23–7.14 ppm which are characteristic of the protons of a phenyl group and signals at δ 12.22–10.21 ppm which are characteristic of a NH group. The disappearance of both NH_2 signals at δ 5.00–6.00 ppm for positions C-5 and C-6 further confirmed the formation of the new compounds. The structures of 6-amino-5-indoloaminopyrimidines **15–17** were characterised by ^{1}H NMR spectra which showed the appearance of NH_2 signals at δ 6.19–5.54 ppm, NH signals at δ 12.37–10.20 ppm and signals for the phenyl group at δ 8.30–7.35 ppm. Indolopteridines **18–20** showed signals at δ 12.63–11.89 for the NH groups and signals at δ 8.23–7.12 ppm for the protons of the phenyl group. The mechanism of formation of **10–14** and **18–20** is shown in Scheme 4.

Biological investigation

Cytotoxic activity

Using as controls the well-known anticancer drugs 5-flourouracil (5-FU) and Imatinib (2-substituted aminopyrimidine derivative; Gleevec®), the *in vitro* growth inhibition (%) and inhibitory growth activity (as measured by IC_{50}) of the synthesised compounds were investigated, using crystal violet colourimetric viability assay. The results are shown in Tables 1 and 2 which reveal that all the tested compounds showed high variation in inhibitory growth and activities towards the tumour cell line in a concentration dependent manner.

Compound **6** had good inhibitory activities with an IC_{50} value of 1.29 μM and it is clear that compounds **4, 9** and **13** were very active at 8 μM against human hepatocellular carcinoma (HepG2) cell line with inhibition ratios between 70% and 90%. The highest activity against human hepatocellular carcinoma (HepG2) cell line was shown by compound **13** with an IC_{50} value 0.98 μM, comparable with the reference drug imatinib. However, compound **14** was almost inactive as shown in Table 2.

Compounds **4, 10** and **17** were also found to be active at 8 μM against human hepatocellular carcinoma (HepG2) cell line with inhibition ratios - 68.35, 63.03 and 71.81%, respectively. The highest detected inhibitory activities against human hepatocellular carcinoma (HepG2) cell line was shown by compound **13**, comparable to the reference drug imatinib, followed by **19**, **16** then **9** with IC_{50} values of 0.98, 1.04, 1.29 and 1.61, activities of all the compounds with different concentrations was statistically significant, $P < 0.001$.

Table 2 The antiproliferative activity against a human hepatocellular carcinoma cell line (HepG2) of ten of the synthesised compounds **4–6,9,10,13,14,17–19** (Schemes 1 and 3)

Compounds	IC_{50} / μM	Compounds	IC_{50} / μM
4	3.67 ± 0.15	**13**	0.98 ± 0.11
5	7.04 ± 0.22	**14**	>8
6	1.29 ± 0.07	**17**	3.09 ± 0.18
9	1.61 ± 0.13	**18**	5.61 ± 0.35
10	3.05 ± 0.19	**19**	1.04 ± 0.12
Imatinib	1.8 ± 0.13	**5-FU**	0.76 ± 0.14

Data analysis

The percentage cell viability was calculated using the equation: percentage of cell viability = [1 − (ODt/ODc)] × 100, where ODt is the mean optical density of wells treated with the tested compound and ODc is the mean optical density of untreated cells. Comparison of the test compounds was made using the IC_{50} value, *i.e.*, the concentration of an individual compound leading to 50% cell death that was estimated from graphical plots of surviving cells versus compound concentrations.

Conclusions

On the basis of our work, we can conclude that most of the indenopyrrolopyrimidines, indolopyrrolopyrimidines, indenopteridines and indolopteridines that we have synthesised showed good anticancer activities against a HepG2 cell line. Compounds **13** and **19**, with an IC_{50} of 0.98 and 1.04 μM, respectively, were found to be the most active.

Experimental

All melting points were determined with an Electrothermal Melting-Temperature II apparatus and were uncorrected. Element analyses were performed at the Regional Center for Mycology and Biotechnology at Al-Azhar University. The infrared (IR) spectra were recorded using potassium bromide disc technique on Nikolet IR 200 FTIR. Mass spectra were recorded on a DI-50 unit of Shimadzu GC/MS-QP 5050A at the Regional Center for Mycology and Biotechnology at Al-Azhar University. NMR spectra were obtained on a Bruker 400 MHz spectrometer (^{1}H NMR at 400 MHz, ^{13}C NMR at 100 MHz) in DMSO-d_6 using TMS as an internal standard at the Applied Nucleic Acid Research Center, Zagazig University, Egypt. All reactions were monitored by TLC using precoated plastic sheets silica gel (Merck 60 F_{254}). Spots were visualised by irradiation with UV light (254 nm). The solvent systems were either chloroform:methanol (9:1) or ethyl acetate:toluene (1:1).

6- Aminouracil, 6-amino-1-methyluracil and 6-amino-1-methyl-2-thiouracil (**1a**, **1b** and **1e**)[26–34]

Compounds **1a**, **1b** and **1e** were prepared according to the reported methods[26–34] by the addition of urea, methylurea and/or methyl thiourea (0.1 mol) to ethyl cyanoacetate (0.1 mol) in absolute ethanol (290 mL) containing sodium (0.2 mol). The mixture was refluxed for 10–12 h, allowed to cool to r.t. and acidified with acetic acid (pH 6). The resulting precipitate was washed with distilled water and dried in a desiccator overnight.

1a: Yield 69%; m.p. > 360 °C (lit. ≥ 360 °C).

1b: Yield 63%; m.p. 309–310 °C (lit. 300 °C).

1e: Yield 59%; m.p. 266 °C (lit. 260 °C).

6- Amino-1-benzyl- and 6-amino-1-[2-chlorobenzyl]uracil (**1c**, **1d**)[30–33]

The compounds **1c** and **1d** were prepared according to the reported methods.[30,32,33]

1c: Yield 72%; m.p. 285–286 °C (lit. 283 °C).

1d: Yield 66%; m.p. 295–296 °C (lit. 295 °C).

5,6- Diaminouracil, 5,6-diamino-1-substituted-uracils or -2-thiouracil (**3a–e**)[30,34]

Compounds **3a–e** were prepared by the same reported method.[33,34] The nitroso analogues **2a–e** of compounds **1a–e** [obtained by the addition of a mixture of acetic acid (3 mL) and sodium nitrite (4.60 mmol) in water (2.0 mL) to compound **1a–e** suspended in water (20 mL) with stirring at room temperature. The formed coloured nitroso analogue **2a–e** was filtered, washed with water and dried in a desiccator] (8.12 mmol) was added over 15 min to $(NH_4)_2S$ solution (40.0 mL) at 70–80 °C with stirring. The resulting precipitate was collected by filtration, washed with ether and dried in a vacuum desiccator to afford the diaminouracil analogues **3a–e**.

5,6-Diaminouracil **3a**: Yield 58%; m.p. 262 °C dec. (lit. > 260 °C dec.).

5,6-Diamino-1-methyluracil **3b**: Yield 73%; m.p. 258 °C dec. (lit. 257 °C dec.).

5,6-Diamino-1-benzyluracil **3c**: Yield 91%; m.p. 253 °C (lit. 250–252 °C).

5,6-Diamino-1-[2-chlorobenzyl]uracil **3d**: Yield 85%; m.p. 250 °C (lit. 245–248 °C).

5,6-Diamino-1-methyl-2-thiouracil **3e**: Yield 68%; m.p. 242 °C (lit. 240 °C).

*4b,9b-Dihydroxy-1-substituted-9b,10-tetrahydroindeno[2′,1′:4,5] pyrrolo[2,3-d]pyrimidine-2,4,5 (1H,3H,4bH)-triones (***4** *and* **5***) and 4b,9b-dihydroxy-1-methyl-9b,10-dihydroindeno[2′,1′:4,5]pyrrolo[2,3-d] pyrimidine-2(1H)-thio-4,5(1H,3H,4bH)-dione (***6***); general procedure*

A mixture of 6-amino-1-substituted-uracils (**1**) (1.2 mmol) and ninhydrin (1.2 mmol) in acetic acid (5 mL) was heated under reflux for 10–15 min. The resulting precipitate was filtered hot, washed with ethanol and crystallised from DMF/ethanol (1:3).

*4b,9b-Dihydroxy-9b,10-dihydroindeno[2′,1′:4,5]pyrrolo[2,3-*d*] pyrimidine-2,4,5(1*H*,3*H*,4b*H*)-trione* (**4**): Yield 73%; m.p. > 300 °C; IR (ν_{max}, cm^{-1}) KBr: 3445 3326 (br.OH), 3210 (NH), 3093 (CH arom.), 2900 (CH aliph.), 1702, 1599 (C=O), 1567 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.14 (s, 1H, NH), 10.05 (s, 1H, NH), 8.70 (s, 1H, NH), 7.97–7.91 (d, 1H, ArH), 7.81–7.77 (m, 1H, ArH), 7.68–7.66 (d, 1H, ArH), 7.57–7.53 (m, 1H, ArH), 6.56 (s, 1H, OH-9b), 5.82 (s, 1H, OH-4b); MS *m/z* (%): 287 ([M]$^+$, 20), 283 (27), 271 (28), 245 (24), 181 (59), 168 (38), 125 (100), 105 (47), 76 (60); Anal. calcd for $C_{13}H_9N_3O_5$: C, 54.36; H, 3.16; N, 14.63; found: C, 54.52; H, 3.21; N, 14.79%.

*4b,9b-Dihydroxy-1-methyl-9b,10-dihydroindeno[2′,1′:4,5] pyrrolo[2,3-*d*]pyrimidine-2,4,5(1*H*, 3*H*,4b*H*)-trione* (**5**): Yield 79%; m.p. > 300 °C; IR (ν_{max}, cm^{-1}) KBr: 3442, 3393 (br., OH), 3250, 3219 (NH), 3051 (CH Ar), 2990 (CH aliph.), 1700, 1654 (C=O), 1549 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.33 (s, 1H, NH), 9.24 (s, 1H, NH), 7.91–7.89 (d, 1H, ArH), 7.83–7.79 (m, 1H, ArH), 7.69–7.67 (d, 1H, ArH), 7.58–7.57 (m, 1H, ArH), 6.74 (s, 1H, OH), 5.89 (s, 1H, OH), 3.12 (s, 3H, CH_3); MS *m/z* (%): 301 ([M]$^+$, 6), 283 ([M – H_2O], 9), 244 (17), 168 (41), 104 (100), 76 (88); Anal. calcd for $C_{14}H_{11}N_3O_5$: C, 55.82; H, 3.68; N, 13.95; found: C, 55.97; H, 3.74; N, 14.04%.

*4b,9b-Dihydroxy-1-methyl-9b,10-dihydroindeno[2′,1′:4,5] pyrrolo[2,3-*d*]pyrimidine-2(1*H*)-thio-4,5(1*H*,3*H*,4b*H*)-dione* (**6**): Yield 71%; m.p. > 300 °C; IR (ν_{max}, cm^{-1}) KBr: 3400 (br., OH), 3198 (NH), 3052 (CH Ar), 2959 (CH aliph.), 1695, 1647 (C=O), 1537 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.84 (s, 1H, NH), 9.43 (s, 1H, NH), 7.93–7.91 (d, 1H, ArH), 7.85–7.81 (m, 1H, ArH), 7.71–7.69 (d, 1H, ArH), 7.60–7.56 (m, 1H, ArH), 6.89 (s, 1H, OH), 6.08 (s, 1H, OH), 3.53 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 35.6, 83.0, 88.5, 92.3, 122.8, 130.2, 134.3, 135.8, 149.4, 153.9, 155.8, 172.0, 176.6, 197.1; MS *m/z* (%): 317 ([M]$^+$, 31), 315 (6), 299 ([M – H_2O], 25), 256 (24), 240 (20), 224 (32), 192 (46), 184 (40), 160 (51), 128 (35), 104 (100), 76 (96); Anal. calcd for $C_{14}H_{11}N_3O_4S$: C, 52.99; H, 3.49; N, 13.24; found: C, 53.08; H, 3.53; N, 13.37%.

Dihydroxy-1-substituted-indolopyrrolopyrimidines (**7–9**)*; general procedure*

A mixture of 6-amino-1-substituted-uracils (**1**) (1.2 mmol) and isatin (1.2 mmol) in acetic acid (5 mL) was heated under reflux for 2–2.30 h. The formed hot precipitate was filtered, washed with ethanol and crystallised from DMF/ethanol (1:3).

*1-(2-Chlorobenzyl)-4b,9a-dihydroxy-4b,9,9a,10-tetrahydropyrimido[5′,4′:4,5]pyrrolo-[2,3-*b*] indolo-2,4(1*H*,3*H*)-dione* (**7**): Yield 66%; m.p. > 300 °C; IR (ν_{max}, cm^{-1}) KBr: 3406 (OH), 3177 (NH), 3055 (CH Ar), 2808 (CH aliph.), 1710, 1652 (C=O), 1526 (C=C), 754 (*o*-substituted phenyl); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.69 (s, 1H, NH), 11.20 (s, 1H, NH), 11.01 (s, 1H, OH), 10.66 (s, 1H, OH), 9.42 (s,1H, NH), 7.56–6.97 (m, 8H, ArH), 5.39–5.24 (dd, 2H, NCH_2); MS *m/z* (%): 400 ([M]$^{2+}$, 1), 398 ([M]$^+$, 2), 380 ([M – H_2O], 2), 342 (72), 271 (14), 181 (31), 125 (100), 102 (24), 76 (14); Anal. calcd for $C_{19}H_{15}ClN_4O_4$: C, 57.22: H, 3.79; N, 14.05; found: C, 57.43; H, 3.81; N, 14.13%.

*1-Benzyl-4b,9a-dihydroxy-4b,9,9a,10-tetrahydropyrimido[5′,4′:4,5] pyrrolo[2,3-*b*]indolo-2,4 (1*H*,3*H*)-dione* (**8**): Yield 68%; m.p. > 300°C; IR (ν_{max}, cm^{-1}) KBr: 3579, 3339 (OH), 3154 (NH), 3065 (CH Ar), 2860 (CH aliph.), 1720, 1654 (C=O), 1527 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.95 (s, 1H, NH), 11.69 (s, 1H, NH), 11.11 (s,1H, OH), 10.89 (s,1H, OH), 9.39 (s, 1H, NH), 7.40–6.87 (m, 9H, ArH), 5.42–5.27 (dd, 2H, NCH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 50.4, 83.5,98.3, 116.7, 121.0, 123.9, 126.5, 128.5, 135.5, 136.5, 146.7, 150.0, 153.0, 157.8, 160.8, 172.0, 181.5; MS *m/z* (%): 364 ([M]$^+$, 5), 348 (4), 302 (7), 276 (8), 263 (7), 208 (18), 119 (100), 106 (19), 92 (60); Anal. calcd for $C_{19}H_{16}N_4O_4$: C, 62.63; H, 4.43; N, 15.38; found: C, 62.80; H, 4.49; N, 15.47%.

*4b,9a-Dihydroxy-1-methyl-4b,9,9a,10-tetrahydropyrimido[5′,4′:4,5] pyrrolo-[2,3-*b*]indolo-2,4 (1*H*,3*H*)-dione* (**9**): Yield 59%; m.p. > 300°C; IR (ν_{max}, cm^{-1}) KBr: 3524, 3385 (OH), 3159 (NH), 3025 (CH Ar), 2939, 2846 (CH aliph.), 1746, 1688 (C=O), 1555 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.95 (s, 1H, NH), 11.48 (s, 1H, NH), 10.89 (s,1H, OH), 10.73 (s,1H, OH), 9.25 (s, 1H, NH),7.26–6.93 (m, 4H, ArH), 3.42 (s, 3H, CH_3); MS *m/z* (%): 288 ([M]$^+$, 8), 270 ([M – H_2O], 8), 249 (10), 188 (9), 119 (52), 92 (36), 61 (100); Anal. calcd for $C_{13}H_{12}N_4O_4$: C, 54.17; H, 4.20; N, 19.44; found: C, 54.25; H, 4.26; N, 19.58%.

1-Substituted-2H-indeno[2,1-g]pteridine-2,4,6-(1H,3H)-triones (**10–14**)*; general procedure*

A mixture of 5,6-diamino-1-substituted uracils (**3**) (1.00 mmol) and ninhydrin (1.00 mmol) in acetic acid (5 mL) was heated under reflux for 10–15 min. The resulting precipitate was filtered hot, washed with ethanol and crystallised from DMF/ethanol (1:3).

*2*H*-Indeno[2,1-*g*]pteridine-2,4,6-(1*H*,3*H*)-trione* (**10**): Yield 81%; m.p. > 300°C; IR (ν_{max}, cm^{-1}) KBr: 3193, 3260 (NH), 3089 (CH Ar), 2813 (CH aliph.), 1714, 1642 (C=O), 1562 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.35 (s, 1H, NH), 10.29 (s, 1H, NH), 7.87–7.81 (m, 3H, ArH), 7.74–7.70 (m, 1H, ArH); MS *m/z* (%): 266 ([M]$^+$, 1.10), 239 (27), 182 (8), 154 (13), 135 (100), 98 (59), 75 (25), 74 (52); Anal. calcd for $C_{13}H_6N_4O_3$: C, 58.65; H, 2.27; N, 21.05; found: C, 58.83; H, 2.24; N, 21.19%.

*1-Methyl-2*H*-indeno[2,1-*g*]pteridine-2,4,6-(1*H*,3*H*)-trione* (**11**): Yield 79%; m.p. > 300 °C; IR (ν_{max}, cm^{-1}) KBr: 3176 (NH), 3071 (CH Ar), 2827 (CH aliph.), 1684, 1652 (C=O), 1500 (C=C); ^{1}H NMR

(400 MHz, DMSO-d_6) δ 10.54 (s, 1H, NH), 7.99–7.97 (d, 1H, ArH), 7.86–7.82 (m, 2H, ArH), 7.74–7.71 (m, 1H, ArH), 3.59 (s, 3H, CH_3); MS *m/z* (%): 280 ([M]$^+$, 97), 209 (66), 182 (100), 154 (51), 130 (32), 102 (28), 76 (23); Anal. calcd for $C_{14}H_8N_4O_3$: C, 60.00; H, 2.88; N, 19.99; found: C, 60.14; H, 2.91; N, 20.04%.

*1-Methyl-2*H-*indeno[2,1-*g*]pteridine-2-thio-4,6-(1*H,*3*H*)-dione* (**12**): Yield 82%; m.p. > 300 °C; IR (ν_{max}, cm^{-1}) KBr: 3210 (NH), 3093 (CH Ar), 2900 (CH aliph.), 1702, 1599 (C=O), 1567 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.22 (s, 1H, NH), 8.03–7.73 (m, 4H, ArH), 3.68 (s, 3H, CH_3); MS *m/z* (%): 296 ([M]$^+$, 65), 280 (15), 236 (35), 209 (34), 182 (100), 154 (77), 126 (54), 102 (55), 75 (30); Anal. calcd for $C_{14}H_8N_4O_2S$: C, 56.75; H, 2.72; N, 18.91; found: C, 56.89; H, 2.70; N, 19.07%.

*1-Benzyl-1*H-*indeno[2,1-*g*]pteridine-2,4,6(3*H*)-trione* (**13**): Yield 77%; m.p. > 300°C; IR (ν_{max}, cm^{-1}) KBr: 3168 (NH), 3024 (CH Ar), 2843 (CH aliph.), 1692, 1601 (C=O), 1563 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.21 (s, 1H, NH), 7.95–7.93 (d, 1H, ArH), 7.84–7.80 (m, 2H, ArH), 7.73–7.69 (m, 1H, ArH), 7.50–7.48 (d, 2H, ArH), 7.34–7.25 (m, 3H, ArH), 5.44 (s, 2H, NCH_2); MS *m/z* (%): 356 ([M]$^+$, 18), 284 (11), 232 (13), 230 (17), 132 (10), 91 (100); Anal. calcd for $C_{20}H_{12}N_4O_3$: C, 67.41; H, 3.39; N, 15.72; found: C, 67.59; H, 3.45; N, 15.81%.

*1-(2-Chlorophenyl)methyl-2*H-*indeno[2,1-*g*]pteridine-2,4,6-(1*H,*3*H*)-trione* (**14**): Yield 83%; m.p. > 300 °C; IR (ν_{max}, cm^{-1}) KBr: 3303, 3175 (NH), 3059 (CH Ar), 2842 (CH aliph.), 1695, 1659 (C=O), 1557 (C=C), 751 (*o*-substituted phenyl); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.52 (s, 1H, NH), 7.82–7.69 (m, 2H, ArH), 7.53–7.50 (m, 1H, ArH), 7.34–7.22 (m, 5H, ArH), 5.49 (s, 2H, NCH_2); MS *m/z* (%): 392 ([M]$^{2+}$, 1.63), 390 ([M]$^+$, 0.62), 355 (100), 318 (9), 284 (11), 154 (11), 125 (94), 102 (20), 76 (21); Anal. calcd for $C_{20}H_{11}ClN_4O_3$: C, 61.47; H, 2.84; N, 14.34; found: C, 61.61; H, 2.83; N, 14.51%.

6-Amino-1-substituted-5-{[(3Z)-2-oxo-1,2-dihydro-3H-indol-3-ylidene]amino}pyrimidine-2,4(1H,3H)-diones (**15–17**)*; general procedure*

A mixture of 5,6-diamino-1-substituted-uracils (**3**) (1.00 mmol) and isatin (1.00 mmol) in ethanol (20 mL) in the presence of TEA as a base was heated under reflux for 4–5 h. The resulting precipitate was filtered hot, washed with ethanol and crystallised from DMF/ethanol (1:3) to afford the desired compounds **15–17**.

*6-Amino-1-methyl-5-{[(3Z)-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]amino}pyrimidine-2,4(1*H*, 3*H*)-dione* (**15**): Yield 85%; m.p. > 300 °C; IR (ν_{max}, cm^{-1}) KBr: 3349, 3309, 3260, 3146 (NH_2 and NH), 3054 (CH Ar), 2846 (CH aliph.), 1693, 1643 (C=O), 1575 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.78 (s, 1H, NH), 10.54 (s, 1H, NH), 8.22–8.20 (d, 1H, ArH), 7.63–7.56 (m, 2H, ArH), 7.42–7.38 (m, 1H, ArH), 6.11 (s, 2H, NH_2), 3.56 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.7, 96.1, 120.2, 128.1, 136.5, 143.2, 146.0, 149.3, 151.2, 158.0, 159.4, 160.8, 171.4; MS *m/z* (%) 285 ([M]$^+$,1.13), 156 (100), 139 (46), 98 (15), 84 (21), 57 (51); Anal. calcd for $C_{13}H_{11}N_5O_3$: C, 54.74; H, 3.89; N, 24.55; found: C, 54.91; H, 3.94; N, 24.67%.

*6-Amino-5-{[(3Z)-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]amino}pyrimidine-2,4(1*H,*3*H*)-dione* (**16**): Yield: 88%; m.p > 300 °C; IR (ν_{max}, cm^{-1}) KBr: 3459, 3407, 3360, 3194 (NH_2 and NH), 3093 (CH Ar), 2842 (CH aliph.), 1704, 1647 (C=O), 1564 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.37 (s, 1H, NH), 10.90 (s, 1H, NH), 10.26 (s, 1H, NH), 8.22–8.20 (d, 1H, ArH), 7.69–7.35 (m, 3H, ArH), 5.54 (s, 2H, NH_2); MS *m/z* (%): 271 ([M]$^+$, 2.77) 266 (20), 142 (100), 97 (67), 71 (32), 43 (50); Anal. calcd for $C_{12}H_9N_5O_3$: C, 53.14; H, 3.34; N, 25.82; found: C, 53.42; H, 3.23; N, 26.07%.

*(3Z)-3-[(6-Amino-1-methyl-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)imino]-1,3-dihydro-2*H*-indol-2-one* (**17**): Yield 87%; m.p. > 300 °C; IR (ν_{max}, cm^{-1}) KBr: 3346, 3307, 3257, 3141 (NH_2 & NH), 3026 (CH Ar), 2846 (CH aliph.), 1685, 1634 (C=O), 1572 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.89 (s, 1H, NH), 10.20 (s, 1H, NH), 8.30–8.28 (d, 1H, ArH), 7.78–7.37 (m, 3H, ArH), 6.19 (s, 2H, NH_2), 3.73 (s, 3H, CH_3); MS *m/z* (%): 301 ([M]$^+$, 2.24), 239 (9), 185 (16), 147 (67), 132 (16), 129 (29), 119 (100), 118 (23), 112 (18), 104 (33), 98 (36), 97 (26), 92 (82), 84 (26), 76 (44), 57 (16), 55 (28); Anal. calcd for $C_{13}H_{11}N_5O_2S$: C, 51.82; H, 3.68; N, 23.24; found: C, 51.98; H, 3.71; N, 23.43%.

1-Substituted-indolopteridines (**18–20**)*; general procedure*

Method A: A mixture of 5,6-diamino-1-substituted-uracils (**3**) (1.00 mmol) and isatin (1.00 mmol) in acetic acid (5.0 mL) was heated under reflux for 2–3 h. The resulting precipitate was filtered when hot, washed with ethanol and crystallised from DMF/ethanol (1:3) to give compounds **18** and **19**.

Method B: Compound **15** (1.00 mmol) in acetic acid (5 mL) was heated under reflux for 2 h. The resulting precipitate was filtered when hot, washed with ethanol and crystallised from DMF/ethanol (1:3) to give compound **20**.

*1-(2-Chlorobenzyl-1,10-dihydro-2*H*-indolo[3,2-*g*]pteridine-2,3(3*H*)-dione* (**18**): Yield 68% (Method A); m.p. > 300 °C; IR (ν_{max}, cm^{-1}) KBr: 3250, 3183 (NH), 3050 (CH Ar), 2814 (CH aliph.), 1705, 1638 (C=O), 1562 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.16 (s, 1H, NH), 10.79 (s,1H, NH), 8.23–8.21 (d, 1H, ArH), 7.60–7.14 (m, 7H, ArH), 5.41 (s, 2H, NCH_2); MS *m/z* (%): 379 ([M]$^{2+}$, 12), 377 ([M]$^+$, 17), 360 (25), 287 (19), 166 (23), 104 (21), 63 (27), 51 (100); Anal. calcd for $C_{19}H_{12}ClN_5O_2$: C, 60.41; H, 3.20; N, 18.54; found: C, 60.53; H, 3.18; N, 18.71%.

*1-Benzyl-1,10-dihydro-2*H*-indolo[3,2-*g*]pteridine-2,3(3*H*)-dione* (**19**): Yield 65% (Method A); m.p. > 300 °C; IR (ν_{max}, cm^{-1}) KBr: 3270, 3136 (NH), 3029 (CH Ar), 2920 (CH aliph.), 1712, 1642 (C=O), 1533 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.63 (s, 1H, NH), 11.89 (s, 1H, NH), 8.21–8.20 (d, 1H, ArH), 7.63–7.59 (m, 1H, ArH), 7.54–7.52 (d, 1H, ArH), 7.40–7.21 (m, 6H, ArH), 5.43 (s, 2H, NCH_2); MS *m/z* (%): 343 ([M]$^+$, 8), 271 (10), 181 (15), 125 (12), 102 (12), 91 (100); Anal. calcd for $C_{19}H_{13}N_5O_2$: C, 66.47; H, 3.82; N, 20.40; found: C, 66.63; H, 3.79; N, 20.62%.

*1,10-Dihydro-1-methyl-2*H*-indolo[3,2-*g*]pteridine-2,3(3*H*)-dione* (**20**): Yield 82% (Method B); m.p. > 300 °C; IR (ν_{max}, cm^{-1}) KBr: 3292, 3140 (NH), 3098 (CH Ar), 2942 (CH aliph.), 1694, 1623 (C=O), 1570 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.89 (s, 1H, NH), 11.54 (s, 1H, NH), 8.18–7.35 (m, 4H, ArH), 3.55 (s, 3H, CH_3); MS *m/z* (%): 267 ([M]$^+$, 99), 196 (100), 181 (24), 156 (73), 102 (11), 58 (47); Anal. calcd for $C_{13}H_9N_5O_2$: C, 58.43; H, 3.39; N, 26.21; found: C, 58.70; H, 3.43; N, 26.35%.

Biological investigation

Evaluation of the antitumour activity

Mammalian cell lines: The cell line used in this study was human hepatocellular carcinoma cell line (HepG2 cells) obtained from the tissue culture Unit, VACSERA, Cairo, Egypt.

The mammalian cells were propagated in Dulbecco's modified Eagle's[37] medium (DMEM) or RPMI-1640 depending on the type of cell line, supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50μg/mL gentamycin. All cells were maintained at 37 ºC in a humidified atmosphere with 5% CO_2 and were subcultured twice a week**.**

Antitumour activity evaluation using viability assay

Antitumour activity assay was carried out according to the method described previously.[38] All the experiments concerning the cytotoxicity evaluation were performed and analysed by tissue culture unit at the Regional Centre for Mycology and Biotechnology RCMB, Al-Azhar University, Cairo, Egypt.

The tumor cell lines were seeded in a sterile 96-well plate in 100μl of growth medium at a cell concentration of 1×10^4 cells per well. After 24 h of seeding, the monolayers were then washed with sterile phosphate buffered saline (0.01 M, pH 7.2) and simultaneously the cells were treated with 100 μl from different dilutions of the test sample in fresh maintenance medium and incubated at 37 °C. Different two-fold dilutions of the tested compound (100, 50, 25, 12.5, 6.25, 3.125, 1.56 and 0.78 μg mL^{-1}) were added to confluent cell monolayers

dispensed into a 96-well, flat-bottomed microtitre plates (Falcon, NJ, USA) using a multichannel pipette. The microtitre plates were incubated at 37 ºC in a humidified incubator with 5% CO_2 for a period of 24 h. Untreated cells were served as controls. Three independent experiments were performed each containing six replicates for each concentration of the tested samples. The cytotoxic effects of the tested compounds were then measured using crystal violet staining viability assay. Briefly, after 24 h of treatment, the medium was removed, 100 µL of 0.5% of crystal violet in 50% methanol was added to each well and incubated for 20 minutes at room temperature and subsequently excess dye was washed out gently by distilled water. The plate was allowed to dry, then the viable crystal violet-stained cells were lysed using 33% glacial acetic acid solution. Absorbance at 570 nm was then measured in each well using a microplate reader (SunRise, TECAN, Inc, USA). 5-Fluorouracil was used as a positive control. The absorbance is proportional to the number of surviving cells in the culture plate. Thus, using this colorimetric procedure, the tested compounds-mediated cell lysis and the cytotoxic effect of 5-FU (used as a positive control) were measured and compared to the viability of untreated cells.

Because the stock solutions to prepare the different concentrations of the tested compounds were dissolved in DMSO, controls with DMSO alone were performed in parallel for each concentration.

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