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ORIGINAL RESEARCH

Design and synthesis of novel nucleobase-based barbiturate derivatives as potential anticancer agents

Bhaveshkumar D. Dhorajiya · Ahmed S. Ibrahim · Farid A. Badria · Bharatkumar Z. Dholakiya

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Abstract Cancer today remains one of the most deadly diseases in the world. In search of novel anticancer agents, a series of newly hybrid molecules were designed and synthesized by combining the structural features of nucleobase and barbiturate derivatives using the concept of green chemistry. This approach was accomplished efficiently using the aqueous medium to give the corresponding products in a high yield. The newly synthesized compounds were characterized by spectral analysis FT-IR, ^{1}H NMR, ^{13}C-NMR, HMBC, MASS and elemental analysis. Evaluations of these molecules over four cell lines panel of human cancer cells have identified several compounds with significant anticancer activities against one or more cell lines. Compounds TBC and TBA proved to exhibit a wide cytotoxic effect on the tested four cell lines at (IC_{50} 16–24 μM) and (IC_{50} 25–34 μM), respectively. Other compounds, e.g., 1,3-BA, showed a selective cytotoxicity against HepG2 at 7 μM. Taken together, this study has led to the development of a convenient, a highly yielded, and an environmentally friendly synthetic method of highly promising leads for cancer fighting.

Keywords Green chemistry · Barbiturates · Cytotoxicity

Introduction

Every year almost 9 million cancer cases are newly diagnosed in developing countries where cancer incidence continues to increase at alarming rates. According to cancer society statistics, at least one third of these individuals are not expected to survive the disease, making cancer as the most prevalent cause of death. Systemic chemotherapy has emerged as a very promising strategy in treating a wide variety of cancers. The key drug is still 5-fluorouracil (5-FU). However, the major stumbling blocks for 5-FU therapy includes the multidrug resistance developed by cancer cells; in addition, it causes dose-limiting toxicities. Thus, the search for new ones has been prompted as urgency.

The fundamental role of small chemical entities (like amino acids and nucleobases), and the acceptability of their synthetic analogs in the biological systems may be the reason for the design and development of about 80 % of the drugs. Moreover, a suitable combination of the structural features of two biologically active molecules results in the creation of new molecules in which the characteristics of various components are modulated, amplified or give rise to entirely new properties. Due to the biological significance of nucleobases as well as barbiturates as anticonvulsant (Nicoll and Wojtowicz, 1980), anti-hypnotic (Huang and Barker, 1980), anticancer (Dhorajiya *et al.*, 2012), and anti-inflammatory, we aimed to suitably combine these two molecular entities through *N*-formylation of

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various DNA bases followed by condensation with barbiturates in an attempt for creating new hybrid molecules carrying the critical components of 5-fluorouracil. Thereafter, we pursue to evaluate their tumor growth inhibitory activities over four cell lines panel of human cancer.

In recent years, a number of formylation methods have been reported using various formylating reagents (such as acetic formic anhydride (Green and Wuts, 1999; Strazzolini *et al.*, 1990), chloral (Blicke and Lu, 1952), DCC (Waki and Meienhofer, 1977), EDCI (Chen and Benoiton, 1979), activated formic acid esters (Yale 1971; Kisfaludy and Laszlo, 1987; Nerveux *et al.*, 1991; Duezek *et al.*, 1996) KF–Al_2O_3 (Miharam *et al.*, 2003) ammonium formate (Reddy *et al.*, 2000) and solid supported reagent (Luca *et al.*, 2004) together with a catalyst (such as $ZnCl_2$, $SnCl_2$, $LaCl_3$, $La(OTf)_3$, $FeCl_3$, $AlCl_3$, $NiCl_2$, ZnF_2, and AlF_3) in organic solvent. However, many of these formylation methods have disadvantages of being expensive, producing side products, thermally instable, and difficult accessibility to reagents (Dawane *et al.*, 2011). Thus, a mild, convenient, and high-yield procedure without catalyst would be valuable. In continuation of our interest in the development of newly synthetic anticancer agents, we further report herein a convenient, an environmentally friendly, and novel approach for the synthesis of nucleobase-based barbiturates in aqueous medium with a very good yield using the concept of green chemistry. The significance of this protocol is to avoid the use of organic solvents, catalyst-free as well as does not require special conditions or equipments.

Experimental

The barbituric acid was synthesized by using diethyl malonate and urea using the standard procedure (Jacobson, 1937). Melting points were determined in open capillaries on a Veego electronic apparatus VMP-D (Veego Instrument Corporation, Mumbai, India). IR spectra (4,000–400 cm^{-1}) of synthesized compounds were recorded on a Perkin Elmer-Spectrum RX-IFTIR spectrophotometer using KBr pellets. Thin layer chromatography was performed on the object glass slides (2 × 7.5 cm) coated with silica gel-G and spots were visualized under UV irradiation. ^1H NMR and ^{13}C NMR spectra were recorded on an Avance-II (Bruker) model using DMSO as a solvent and TMS as internal standard with ^1H resonant frequency of 400 MHz and ^{13}C resonant frequency of 100 MHz. The ^1H NMR and ^{13}C NMR chemical shifts were reported as parts per million (ppm) downfield from TMS (Me_4Si). The splitting patterns were designated as follows; s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; and m, multiplet. UV Spectra were recorded on Maya pro 2000 (Ocean Optics USA) using DMSO as a solvent with 10^{-5} M solution.

Synthesis of DNA-based barbiturates

As shown in Scheme 1, a solution of the corresponding DNA-bases (for e.g., adenine 1 mmol) and formic acid (4 mmol) in distilled water, as a green solvent, was allowed to reflux at 60 °C over a period of 2–3 h. Then, it was cooled down until the reaction mixture becomes a clear solution. The completion of reaction was confirmed by TLC. In situ barbiturates were added again and refluxed further for 3–4 h. As the reaction proceeds, the solid products were separated out in the form of suspension and the precipitated DNA-based barbiturates were separated by filtration, washed with water three times followed by *n*-hexane, and then dried in a desiccator (Dhorajiya and Dholakiya, 2013); Scheme 2.

Characterization data of synthesized compounds

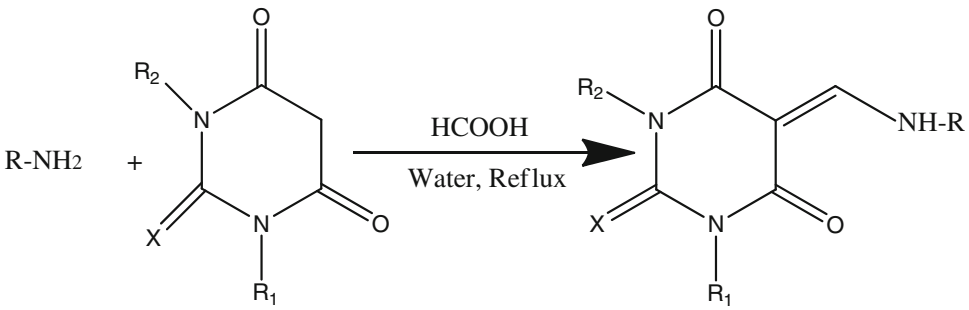
5-[(9H-purin-6-ylamino)-methylene]-pyrimidine-2,4,6-trione (BA)

Yellow powder, Yield 77 %; m.p. > 250 °C; ^1H NMR (400 MHz, DMSO), 4.02 δ ppm (1H, dd, exocyclic N<u>H</u> of purine ring, $j = 16.80$ Hz), 6.88 δ ppm (2H, s, endocyclic –N<u>H</u> of purine ring), 8.08 δ ppm (1H, dd, exocyclic C<u>H</u> of pyrimidine ring, $j = 25.36$ Hz), 8.14 δ ppm (1H, dd, N<u>H</u> of purine ring), 11.21 δ ppm (1H, s, N<u>H</u> of pyrimidine ring), 11.22 δ ppm (1H, s, N<u>H</u> of pyrimidine ring). ^{13}C NMR (400 MHz, DMSO), 77 (C-5), 114.32 (C-9), 114.67 (C-8), 122.95 (C-13), 127.12(C-12, C-14), 129.27 (C-11, C-15), 132 (C-10), 155 (C-7), 163.15(C-4, C-6), 168.05 (C-2) δ ppm. FTIR (KBr) υ_{max} cm^{-1}: 1212 (m, –O–C stretching), 1631 (s, =C–NH aliphatic amine), 1693 (s, C=O), 2826 (exocyclic CH), 3073 (m, CH–NH streching). λ max: 303.22 nm; (€: 1.10×10^5 L mol^{-1} cm^{-1}); M.W.273.21, ESIMS: *m/z* 274.25 (M + 1); Anal. Calcd. For $C_{10}H_7N_7O_3$ (%): C 43.96, H 2.58, N 35.89. Found (%): C 43.94, H 2.57, N 35.91.

5-[(9H-purin-6-ylamino)-methylene]-2-thioxo-dihydro-pyrimidine-4,6-dione (TBA)

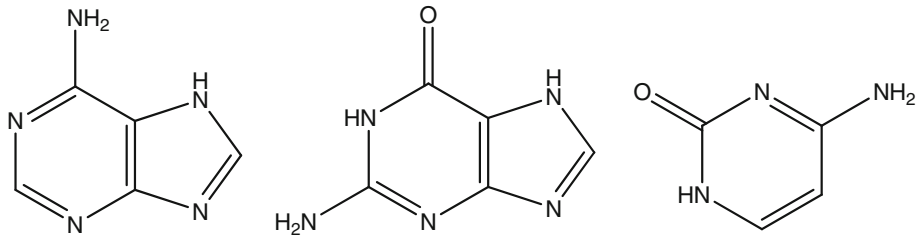
Yellow powder, Yield 58 %; m.p. > 250 °C; ^1H NMR (400 MHz, DMSO), 4.04 δ ppm (1H, dd, exocyclic N<u>H</u> of purine ring, $j = 15.85$ Hz), 6.92 δ ppm (2H, s,–C<u>H</u> of purine ring), 8.12 δ ppm (1H, dd, exocyclic C<u>H</u> of pyrimidine ring, $j = 24.60$ Hz), 8.20 δ ppm (1H, dd, N<u>H</u> of purine ring), 11.18 δ ppm (1H, s, N<u>H</u> of pyrimidine ring), 11.21 δ ppm (1H, s, N<u>H</u> of pyrimidine ring). ^{13}C NMR (400 MHz, DMSO), 76 (C-5), 115.10 (C-9), 115.40 (C-8), 122.82 (C-13, C-14), 126.72 (C-12, C-15), 129.67 (C-11), 131.89 (C-10) 155.15 (C-7), 165.15 (C-4, C-6),

Scheme 1 Synthesis of DNA base and heterocyclic primary amines-based barbiturates



Where X= O or S and R1 & R2= -H or -CH3

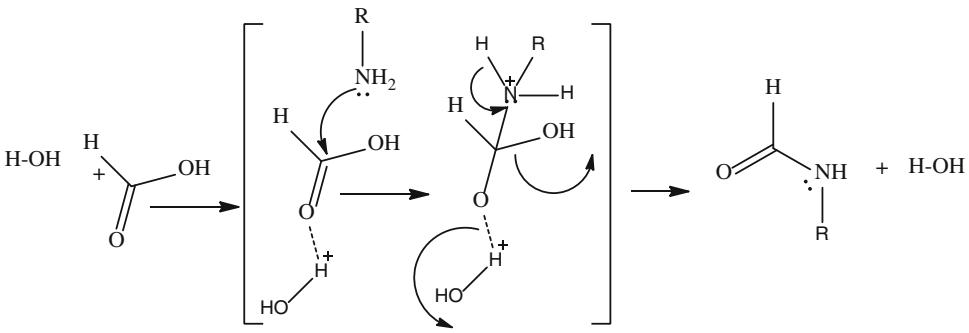
Where R are different Nucleo-base Amines



adenine Guanine Cytosine

2-amino-pyridine 2-amino-5-Methyl-Thiazole

Scheme 2 Plausible reaction mechanism pathway for *N*-formylation of aromatic amines with formic acid using water



168.25 (C-2) δ ppm. FTIR (KBr) υ_{max} cm^{-1}: 1216 (m, –O–C stretching), 1628 (s, = C–NH aliphatic amine), 1698 (s, C=O), 2808 (exocyclic CH), 3075(m, CH–NH streching). λ max: 299 nm; (€: 1.03×10^5 L mol^{-1} cm^{-1}); M.W.289. 27, ESIMS: *m/z* 289.05 (M); Anal. Calcd. For $C_{10}H_7N_7O_2S$ (%): C 41.52, H 2.44, N 33.89. Found (%): C 42.48, H 2.48, N 33.90.

1,3-Dimethyl-5-[(9H-purin-6-ylamino)-methylene]-pyrimidine-2,4,6-trione (1,3-BA)

Yellow powder, Yield 82 %; m.p. > 250 °C; ^{1}H NMR (400 MHz, DMSO), 2.72 (6H, s, two $C\underline{H_3}$ group of pyrimidine ring), 5.67 δ ppm (1H, dd, exocyclic N$\underline{H}$ of purine ring, $j = 14.08$ Hz), 6.85 δ ppm (2H, s,–C$\underline{H}$

of purine ring), 8.09 δ ppm (1H, dd, exocyclic CH of pyrimidine ring, $j = 24.94$ Hz), 8.16 δ ppm (1H, dd, NH of purine ring). ^{13}C NMR (400 MHz, DMSO), 78(C-5), 113.10 (C-9), 113.38 (C-8), 121.39 (C-13), 126.56 (C-12, C-14), 129.60 (C-11, C-15), 133.29 (C-10), 157.55 (C-7), 163.15 (C-4, C-6), 168.05 (C-2) δ ppm. FTIR (KBr) υ_{max} cm^{-1}: 1208 (m, –O–C stretching), 1628 (s, =C–NH aliphatic amine), 1695 (s, C = O), 2805 (m,–N–CH_3 streching), 2816 (exocyclic CH), 3070(m, CH–NH streching). λ max: 303.22 nm; (€: 1.00×10^5 L mol^{-1} cm^{-1}); M.W. 301.26, ESIMS: *m/z* 302.25 (M + 2); Anal. Calcd. For $C_{12}H_{11}N_7O_3$ (%): C 47.84, H 3.68, N 32.55. Found (%): C 47.81, H 3.70, N 32.56.

1,3-Dimethyl-5-[(9H-purin-6-ylamino)-methylene]-2-thioxo-dihydro-pyrimidine-4,6-dione (1,3-TBA)

Dark orange powder, Yield 62 %; m.p. > 250 °C; ^{1}H NMR (400 MHz, DMSO), 2.78 (6H, s, two CH_3 group of pyrimidine ring), 4.03 δ ppm (1H, dd, exocyclic NH of purine ring, $j = 15.28$ Hz), 6.90 δ ppm (2H, s,–CH of purine ring), 8.14 δ ppm (1H, dd, exocyclic CH of pyrimidine ring, $j = 23.34$ Hz), 8.18 δ ppm (1H, dd, NH of purine ring). ^{13}C NMR (400 MHz, DMSO), 58.08 (C-18), 78.85 (C-5), 113.30 (C-9), 113.47 (C-8), 122.95 (C-11, C-15), 129.27 (C-12, C-14), 131.17 (C-10, C-13), 157 (C-7), 162.66 (C-4, C-6), 172.05 (C-2) δ ppm. λ max: 288.21 nm; (€: 0.90×10^5 L mol^{-1} cm^{-1}); M.W.317.33, FTIR (KBr) υ_{max} cm^{-1}: 1220 (m, –O–C stretching), 1635 (s, =C–NH aliphatic amine), 1702 (s, C=O), 2796 (m, –N–CH_3 streching), 2812 (exocyclic CH), 3081(m, CH–NH streching). M.W. 317.07, ESIMS: *m/z* 309.06 (M + 2); Anal. Calcd. $C_{12}H_{11}N_7O_2S$ (%): C 45.42, H 3.49, N 30.90. Found (%): C 42.40, H 3.52, N 30.92.

5-[(2-Hydroxy-9H-purin-6-ylamino)-methylene]-pyrimidine-2,4,6-trione (BG)

Yellow powder, Yield 74 %; m.p. 250 °C; ^{1}H NMR (400 MHz, DMSO), 4.04 δ ppm (1H, dd, exocyclic NH of purine ring, $j = 15.68$ Hz), 6.97 δ ppm (1H, s, –CH of purine ring), 8.06 δ ppm (1H, dd, exocyclic CH of pyrimidine ring, $j = 24.76$ Hz), 8.15 δ ppm (1H, dd, NH of purine ring), 11.15 δ ppm (1H, s, NH of pyrimidine ring), 11.27 δ ppm (1H, s, NH of pyrimidine ring), 12.30 δ ppm (1H, s, OH of purine ring). ^{13}C NMR (400 MHz, DMSO), 58.08 (C-18, C-17) 75.35 (C-5), 114.56 (C-9), 114.87 (C-8), 122.95 (C-11, C-15), 129.27 (C-12, C-14), 131.17 (C-10, C-13), 158 (C-7), 162.66 (C-4, C-5), 167.05 (C-2) δ ppm. FTIR (KBr) υ_{max} cm^{-1}: 1221 (m, –O–C stretching), 1629 (s, =C–NH aliphatic amine), 1691 (s, C=O), 3070 (m, CH–NH streching) 3345 (C–OH). λ max: 276 nm; (€: 0.95×10^5 L mol^{-1} cm^{-1}); M.W. 289.21, ESIMS: *m/z* 289.20 (M); Anal. Calcd. For $C_{10}H_7N_7O_4$ (%): C 41.53, H 2.44, N 33.90. Found (%): C 42.49, H 2.46, N 33.89.

5-[(2-Hydroxy-9H-purin-6-ylamino)-methylene]-2-thioxo-dihydro-pyrimidine-4,6-dione (TBG)

Off white, Yield 84 %; m.p. > 250 °C; ^{1}H NMR (400 MHz, DMSO), 4.09 δ ppm (1H, dd, exocyclic NH of purine ring, $j = 14.98$ Hz), 6.93 δ ppm (2H, s,–CH of purine ring), 8.03 δ ppm (1H, dd, exocyclic CH of pyrimidine ring, $j = 23.26$ Hz), 10.97 δ ppm (1H, dd, NH of purine ring), 11.17 δ ppm (1H, s, NH of pyrimidine ring), 11.26 δ ppm (1H, s, NH of pyrimidine ring), 12.36 δ ppm (1H, s, OH of purine ring). ^{13}C NMR (400 MHz, DMSO), 74.53 (C-5), 113.26 (C-9), 113.59 (C-8), 122.92 (C-13, C-14), 126.51 (C-12, C-15), 129.39 (C-11), 131.78 (C-10) 157.23 (C-7), 162.66 (C-4, C-6), 168.62 (C-2) δ ppm. FTIR (KBr) υ_{max} cm^{-1}: 1215 (m, –O–C stretching), 1362 (s, C–NH, aromatic amine), 1632 (s, =C–NH aliphatic amine), 1698 (s, C=O), 2821 (exocyclic CH), 3076 (m, CH–NH streching), 3342 (b, C–OH). λ max: 291.03 nm; (€: 0.95×10^5 L mol^{-1} cm^{-1}); M.W. 305.27, ESIMS: *m/z* 306.29 (M + 1); Anal. Calcd. $C_{10}H_7N_7O_3S$ (%): C 39.34, H 2.31, N 32.12. Found (%): C 39.31, H 2.35, N 32.09.

5-(((2-Hydroxypyrimidin-4-yl)amino)methylene) pyrimidine-2,4,6-(1H,3H,5H)-trione (BC)

Yellow powder, Yield 82 %; m.p. > 250 °C; ^{1}H NMR (400 MHz, DMSO), 4.06 δ ppm (1H, dd, exocyclic NH of purine ring, $j = 15.68$ Hz), 6.98 δ ppm (2H, s, –CH of cytosine ring), 8.11 δ ppm (1H, dd, exocyclic CH of pyrimidine ring, $j = 23.60$ Hz), 8.16 δ ppm (1H, dd, NH of cytosine ring), 11.18 δ ppm (1H, s, NH of pyrimidine ring), 11.26 δ ppm (1H, s, NH of pyrimidine ring). ^{13}C NMR (400 MHz, DMSO), 78.53(C-7), 113.26 (C-9), 113.68 (C-8), 121.29 (C-13), 126.43(C-12, C-14), 129.58 (C-11, C-15), 133.18 (C-10) 157.53 (C-7), 161.35 (C-4, C-6), 169.34 (C-2) δ ppm. FTIR (KBr) υ_{max} cm^{-1}: 1209 (m, –O–C stretching), 1354 (s, C–NH, aromatic amine), 1632 (s, =C–NH aliphatic amine), 1694 (s, C=O aromatic and α,β-unsaturated ketone), 3072(m, CH–NH streching). λ max: 286.34 nm; (€: 1.14×10^5 L mol^{-1} cm^{-1}); M.W. 249.18, ESIMS: *m/z* 251.14 (M + 2); Anal. Calcd. for $C_9H_7N_5O_4$ (%):C 43.38, H 2.83, N 28.11. Found (%): C 43.40, H 2.79, N 28.12.

5-((2-Hydroxypyrimidine-4-ylamino)methylene)-dihydro-2-thioxopyrimidine-4,6(1H,5H)-dione (TBC)

Dark orange powder, Yield 75 %; m.p. > 250 °C; ^{1}H NMR (400 MHz, DMSO), 4.09 δ ppm (1H, dd, exocyclic NH of purine ring, $j = 13.8$ Hz), 6.95 δ ppm (2H,

s, –CH of cytosine ring), 8.07 δ ppm (1H, dd, exocyclic CH of pyrimidine ring, $j = 23.66$ Hz), 8.18 δ ppm (1H, dd, NH of cytosine ring), 11.12 δ ppm (1H, s, NH of pyrimidine ring), 11.19 δ ppm (1H, s, NH of pyrimidine ring). ^{13}C NMR (400 MHz, DMSO), 77 (C-5), 113.45 (C-9), 113.76 (C-8), 121.78 (C-13), 126.34(C-12, C-14), 129.87(C-11, C-15), 132.22 (C-10), 158.25 (C-7), 166.15(C-4, C-6), 169.26 (C-2) δ ppm. FTIR (KBr) υ_{max} cm^{-1}: 1213 (m, –O–C stretching), 1626 (s, =C–NH aliphatic amine), 1699 (s, C=O), 2809 (exocyclic CH), 3078 (m, CH–NH streching). λ max: 284.46 nm; ESIMS: *m/z* 284.45 (M); (€: 1.07×10^5 L mol^{-1} cm^{-1}); M.W. 265.25, Anal. Calcd. $C_9H_7N_5O_3S$ (%): C 40.75, H 2.66, N 26.40. Found (%): C 40.71, H 2.69, N 26.42.

5-((1,6-Dihydro-2-hydroxypyrimidine-4-ylamino)-methylene)-1,3 dimehylpyrimidine-2,4,6(1H,3H,5H)-trione (1,3-BC)

Yellow powder, Yield 65 %; m.p. > 250 °C; ^{1}H NMR (400 MHz, DMSO), 2.73 (6H, s, two CH_3 group of pyrimidine ring), 4.07 δ ppm (1H, dd, exocyclic NH of purine ring, $j = 14.52$ Hz), 6.92 δ ppm (2H, s, –CH of cytosine ring), 8.13 δ ppm (1H, dd, exocyclic CH of pyrimidine ring, $j = 23.62$ Hz), 8.15 δ ppm (1H, dd, NH of cytosine ring). ^{13}C NMR (400 MHz, DMSO), 26.6 (N-CH_3), 35.9 (C-11), 79.5 (C-5), 96.5 (C-12), 140.9 (C-8), 146.1 (C-7), 162.4 (C-4, C-6), 169.54 (C-10), 172.6 (C-2) δ ppm. FTIR (KBr) υ_{max} cm^{-1}: 1217 (m, –O–C stretching), 1634 (s, =C–NH aliphatic amine), 1708 (s, C=O), 2809 (m, –N–CH_3 streching), 2824 (exocyclic CH), 3082 (m, CH–NH streching). λ max: 292.44 nm; (€: 1.05×10^5 L mol^{-1} cm^{-1}); M.W. 277.24, ESIMS: *m/z* 278.22 (M + 1); Anal. Calcd. $C_{11}H_{13}N_5O_4$ (%): C 47.31, H 4.69, N 25.08. Found (%): C 47.29, H 4.74, N 25.09.

5-((1,6-Dihydro-2-hydroxypyrimidin-4-ylamino)methylene)-dihydro-1,3-dimethyl-2-thioxopyrimidine-4,6(1H,5H)-dione (1,3-TBC)

Orange powder, Yield 65 %; m.p. > 250 °C; ^{1}H NMR (400 MHz, DMSO), 2.78 (6H, s, two CH_3 group of pyrimidine ring), 4.04 δ ppm (1H, dd, exocyclic NH of purine ring, $j = 16.28$ Hz), 6.89 δ ppm (2H, s, –CH of cytosine ring), 8.17 δ ppm (1H, dd, exocyclic CH of pyrimidine ring, $j = 24.62$ Hz), 8.20 δ ppm (1H, dd, NH of cytosine ring). ^{13}C NMR (400 MHz, DMSO), 34.6 (N–CH_3), 39.7 (C-11), 78.8 (C-5), 97.5 (C-12), 139.6 (C-8), 144.5 (C-7), 161.7 (C-4, C-6), 169.54 (C-8), 173.6 (C-2) δ ppm. FTIR (KBr) υ_{max} cm^{-1}: 1212 (m, –O–C stretching), 1628 (s, =C–NH aliphatic amine), 1691 (s, C=O), 2797 (m, –N–CH_3 streching), 2813(exocyclic CH), 3068 (m, CH–NH streching). λ max: 281.17 nm; (€: 0.95×10^5 L mol^{-1} cm^{-1}); M.W. 293.30, ESIMS: *m/z* 295.27 (M + 2); Anal. Calcd. $C_{11}H_{13}N_5O_3S$ (%): C 44.74, H 4.44, N 23.71, Found (%): C 44.71, H 4.46, N 23.70.

5-((Pyridin-2-ylamino)methylene)pyrimidine-2,4,6(1H,3H,5H)-trione (2-BAP)

Yellow powder, Yield 65 %; m.p. > 250 °C; ^{1}H NMR (400 MHz, DMSO), 4.05 δ ppm (1H, dd, exocyclic NH of pyridine ring, $j = 16.38$ Hz), 6.59–8.09 δ ppm (4H, m, –CH of Pyridine ring), 8.23 δ ppm (1H, dd, exocyclic CH of pyrimidine ring, $j = 23.68$ Hz), 10.89 δ ppm (1H, s, NH of pyrimidine ring), 11.21 δ ppm (1H, s, NH of pyrimidine ring). ^{13}C NMR (400 MHz, DMSO), 80.2 (C-5), 108.5 (C-13), 113.7 (C-11), 138.2 (C-12), 147.8, (C-10), 151.3 (C-2), 154.4 (C-7), 164.7 (C-4, C-6) δ ppm. FT-IR (KBr) υ_{max} cm^{-1}: 1218 (m, –O–C stretching), 1622 (=C–NH aliphatic amine), 1697 (C=O), 2815 (exocyclic CH), 3078 (CH–NH streching). λ max: 288.17 nm; (€: 0.95×10^5 L mol^{-1} cm^{-1}); M.W. 232.20, ESIMS: *m/z* 232.18 (M); Anal. Calcd. $C_{10}H_8N_4O_3$ (%): C 51.73, H 3.47, N 24.13, Found (%): C 51.74, H 3.45, N 24.15.

5-((5-Methylthiazol-2-ylamino)methylene)pyrimidine-2,4,6(1H,3H,5H)-trione (BAMT)

Orange powder, Yield 65 %; m.p. > 250 °C; ^{1}H NMR (400 MHz, DMSO), 2.39 (3H, s, –CH_3 group of thiazol ring), 4.06 δ ppm (1H, dd, exocyclic NH of thiazol ring, $j = 15.38$ Hz), 7.17 δ ppm (1H, s, –CH of thiazol ring), 8.17 δ ppm (1H, dd, exocyclic CH of pyrimidine ring, $j = 24.36$ Hz), 10.96 δ ppm (1H, s, NH of pyrimidine ring), 11.26 δ ppm (1H, s, NH of pyrimidine ring). ^{13}C NMR (400 MHz, DMSO), 17.6 (–CH_3), 78.8 (C-5), 120.7 (C-11), 134.9 (C-12), 150.4 (C-2), 161.7 (C-9), 165.3 (C-4, C-6), 167.5 (C-7) δ ppm. FT-IR (KBr) υ_{max} cm^{-1}: 1215 (m, –O–C stretching), 1256 (C–S), 1625 (=C–NH aliphatic amine), 1693 (C=O), 2236 (C–N of thiazol ring), 2794 (–CH_3 streching), 2813(exocyclic CH), 3073 (CH–NH streching). λ max: 281.17 nm; (€: 0.95×10^5 L mol^{-1} cm^{-1}); M.W. 252.25, ESIMS: *m/z* 253.05 (M + 1); Anal. Calcd. $C_9H_8N_4O_3S$ (%): C 42.85, H 3.20, N 22.21, Found (%): C 42.82, H 3.21, N 22.23.

Chemistry

The synthetic strategy adopted to obtain the target compounds is depicted in Scheme 1. Various nucleic acid bases such as adenine, guanine, cytosine, and heterocyclic amines were refluxed with formic acid in aqueous medium at 60 °C to yield formamide derivatives of corresponding DNA bases. Thereafter, in situ barbituric acid and its

Table 1 List of compounds with their different functional groups

Sr. no.	Compound code	R_1	R_2	X	R
1	BA	–H	–H	–O	Adenine
2	TBA	–H	–H	–S	Adenine
3	1,3-BA	$–CH_3$	$–CH_3$	–O	Adenine
4	1,3-TBA	$–CH_3$	$–CH_3$	–S	Adenine
5	BG	–H	–H	–O	Guanine
6	TBG	–H	–H	–S	Guanine
7	BC	–H	–H	–O	Cytosine
8	TBC	–H	–H	–S	Cytosine
9	1,3-BC	$–CH_3$	$–CH_3$	–O	Cytosine
10	1,3-TBC	$–CH_3$	$–CH_3$	–S	Cytosine
11	2-BAP	–H	–H	–O	2-Amino pyridine
12	BAMT	–H	–H	–O	2-Amino 5-methyl thiazole

analogs were refluxed giving the corresponding DNA-based barbiturates Table 1.

Result and discussion

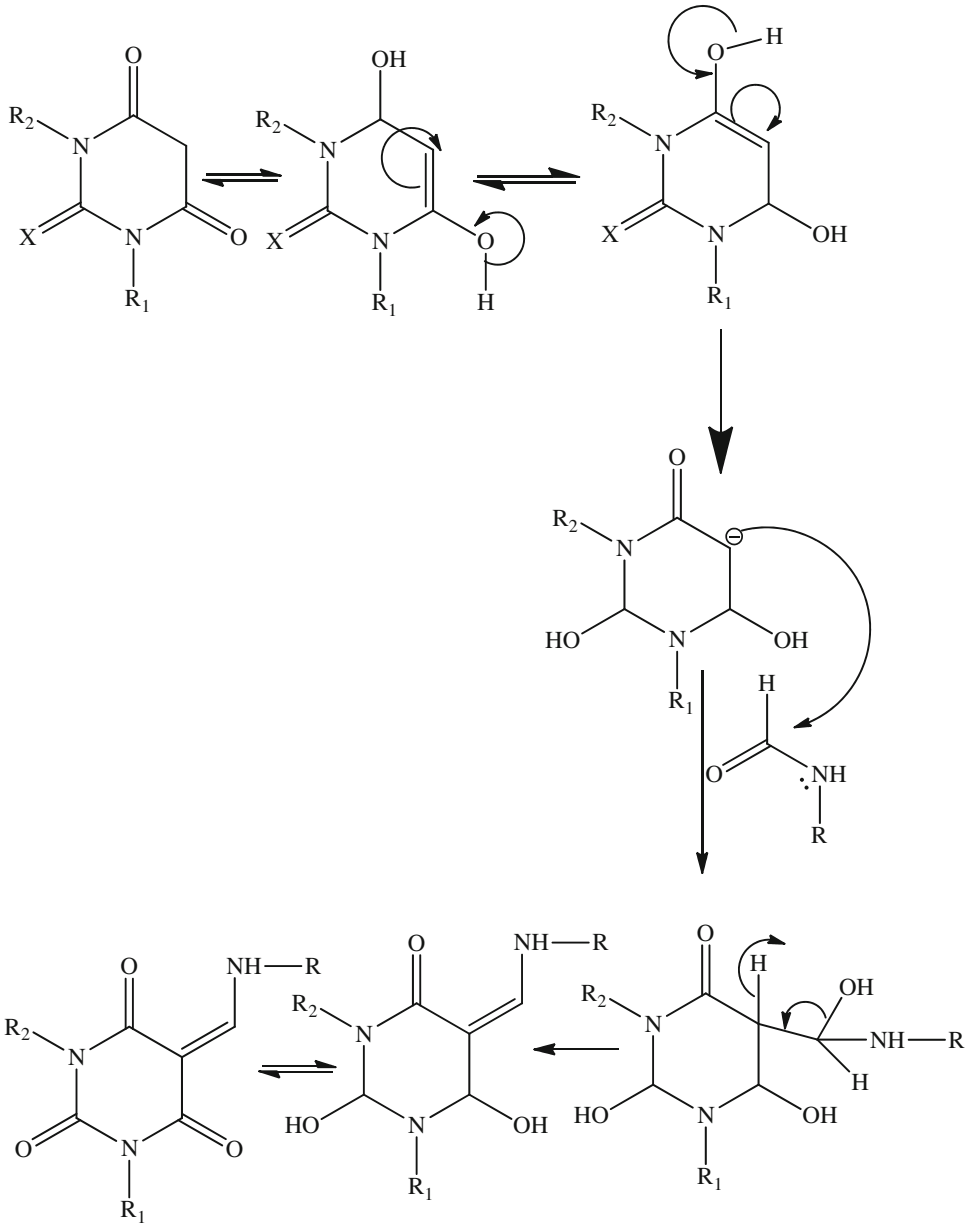
IR, ^{1}H-NMR, ^{13}C-NMR, and UV spectral studies

The important infrared spectral bands and their tentative assignments for DNA-based barbiturates were recorded using KBr disks. IR spectrum of the synthesized compounds showed a characteristic bands between 1,691 and 1,708 cm^{-1} confirming the presence of C=O groups. ^{1}H NMR spectra revealed signals at 3.50 δ ppm for DMSO solvent, between 4.2 and 4.09 δ ppm for NH of the DNA base, between 8.03 and 8.17 δ ppm for exocyclic CH group, and between 8.09 and 11.27 δ ppm for NH of pyrimidine ring. From ^{13} C NMR spectra, exocyclic CH signal was observed between 144.5 and 158.25 δ ppm. The UV absorption spectra were made using DMSO as a solvent in concentrations (10^{-5} M). All synthesized new DNA-based barbiturates derivatives showed the strong absorption bands (λ max) in the range 275–305 nm owing to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions as well as presence of chromophoric exocyclic CH of pyrimidine ring in their UV spectra (Silverstein and Webster, 1997); Scheme 3.

Cytotoxicity and antitumor evaluation of the synthesized compounds

Samples were prepared for assay by dissolving in 50 ml of dimethyl sulfoxide (DMSO), and diluting aliquots into sterile culture medium at 0.4 mg ml^{-1}. These solutions were sub-diluted to 0.02 mg ml^{-1} in sterile medium, and the two solutions were used as stocks to test samples at 100, 50, 20, 10, 5, 2, and 1 mg ml^{-1} in triplicate in the wells of microtiter plates. The compounds were assayed in triplicate on monolayers grown in Dulbecco's modified Eagle's medium supplemented with 10 % (v/v) calf serum (HyClone Laboratories, Ogden, UT), and 60 mg ml^{-1} Penicillin G, and 100 mg ml^{-1} streptomycin sulfate maintained at 37 °C in a humidified atmosphere containing about 5 % CO_2 in air. All medium components were obtained from Sigma Chemical Co., St. Louis, MO. Subcultures of cells for screening were grown in the wells of microtiter trays (Falcon Microtest III 96-wells trays, Becton–Dickinson Labware, Lincoln Park, NJ) by suspending cells in medium following trypsin–EDTA treatment, counting the suspension with a hemocytometer, diluting in medium containing 10 % calf serum to 2×10^4 cells per 200 ml culture, aliquoting into each well of a tray, and culturing until confluent. Microtiter trays with confluent monolayer cultures of cells were inverted, the medium shaken out, and replaced with serial dilutions of sterile compounds in triplicate. In each tray, the last row of wells was reserved for controls that were not treated with compounds. Trays were cultured for 96 h. Trays were inverted onto a paper towel pad, the remaining cells rinsed carefully with medium, and fixed with 3.7 % (v/v) formaldehyde in saline for at least 20 min. The fixed cells were rinsed with water, and examined visually. The IC_{50} was estimated as the concentration that caused approximately 50 % loss of the monolayer. 5-Fluorouracil was used as a positive control. (Fadda *et al.*, 2012) The results of this preliminary screening indicated that **1,3-BA** showed the strongest cytotoxicity activity against HEPG-2 and lower but moderate cytotoxicity activity against WI-38, VERO, and MCF-7 cell lines; whereas **TBC** showed the strongest cytotoxicity activity against all four cell lines, followed by TBA which showed a moderate cytotoxicity against all

Scheme 3 Plausible reaction mechanism pathway for Knoevenagel condensation in water



four cancer cell lines. On the hand, **1,3-TBC** and **BPA** exhibited a moderate cytotoxicity against MCF-7 and HEPG-2 cell lines, and weak but significant activity against WI-38, VERO. Following this further **BA, 1,3-TBA, BG, TBG, BC, 1,3-BC DMBA,** and **BPA** showed a weak cytotoxicity activity against all four cancer cell lines (Table 2).

Structure–activity trends within the synthesized library

Subsequently, we may conclude the following structure–activity relationship (SAR). The presence of the barbiturate moiety is necessary for the broad spectrum cytotoxic activity of the synthesized compounds toward different cell lines (HepG2, WI-38, VERO and MCF-7). Compounds having adenine, guanine, cytosine, 2-amino pyridine, or 2-amino, 5-methyl thiazole as a nucleobase, with (R_1 & R_2 =H) and (X=O), show a weak cytotoxicity activity with respect to the standard drug (5-Fluorouracil) against cancer cell lines (WI-38, VERO, MCF-07, and HEPG-2), while compounds having adenine as a DNA base with (R_1 and R_2 =H) and (X=S), show a moderate cytotoxicity activity against the aforementioned cancer cell lines. Thus, **TBA** is more potent than **BA** due to presence of more electronegative group.

Following this further, compounds having adenine as a DNA base with (R_1 and R_2 =Me) and (X=O), show a differential cytotoxic activity. They have a very strong cytotoxic activity against (HEPG-2), while they have a weak cytotoxic activity against (WI-38, VERO, and MCF-7).

Table 2 Cytotoxicity of the synthesized compounds

Compound code	IC_{50} (μM)[a]			
	WI-38	VERO	MCF-7	HEPG-2
BA	98 ± 0.08	84 ± 0.14	74 ± 1.34[b]	69 ± 0.29
TBA	25 ± 0.17	28 ± 0.12	32 ± 1.32[b]	34 ± 0.29
1,3-BA	62 ± 0.05	59 ± 0.13	55 ± 1.30[b]	7 ± 0.15
1,3-TBA	95 ± 0.19	88 ± 0.15	78 ± 1.35[b]	63 ± 0.21
BG	75 ± 0.34	85 ± 0.11	80 ± 0.35	75 ± 0.35
TBG	90 ± 0.14	89 ± 0.18	78 ± 1.24[b]	72 ± 0.21
BC	97 ± 0.13	90 ± 0.12	60 ± 1.32[b]	56 ± 0.21
TBC	18 ± 0.12	24 ± 0.15	16 ± 1.74[b]	16 ± 0.23
1,3-BC	82 ± 0.12	70 ± 0.15	64 ± 1.25[b]	70 ± 0.23
1,3-TBC	110 ± 0.05	95 ± 0.05	30 ± 0.05	25 ± 0.32
2-BAP	105 ± 0.12	85 ± 0.04	30 ± 0.01	25 ± 0.05
BAMT	80 ± 0.25	70 ± 0.22	85 ± 0.22	82 ± 3.01
5-FU	10 ± 0.05	08 ± 1.02	10 ± 0.48	05 ± 1.36

[a] (IC_{50}, μM): 1–10 (very strong), 11–25 (strong), 26–50 (moderate), 51–100 (weak), 100–200 (very weak), above 200 (non-cytotoxic)

[b] Values are means of three replicates ± SD, and significant difference at $p < 0.05$ by Student's t test

Accordingly, **1,3 BA** is more potent than **BA** due to presence of electron releasing group (Me group), but less potent than **TBA** due to thioxo group. Surprisingly, the inclusion of methyl groups at R_1 and R_2 together with sulphur group at X position on adenine-based barbiturate derivatives, as shown in compound **1,3 TBA**, significantly attenuated the cytotoxic activity against all four cell lines.

Substitution of adenine base in **TBA** by guanine nucleobase to give **TBG** compound significantly diminished the cytotoxicity against all the four cancer cell lines, while the substitution by cytosine nucleobase as shown in **TBC** significantly enhanced the cytotoxic activity of **TBA**. Introducing methyl group at R_1 and R_2 positions in **TBC** to give compound (**1,3-TBC**) had showed a variable activity ranging from a weak cytotoxicity against both WI-38, VERO to strong cytotoxicity against MCF-7 and HEPG-2 cell lines.

Conclusion

In conclusion, we have designed hybrid molecules on the basis of the biological significance of nucleobase and barbituric acid, and evaluated for their anticancer activities. Out of a set of 12 molecules, three compounds exhibit significant anticancer activities and could be used as leads for further investigations.

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