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ARTICLE

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Synthesis of Pyrano[2,3-*d*]pyrimidine-2,4-diones and Pyridino[2,3-*d*]pyrimidine-2,4,6,8-tetraones: Evaluation Antitumor Activity

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Abstract

A series of some novel pyrano[2,3-*d*]pyrimidine **4–10** with 75–95% yields and pyridino[2,3-*d*]pyrimidine derivatives **11–16** with 75–92% yields is reported. The building structures have been achieved by reaction of 5-arylidene barbituric acid **2** and active nucleophile carbon or nitrogen as examples barbituric acid and acetyl acetone or refluxing of bispyrimidine-2,4,6-trione derivative **3** with cyclizing reagents such as phosphorous pentoxide, hydrazine hydrate and aminothiazole. The newly synthesized structures have been elucidated on the basis of their spectral analysis. Some selected members were screened for antitumor activity. Among the screened compounds **9**, **16**, **7**, **10** and **8** exhibited high antitumor activity.

1 | INTRODUCTION

Barbituric acid is a pyrimidine heterocyclic ring with an active methylene group that can be involved in condensation reactions with aldehydes, ketones and δ, β-unsaturated carbonyl compounds forming other heterocycles compounds with an outstanding biological activity. Barbituric acid and its derivatives have been attracting considerable attention due to their wide range of pharmacological activities such as *in-vitro* antitubercular and *in-vivo* anticonvulsant,[1–5] antibacterial, antiurease and antioxidant properties.[6,7] On the other hand, the barbiturates possess several pharmacological applications, like anticancer,[8] antituberculosis,[9] antiviral,[10] sedative, anxiolytic and urease inhibition[11] and anti-proliferative activities.[12] Also, 5-alkyl- and 5,5-dialkylbarbituric acids are widely used as medical agents for the treatment of neurological diseases, depressions, and memory impairment.[13] Pyrimido[4,5-*d*]pyrimidines barbituric acid derivatives are generally used as antitumors.[14–17] Barbitone (**I**) is a barbituric acid derivative acts as a popular hypnotic while phenobarbitone (**II**) that is marketed under the trade name of luminal is still a popular anticonvulsant both in the oral and injectable forms.[18] hexobarbitone is the first rapidly acting intravenous anaesthetic agent (**III**) (Figure 1).[18]

Recently, some new procedures for preparation and reactions of barbituric acid derivatives are presented. As examples, synthesis and dynamics studies of barbituric acid derivatives as urease inhibitors are reported.[19] The synthesis, tautomerism and antibacterial activity with SAR study of novel barbiturates is presented.[6] Novel Y-shaped barbituric acid derivatives including molecular docking have been designed.[20] A reproducible synthesis of several new disubstitutedarylazo-barbituric and thiobarbituric acids is described.[21] A review about enantioselective catalytic transformations of barbituric acid derivatives is achieved.[22] As continuing our project towards chemistry of pyrimidines, a series of condensed heterocycles was built utilizing barbituric acid as starting materials.[23] In view of these interesting studies it has been planned to synthesize more heterocycles involving barbituric acid skeleton with evaluation their anticancer activities.

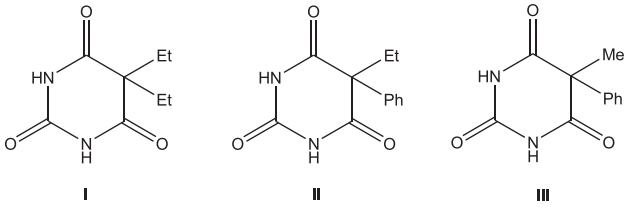


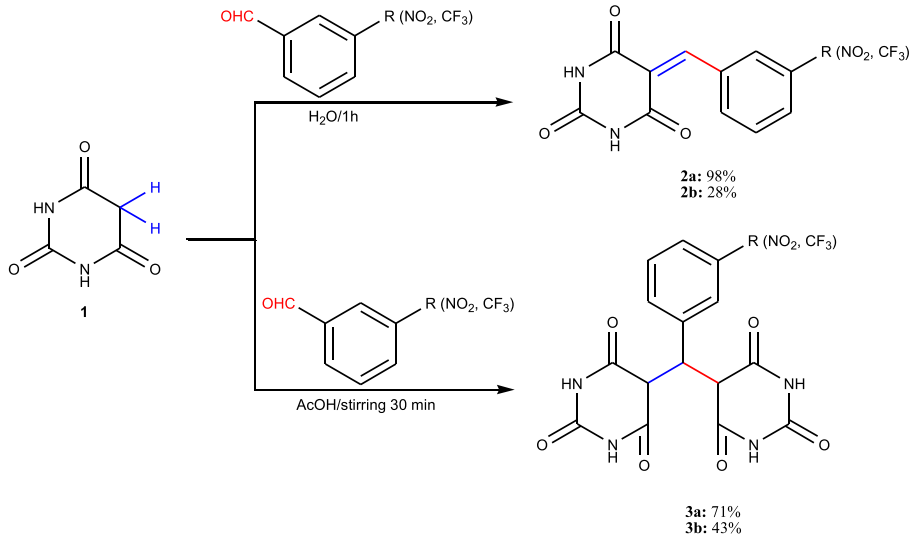
FIGURE 1 Structure of barbitone (**I**), phenobarbitone (**II**) and hexobarbitone (**III**)

2 | RESULTS AND DISCUSSION

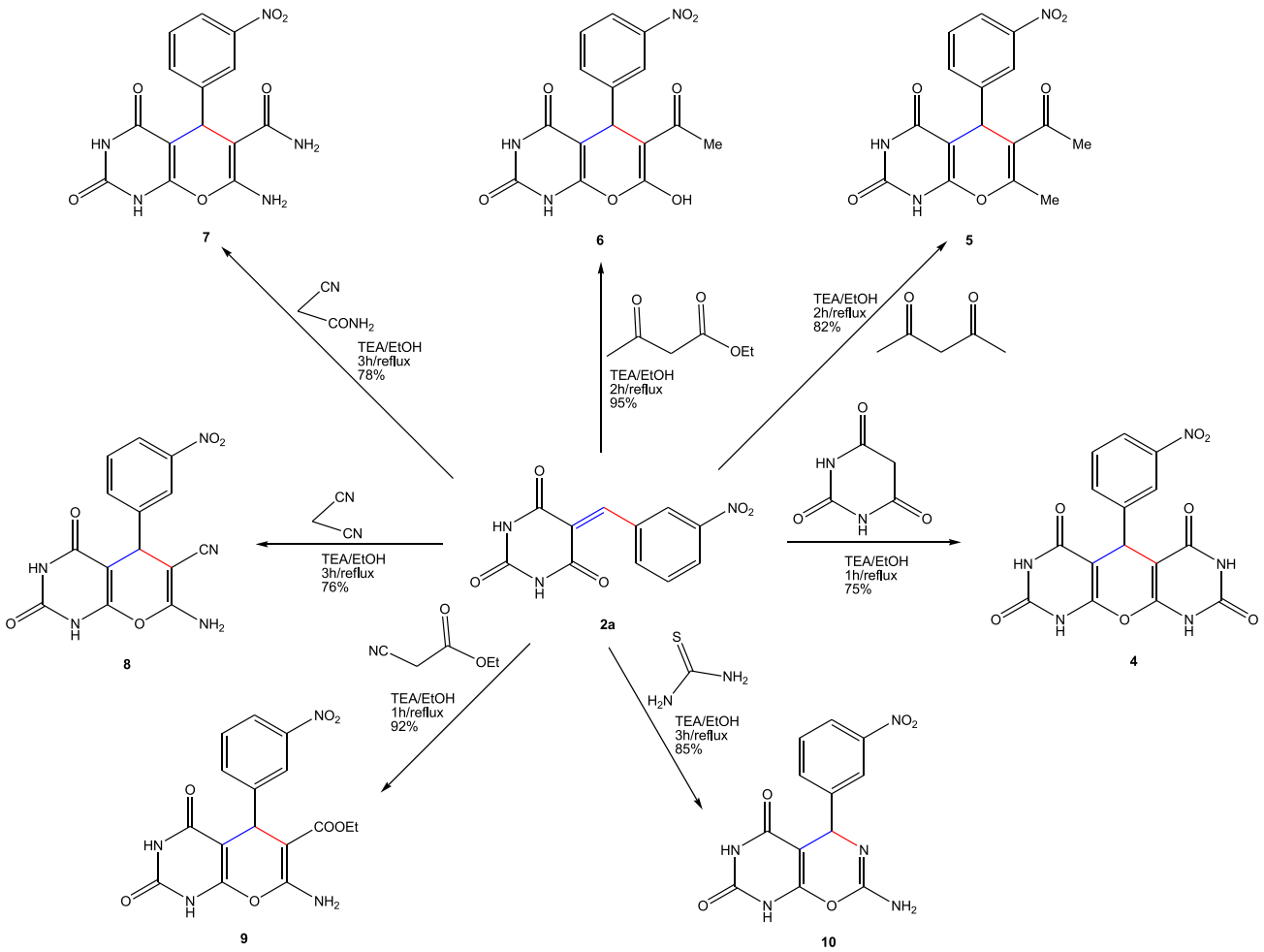
In this publication,we report on synthesis of several derivatives of pyrano[2,3-*d*]pyrimidine derivatives, pyridino[2,3-*d*]pyrimidines and studying their anticancer activity. The starting materials 5-arylidene barbituric acid **2a,b** and bispyrimidine-2,4,6-trione derivative **3a,b** were synthesized via 3- substituted benzaldhyde as a substrate. Knoevenagel type condensation reaction of barbituric acid (**1**) with 3-nitrobenzaldhyde or 3-trifluoro-methylbenzaldhyde in aqueous medium without catalyst and at room temperature took place according to the method described in the literature[24–26] (Scheme 1). The condensation occurred easily and completed through just an hour, affording compound **2a** and **2b** in 98% and 28% yields, respectively. On the other hand, we have found that condensation of two equiv. of barbituric acid (**1**) with 3-nitrobenzaldhyde or 3-trifluoromethylbenzaldhyde in acetic acid under stirring for 30 min gave **3a** with high yield (71%) and **3b** with low yield (43%)[27] (Scheme 1).

From the obtained results, we decided to deal with the nitro derivative **2a** not only owing to its high yield, but also this is in agree with the published data.[28,29] Here we present the behaviour of activated exocyclic double bond of 5-aylidene barbituric acid **2a** towards the carbon and nitrogen nucleophiles. Upon heating compound **2a** with active methylene compounds such as cyanoacetamide, malononitrile and ethylcyanoacetate in ethanol and few drops of trimethylamine for 1–3 h, pyrano[2,3-*d*]pyrimidine derivatives **4**–**10** are formed with yields in the range in between 75 and 95% (Scheme 2). The symmetrical structure of pyrano[2,3-*d*] pyrimidine-2,4,6,8-tetraone **4** was established through its spectral data. Its IR spectrum exhibited absorption bands at 3204, 3182 cm^{-1} for 2NH and 1716, 1680 cm^{-1} for 2C=O groups. The ^{1}H NMR spectrum of **4** displayed a singlet signal at δ = 4.41 ppm for CH pyran and two broad singlets at δ = 11.14, 11.22 ppm for 4NH protons which are D_2O exchangeable. This indicates that it is a symmetrical compound. Its ^{13}C NMR spectrum showed that two signals at δ = 150.6 and 165.4 ppm for the 4C=O groups (Scheme 2). The ^{1}H NMR spectrum of pyrano[2,3-*d*]pyrimidine-2,4-dione **5** showed three singlets at δ = 2.23, 2.61 and 4.37 ppm for the two methyl groups and the CH pyran. Also, the two broad singlets at δ = 10.30, 11.33 ppm for 2NH protons are D_2O exchangeable. Moreover, its ^{13}C NMR spectrum displayed characteristic signals at δ = 15.8 ppm for (CH_3), 28.6 ppm for ($CO\underline{CH_3}$) and 36.1 ppm for (CH pyran) as shown in (Scheme 2). Elucidation the structure of compound **6** was explained in details (see the experimental section).

The IR spectrum of pyrano[2,3-*d*]pyrimidine-6-carboxamide **7** showed str. Absorption bands at 3385, 3381 and 1671 cm^{-1} for NH_2 and amide carbonyl. The ^{1}H NMR spectrum revealed two singlet signals at δ = 7.61, 8.12 ppm for $2NH_2$ and two broad signals at δ = 10.98, 11.12 ppm 2NH protons which are D_2O exchangeable. The ^{1}H NMR spectrum of pyrano[2,3-*d*] pyrimidine carboxylate **9** gave triplet at δ = 1.32 ppm and quartet at δ = 4.85 ppm with coupling costant



SCHEME 1 Synthesis of the starting materials **2a**,**b** and **3a**,**b** [Color figure can be viewed at wileyonlinelibrary.com]



SCHEME 2 Reaction of 5-arylidene barbituric acid **2a** with different active carbon and nitrogen nucleophiles reagents [Color figure can be viewed at wileyonlinelibrary.com]

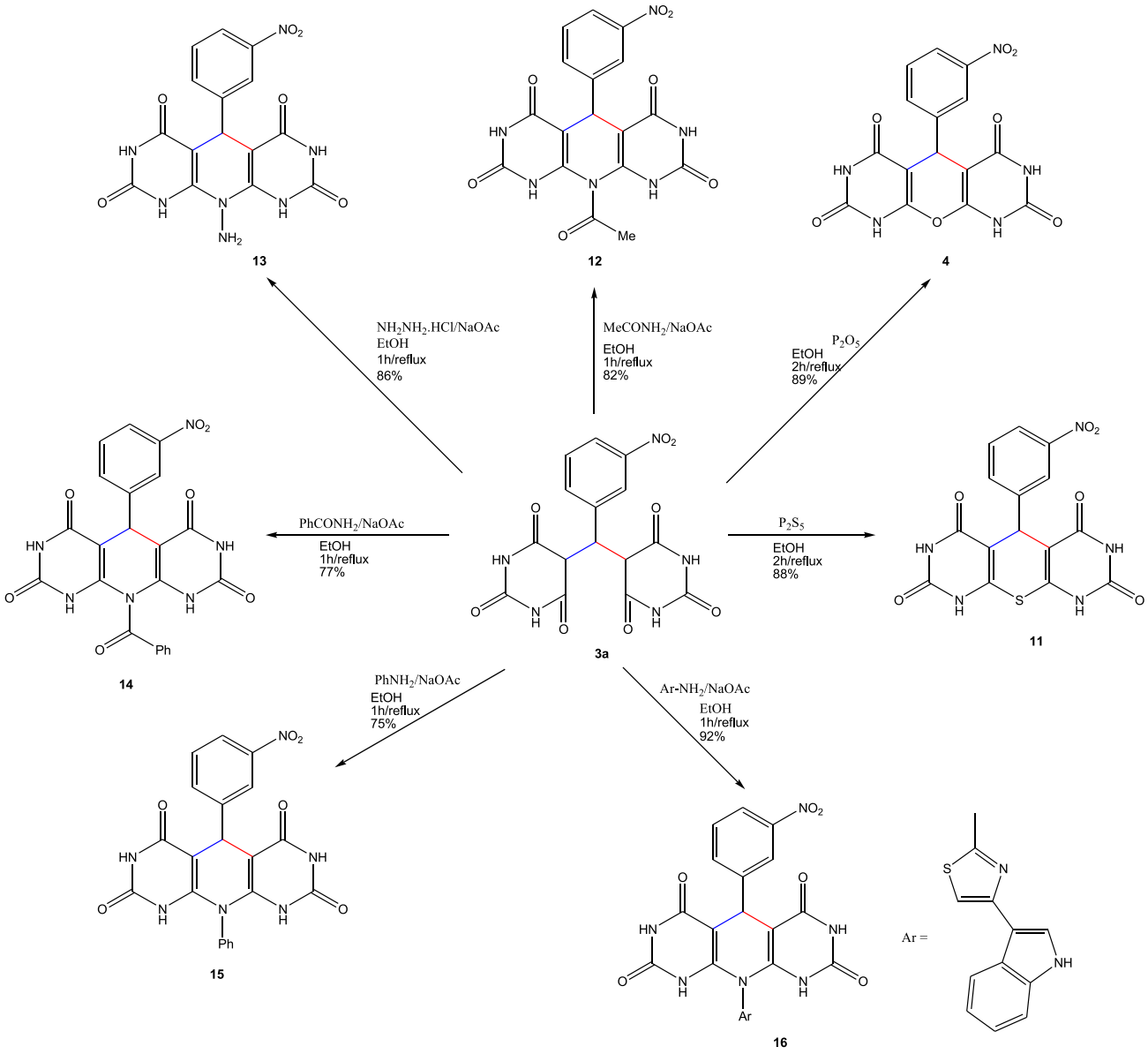
J = 6.3 Hz responsible for CH_3 and CH_2 groups, respectively (Scheme 2). The structure confirmation of compounds **8** and **10** are mentioned in the experimental section.

Upon refluxing of bispyrimidine-2,4,6-trione derivative **3a**[27] and heterocyclizing reagents such as P_2O_5, acetamide, hydrazine hydrate and aminothiazole in presence of anhydrous sodium acetate in ethanol (30–50 mL) for 1–2 h produced pyridino[2,3-*d*]pyrimidine derivatives **11–16** with 75–92% yields (Scheme 3). The IR spectrum of acetyl-pyridino[2,3-*d*]pyrimidine **12** showed str. Absorption bands at 1702, 1667, 1664 cm^{-1} for amide carbonyl groups. The ^1H NMR spectrum of **12** revealed two singlets at δ = 1.95 and 4.51 ppm for methyl protons and and the CH pyran. Moreover, the broad singlets at δ = 11.24, 11.38 ppm for 4NH protons are D_2O exchangeable (Scheme 3). The ^1H NMR spectrum of compound **13** displayed two singlets at δ = 8.21 ppm characteristic for amino group and at δ = 4.47 ppm for the CH pyran. To prove the structure of compounds **14** and **15** see the experimental section). The ^1H NMR spectrum of compound **16** gave singlet at δ = 10.23 ppm for the NH indole moiety. The broad singlets at δ = 10.23, 11.28 and 11.37 ppm for NH protons are D_2O exchangeable as well (Scheme 3).

3 | ANTITUMOR ACTIVITY

3.1 | Tumor cell growth assay

The effects of compounds **5, 7, 8, 9, 10, 11, 13** and **16** on the *in vitro* growth of human tumor cell lines were evaluated according to the procedure adopted by the National Cancer Institute (NCI, USA) in the '*In vitro Anticancer Drug Discovery Screen*' that uses the protein-binding dye sulforhodamine B to assess cell growth. Briefly, exponentially, cells growing in 96-well plates were then exposed for 48 h to five serial concentrations of each compound, starting from a maximum concentration of 150 μM. Following this exposure period adherent cells were fixed, washed, and stained. The bound stain was solubilized



SCHEME 3 Reaction of bispyrimidine-2,4,6-trione derivative **3a** and heterocyclizing reagents [Color figure can be viewed at wileyonlinelibrary.com]

and the absorbance was measured at 492 nm in a plate reader (Bio-Tek Instruments Inc., Power wave XS, Wincoski, USA). For each tested compound and cell line, a dose–response curve was obtained and the minium inhibitor concentration inhibition of 50% (IC_{50}), corresponding to the concentration of the compounds that inhibited 50% of the cancer cell lines, was calculated as described elsewhere. Doxorubicin was used as a positive control and tested in the same manner.

It is clearly notable that, all the tested compounds showed significant inhibitory activities against the three human tumor cell lines, Liver Carcinoma (HepG-2), Colon Carcinoma (HCT-116) and Prostate Cancer (PC-3). The obtained results showed that the compounds **9** and **16** exhibited an amazing cytotoxicity effect, to the investigated cell lines (HCT-116, HepG-2 and PC-3, which is higher than that of the standard drug (doxorubicin) as shown in Table 1 and Figure 2. In addition to, compounds **7, 10** and **8** displayed high cytotoxicity effect for the three tumor cell lines as well. Moreover, compounds **13, 5** and **11** gave moderate inhibitory effect on the cancer cell lines.

4 | CONCLUSION

In conclusion, we reported a series of some new derivatives of pyrano[2,3-*d*]pyrimidine and pyridino[2,3-*d*] pyrimidine that are synthesized with very good yields starting with 5-arylidene barbituric acid and

TABLE 1 Cytotoxicity of the screened compounds **5, 7, 8, 9, 10, 11, 13** and **16** (IC_{50}, μg/mL)

	IC_{50} (μg/mL)		
Cpd. Nu.	**HCT-116**	**Hep-G2**	**PC-3**
5	7.65	5.21	4.80
7	1.40	0.90	1.70
8	3.4	2.80	3.14
9	0.34	0.25	0.53
10	2.3	1.50	3.30
11	11.3	9.9	9.7
13	5.7	8.5	6.5
16	0.28	0.39	0.64
Doxorubicin standard	0.471	0.467	0.73

bispyrimidine-2,4,6-trione derivative via easy approaches. By studying the antitumor activity for some selected compounds, we found that compounds **9** and **16** exhibited antitumor activity, which is higher than the standard drug (doxorubicin), against the human tumor cell lines, (HCT-116, HepG-2 and PC-3). The reason of the activity may be owing to the presence of the polar amino group that increases the ability of the chemotherapeutic agent to pentrate the cell wall of the microorganism. These wonderful results encourage us to do further research on such heterocycles.

5 | EXPERIMENTAL

All melting points were measured via electro thermal IA 9100 series digital melting point apparatus with open capillary tube and are uncorrected. The experiments were carried out using drying solvents. TLC was performed on Merck Silica Gel 60F254 have been detection by UV light. The IR spectra were recorded on a Parkin-Elmer model 1600 FTIR spectrometer as KBr discs (USA). The ^{1}H NMR spectra were recorded with Brucker AC-400 MHz spectrometer at nucleic acid unit, Zagazig University. Chemical shift are expressed as δ (ppm) scale relative to TMS as an internal standard and DMSO-d6 as solvent. Elemental analyses were determined on a Parkin-Elmer model 240 at Micro-analytical Center Cairo University.

5.1 | General synthetic procedure of compounds 4–10

A mixture of compound 5-aylidene barbituric acid **2a** (2.00 mmol) and compounds such as barbituric acid, acetyl acetone, ethylacetoactate, cyanoacetamide, malononitrile, ethylcyanoacetate and thiourea (2. 00 mmol) in ethanol (50 mL) and few drops of triethylamine was refluxed for 1–3 h. The reaction mixture was cooled at room temperature and poured into ice cold water. The crude product was filtered off and crystallized from a proper solvent to deliver the compounds **4–10** with 75–95% yields.

5.2 | 5-(3-Nitrophenyl)-1,3,7,9-tetrahydro-5H-pyrimidino[5′,4′-5,6]pyrano[2,3-d] pyrimidine-2,4,6,8-tetraone (4)

The crude product was crystallized from ethanol to give compound **4** in 75% yield as white crystals, mp 260–261°C, ir (KBr): NH 3204, 3182, 2C=O 1716, 1680, 1621, 1507 C=N and C=C cm^{-1}. ^{1}H nmr (400 MHz, DMSO-d_6): δ 4.41 (s, 1H, CH pyran), 7.27–7.54 (m, 4H, ArH's), 11.14 (brs, 2H, 2NH), 11.22 (brs, 2H, 2NH). ^{1}H nmr (400 MHz, DMSO-d_6, D_2O exchangeable): δ 4.41 (s, 1H, CH pyran), 7.27–7.54 (m, 4H, ArH's). ^{13}C nmr (75 MHz, DMSO-d_6): δ 35.7 (CH pyran), 90.3 (2C=C alkene), 119.4, 124.3, 130.1, 135.2, 144.1, 147.7 (Ph-carbons), 150.6 (2NC=ON), 157.2 (2C-O), 165.4, (2C=O); Anal. Calcd for $C_{15}H_9N_5O_7$ (371.26): C, 48.63; H, 2.57; N, 18.89. Found: C, 48.53; H, 2.44; N, 18.86.

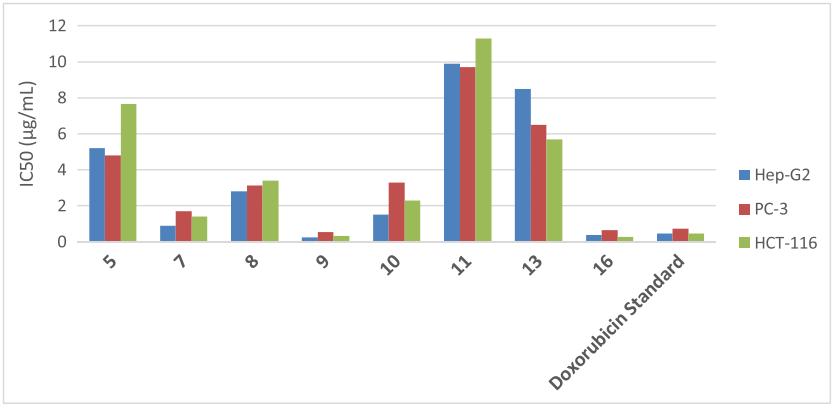


FIGURE 2 Cytotoxicity chart of the screened compounds **5, 7, 8, 9, 10, 11, 13** and **16** (IC_{50}, μg/mL) [Color figure can be viewed at wileyonlinelibrary.com]

5.3 | 6-Acetyl-7-methyl-5-(3-nitrphenyl)-1H-pyrano[2,3-d]pyrimidine-2,4(3H,5H)-dione (5)

The crude product was crystallized from ethanol to give compound **5** in 82% yield as yellow crystals, mp 245–246°C, ir (KBr): NH 3219, 3213, 3C=O 1710, 1703, 1686, 1568 C=C cm^{-1}. ^1H nmr (400 MHz, DMSO-d_6): δ 2.23 (s, 3H, CH_3), 2.61 (s, 3H, CH_3), 4.37 (s, 1H, CH pyran), 7.71–8.19 (m, 4H, ArH's), 10.30, 11.33 (brs, 2H, 2NH). ^1H nmr (400 MHz, DMSO-d_6, D_2O exchangeable): δ 2.23 (s, 3H, CH_3), 2.61 (s, 3H, CH_3), 4.37 (s, 1H, CH pyran), 7.71–8.19 (m, 4H, ArH's). ^{13}C nmr (75 MHz, DMSO-d_6): δ 15.8 (CH_3), 28.6 ($CO\underline{CH_3}$), 36.1 (CH pyran), 88.1, 92.5, 119.2, 120.1, 121.6, 129.4, 135.6, 143.2, 148.1, 151.2 (C=O), 155.2, 159.6, 164.6 (C=O), 195.2 ($CO\underline{CH_3}$); *Anal*. Calcd. for $C_{16}H_{13}N_3O_6$ (343.29): C, 56.20; H, 3.89; N, 12.29. Found: C, 55.98; H, 3.82; N, 12.24.

5.4 | 6-Acetyl-7-hydroxy-5-(3-nitrphenyl)-1H-pyrano[2,3-d]pyrimidine-2,4(3H,5H)-dione (6)

The crude product was crystallized from acetic acid to give compound **6** in 95% yield as pale white crystals, mp 275–274°C, ir (KBr): OH 4415, NH 3222, 3214, 3C=O 1705, 1680, 1664, 1622 C=C cm^{-1}. ^1H nmr (400 MHz, DMSO-d_6): δ 2.75 (s, 3H, CH_3), 4.25 (s, 1H, CH pyran), 7.68–7.89 (m, 4H, ArH's), 11.10, 11.31 (brs, 2H, 2NH), 11.85 (s, 1H, OH). ^1H nmr (400 MHz, DMSO-d_6, D_2O exchangeable): δ 2.75 (s, 3H, CH_3), 4.25 (s, 1H, CH pyran), 7.68–7.89 (m, 4H, ArH's). ^{13}C nmr (75 MHz, DMSO-d_6): δ 27.5 ($CO\underline{CH_3}$), 36.4 (CH pyran), 87.3, 93.5, 118.2, 119.6, 122.0, 130.1, 135.2, 143.7, 148.5, 151.6 (C=O), 158.3, 164.3 (C=O), 194.3 ($CO\underline{CH_3}$), 198.2; *Anal*. Calcd. for $C_{15}H_{11}N_3O_7$ (345.26): C, 52.26; H, 3.29; N, 12.25. Found: C, 52.18; H, 3.21; N, 12.17.

5.5 | 7-Amino-5-(3-nitrphenyl)-2,4-dioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d] pyrimidine-6-carboxamide (7)

The crude product was crystallized from ethanol to give compound **7** in 78% yield as yellowish powder, mp 267–268°C, ir (KBr): NH_2 3385, 3381, NH 3184, 3162, 3C=O 1712, 1685, 1671, 1511 C=C cm^{-1}. ^1H nmr (400 MHz, DMSO-d_6): δ 4.32 (s, 1H, CH pyran), 7.43–7.57 (m, 4H, ArH's), 7.61, 8.12 (s, 4H, $2NH_2$), 10.98, 11.12 (brs, 2H, 2NH). ^1H nmr (400 MHz, DMSO-d_6, D_2O exchangeable): δ 4.32 (s, 1H, CH pyran), 7.43–7.57 (m, 4H, ArH's). ^{13}C nmr (75 MHz, DMSO-d_6): δ 35.6 (CH pyran), 83.6, 88.9, 118.8, 119.2, 122.8, 130.3, 135.6, 144.6, 148.2, 150.8 (C=O), 155.1, 158.7, 164.1 (C=O); *Anal*. Calcd. for $C_{14}H_{11}N_5O_6$ (345.27): C, 48.76; H, 3.29; N, 20.33. Found: C, 48.70; H, 3.21; N, 20.28.

5.6 | 7-Amino-5-(3-nitrphenyl)-2,4-dioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d] pyrimidine-6-carbonitrile (8)

The crude product was crystallized from ethanol to give compound **8** in 76% yield as yellow crystals, mp 286–287°C, ir (KBr): NH_2 3381, 3378, NH 3184, 3162, CN 2235, 2C=O 1708, 1681, 1518 C=C cm^{-1}. ^1H nmr (400 MHz, DMSO-d_6): δ 4.27 (s, 1H, CH pyran), 7.38–7.45 (m, 4H, ArH's), 7.53, (s, 2H, NH_2), 11.23, 11.40 (brs, 2H, 2NH). ^{13}C nmr (75 MHz, DMSO-d_6): δ 35.5 (CH pyran), 58.6, 88.5, 118.5, 119.6 (CN), 123.8, 130.2, 135.3, 145.3, 148.1, 150.4 (C=O), 153.7, 157.7, 163.8 (C=O); *Anal*. Calcd. for $C_{14}H_9N_5O_5$ (327.25): C, 51.44; H, 2.80; N, 21.43. Found: C, 51.38; H, 2.77; N, 21.40.

5.7 | Ethyl7-amino-5-(3-nitrphenyl)-2,4-dioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d] pyrimidine-6-carboxylate (9)

The crude product was crystallized from ethanol to give compound **9** in 92% yield as yellow crystals, mp 201–202°C, ir (KBr): NH_2 3396, 3388, NH 3181, 3176, 3C=O 1738, 1716, 1684, C-O 1150, 1527 C=C cm^{-1}. ^1H nmr (400 MHz, DMSO-d_6): δ 1.32 (t, 3H, J = 6.3 Hz, CH_3), 4.85 (q, 2H, J = 6.3 Hz, CH_2), 4.23 (s, 1H, CH pyran), 7.42–7.48 (m, 4H, ArH's), 7.51, (s, 2H, NH_2), 10.94, 11.36 (brs, 2H, 2NH). ^1H nmr (400 MHz, DMSO-d_6, D_2O exchangeable): δ 1.32 (t, 3H, J = 6.3 Hz, CH_3), 4.23 (s, 1H, CH pyran), 4.85 (q, 2H, J = 6.3 Hz, CH_2), 7.42–7.48 (m, 4H, ArH's). ^{13}C nmr (75 MHz, DMSO-d_6): δ 15.4 (CH_3), 35.4 (CH pyran), 65.8 (OCH_2), 89.3, 98.5, 118.2, 120.4, 122.8, 130.6, 134.3, 144.7, 148.5, 150.8 (C=O), 154.3, 158.4, 163.9 (C=O); *Anal*. Calcd. for $C_{14}H_9N_5O_5$ (374.30): C, 51.40; H, 3.81; N, 15.03. Found: C, 51.34; H, 3.77; N, 14.97.

5.8 | 7-Amino-4-(3-nitrphenyl)-4H-pyrimido[5,4-e][1,3]oxazine-5,7-(1H,8H)-dione (10)

The crude product was crystallized from ethanol to give compound **10** in 85% yield as yellow crystals, mp 240–241°C, ir (KBr): NH_2 3410, 3398, NH 3154, 3132, 2C=O 1709, 1664, 1533 C=C cm^{-1}. ^1H nmr (400 MHz, DMSO-d_6): δ 4.42 (s, 1H, CH pyran), 7.35–7.47 (m, 4H, ArH's), 7.49, (s, 2H, NH_2), 11.39, 11.50 (brs, 2H, 2NH). ^{13}C nmr

(75 MHz, DMSO-d_6): δ 35.7 (CH pyran), 89.7, 119.5, 121.1, 123.4, 130.4, 135.7, 143.2, 148.7, 152.0 (C=O), 155.3, 158.0, 165.1 (C=O); *Anal*. Calcd. for $C_{12}H_9N_5O_5$ (303.23): C, 47.55; H, 3.20; N, 23.14. Found: C, 47.53; H, 2.99; N, 23.10.

5.9 | General synthetic procedure of compounds 11–16

A mixture of bispyrimidine-2,4,6-trione derivative **3a** (2.00 mmol)[27] and an reagents like P_2S_5, P_2O_5, acetamide, hydrazine hydrate, benzamide, aniline and aminothiazole[30] (2. 00 mmol) ethanol and NaOAc (30–50 mL) was refluxed for 1–2 h after TLC showed disappearance of the starting materials. The reaction mixture was cooled and poured into ice cold water. The crude product was filtered off and crystallized from a proper solvent to result in the compounds **11**–**16** with yields in between 75 and 92%.

5.10 | 5-(3-Nitrophenyl)-1,3,7,9-tetrahydro-5H-hydropyrimidino[5′,4′-5,6]thiino[2,3-d]pyrimidine-2,4,6,8-tetraone (11)

The crude product was crystallized from ethanol to give compound **11** in 88% yield as pale white crystals, mp 255–256°C, ir (KBr): NH 3173, 3159, 4C=O 1717, 1665, 1634 C=C cm^{-1}. ^{1}H nmr (400 MHz, DMSO-d_6): δ 4.45 (s, 1H, CH pyran), 7.28–7.52 (m, 4H, ArH's), 11.16 (brs, 2H, 2NH), 11.29 (brs, 2H, 2NH).^{13}C nmr (75 MHz, DMSO-d_6): δ 36.2 (CH pyran), 88.4 (2C=C alkene), 119.4, 124.3, 130.1, 135.2, 144.1, 147.7 (Ph-carbons), 150.6 (2NC=ON), 153.6 (2C-S), 163.4, (2C=O); *Anal*. Calcd. for $C_{15}H_9N_5O_6S$ (387.33): C, 46.62; H, 2.38; N, 18.21. Found: C, 46.51; H, 2.34; N, 18.08.

5.11 | 10-Acetyl-5-(3-nitrophenyl)-1,3,5,7,9-pentahydropyrimidino[5′,4′-5,6]pyridino[2,3-d]pyrimidine-2,4,6,8-tetraone (12)

The crude product was crystallized from ethanol to give compound **12** in 82% yield as pale white crystals, mp 274–275°C, ir (KBr): NH 3165, 3151, 5C=O 1702, 1667, 1664, 1648 C=C cm^{-1}. ^{1}H nmr (400 MHz, DMSO-d_6): δ 1.95 (s, 3H, CH_3), 4.51 (s, 1H, CH pyran), 7.23–7.39 (m, 4H, ArH's), 11.24 (brs, 2H, 2NH), 11.38 (brs, 2H, 2NH). ^{13}C nmr (75 MHz, DMSO-d_6): δ 35.8 (CH pyran), 89.6 (2C=C alkene), 118.4, 123.7, 129.2, 134.5, 143.8, 148.1 (Ph-carbons), 152.3 (2NC=ON), 154.6 (2C-N), 164.7, (2C=O); *Anal*. Calcd. for $C_{17}H_{12}N_6O_7$ (412.31): C, 49.58; H, 2.97; N, 20.45. Found: C, 49.52; H, 2.93; N, 20.38.

5.12 | 10-Amino-5-(3-nitrophenyl)-1,3,5,7,9,10-hexahydropyrimidino[5′,4′-5,6]pyridino[2,3-d]pyrimidine-2,4,6,8-tetraone (13)

The crude product was crystallized from ethanol to give compound **13** in 86% yield as pale white crystals;, mp 286–287°C, ir (KBr): NH_2, NH 3429, 3223, 3174, 3156, 4C=O 1695, 1668, 1645 C=C cm^{-1}. ^{1}H nmr (400 MHz, DMSO-d_6): δ 4.47 (s, 1H, CH pyran), 7.11–7.35 (m, 4H, ArH's), 8.21 (s, 2H, NH_2), 11.17 (brs, 2H, 2NH), 11.35 (brs, 2H, 2NH). ^{1}H nmr (400 MHz, DMSO-d_6, D_2O exchangeable): δ 4.47 (s, 1H, CH pyran), 7.11–7.35 (m, 4H, ArH's). ^{13}C nmr (75 MHz, DMSO-d_6): δ 36.3 (CH pyran), 88.3 (2C=C alkene), 119.6, 124.3, 129.8, 135.7, 143.2, 147.5 (Ph-carbons), 151.1 (2NC=ON), 154.2 (2C-N), 165.7, (2C=O), 174.3(CH_3C=O); *Anal*. Calcd. for $C_{15}H_{11}N_7O_6$ (385.29): C, 46.78; H, 2.90; N, 25.55. Found: C, 46.76; H, 2.88; N, 25.45.

5.13 | 10-Benzoyl-5-(3-nitrophenyl)-1,3,5,7,9-pentahydropyrimidino[5′,4′-5,6]pyridino[2,3-d]pyrimidine-2,4,6,8-tetraone (14)

The crude product was crystallized from ethanol to give compound **14** in 77% yield as pale white crystals, mp 278–279°C, ir (KBr): NH 3161, 3157, 5C=O 1708, 1674, 1665, 1649 C=C cm^{-1}. ^{1}H nmr (400 MHz, DMSO-d_6): δ 4.46 (s, 1H, CH pyran), 7.45–8.13 (m, 9H, ArH's), 11.09 (brs, 2H, 2NH), 10.94 (brs, 2H, 2NH). ^{13}C nmr (75 MHz, DMSO-d_6): δ 37.3 (CH pyran), 92.6 (2C=C alkene), 118.4, 123.7, 129.2, 134.5, 143.8, 148.1 (Ph-carbons), 150.9 (2NC=ON), 156.6 (2C-N), 166.0, (2C=O), 181.2 (PhC=O); *Anal*. Calcd. for $C_{22}H_{14}N_6O_7$ (474.38): C, 55.79; H, 3.20; N, 17.77. Found: C, 55.70; H, 2.97; N, 17.72.

5.14 | 10-Phenyl-5-(3-nitrophenyl)-1,3,5,7,9-pentahydropyrimidino[5′,4′-5,6]pyridino[2,3-d]pyrimidine-2,4,6,8-tetraone (15)

The crude product was crystallized from acetic acid to give compound **15** in 75% yield as pale white crystals, mp 242–243°C, ir (KBr): NH 3166, 3154, 4C=O 1697, 1669, 1655 C=C cm^{-1}. ^{1}H nmr (400 MHz, DMSO-d_6): δ 4.48 (s, 1H, CH pyran), 7.48–8.24. (m, 9H, ArH's), 11.08 (brs, 2H, 2NH), 11.26 (brs, 2H, 2NH); ^{13}C nmr (75 MHz, DMSO-d_6): δ 37.5 (CH pyran), 92.3 (2C=C alkene), 118.6, 122.3, 123.2, 128.7, 129.0, 129.5, 129.8, 134.3, 143.6, 148.2 (Ar-carbons), 151.3 (2NC=ON), 156.2 (2C-N), 166.4, (2C=O), *Anal*. Calcd. for $C_{21}H_{14}N_6O_6$

(446.37): C, 46.78; H, 2.90; N, 25.55. Found: C, 56.51; H, 3.16; N, 18.83.

5.15 | 10-[4-(1H-indol-3-yl)thiazol-2-yl]-5-(3-nitrophenyl)-1,3,5,7,9-pentahydropyrimidino[5′,4′-5,6] pyridino[2,3-d]pyrimidine-2,4,6,8-tetraone (16)

The crude product was crystallized from ethanol to give compound **15** in 92% yield as pale yellow crystals, mp 282–283°C, ir (KBr): NH 3160, 3155, 4C=O 1714, 1669, 1653 C=C cm^{-1}. ^1H nmr (400 MHz, DMSO-d_6): δ 4.48 (s, 1H, CH pyran), 7.14–7.63. (m, 10H, ArH's), 10.23 (s, 1H, NH) 11.28 (brs, 2H, 2NH), 11.37 (brs, 2H, 2NH); ^1H nmr (400 MHz, DMSO-d_6, D_2O exchangeable): δ 4.48 (s, 1H, CH pyran), 7.14–7.63. (m, 10H, ArH's); ^{13}C nmr (75 MHz, DMSO-d_6): δ 36.1 (CH pyran), 92.1 (2C=C alkene), 111.8, 112.1, 115.4, 118.0, 119.1, 121.3, 122.4, 123.4, 127.7, 129.6, 131.7, 134.3, 135.5, 140.3, 144.2, 148.6 (Ar-carbons), 150.7 (2NC=ON), 156.5 (2C-N), 166.3, (2C=O). *Anal*. Calcd. for $C_{26}H_{16}N_8O_6S$ (568.52): C, 54.98; H, 2.95; N, 19.83. Found: C, 54.93; H, 2.84; N, 19.71.

6 | ANTITUMOR ACTIVITY

6.1 | Reagents

Fetal bovine serum (FBS) and L-glutamine, were from Gibco Invitrogen Co. (Scotland, UK). RPMI-1640 medium was from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and sulforhodamine B (SRB) were from Sigma Chemical Co. (Saint Louis, USA).

6.2 | Cell cultures

Three human tumor cell lines, Liver Carcinoma (HepG-2), Colon Carcinoma (HCT-116) and Prostate Cancer (PC-3). All the experiments concerning the cytotoxicity evaluation were performed and analyzed by tissue culture unit at the Regional Center for Mycology and Biotechnology RCMB, AL-Azhar University, Cairo, Egypt. Antitumor viability assay was carried according to method described by.[31] They grow as monolayer and routinely maintained in RPMI-1640 medium supplemented with 5% heat inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100 μg/mL), at 37°C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5 × 105 cells/mL for BBC and 0.75 × 104 cells/mL for MGC-803, followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all the experiments by exposing untreated control cells to the maximum concentration (0.5%) of DMSO used in each assay.

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