Journal of Research in Chemistry

E-ISSN: 2709-9423
P-ISSN: 2709-9415
JRC 2020; 1(1): 26-35

www.chemistryjournal.net
Received: 18-11-2020
Accepted: 23-12-2020

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Nucleobases-Based Barbiturates: Synthesis, Characterization and study of their towards Control on Cell Proliferation

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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 probes were designed and synthesized by combining the structural features of nucleobases 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. The newly synthesized 12 compounds were evaluated for their Cell Proliferation assay against Breast cancer cell lines (MCF-07) and (ZR-75). All compounds shows good to moderate activity against both cell lines.

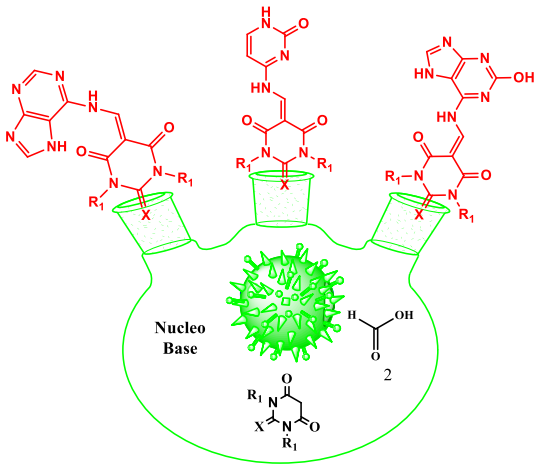


Fig 1: Graphical abstract

Keywords: Green Chemistry, Barbiturates, Cell proliferation assay

1. Introduction
The importance of barbiturates and thiobarbiturates is well established in pharmaceutical chemistry and drug design [1]. The medicinal importance of pyrimidine derivatives barbituric acid and thiobarbituric are significant among various heterocyclic compounds owing to anti-neoplastic, antiviral, antibiotic and anti-inflammatory including other biological activities [2]. Barbiturates are general central nervous system depressants that are used clinically for anesthetic, sedative-hypnotic, and anticonvulsant actions. The site (or sites) of action of these drugs is generally considered to be the excitable nerve membrane, perhaps involving a decrease in excitatory synaptic transmission or an increase in inhibitory synaptic transmission [3]. 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 is 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.

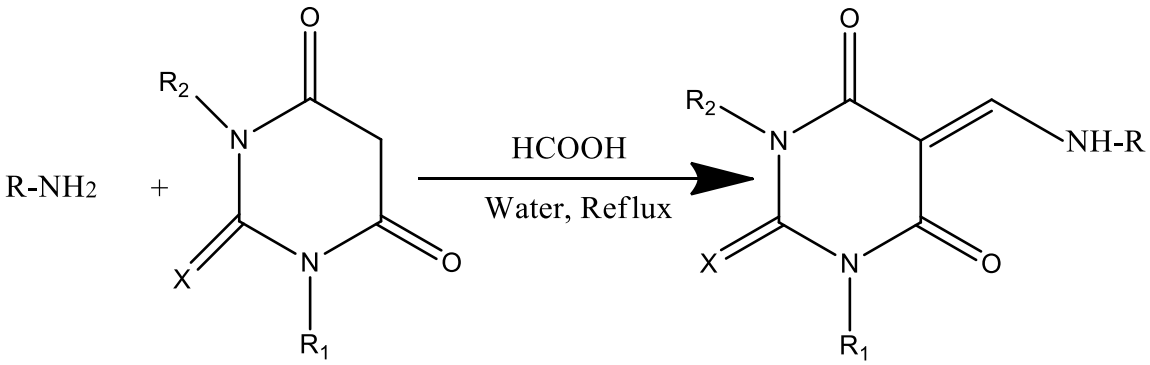
The fundamental role of small chemical leads (such as amino acids and nucleobases) and the acceptability of their synthetic analogues in the biological systems may be the reason for the design and development of about 80% of the drugs. Moreover, a suitable combination of the structural features of two 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 anti-conversant, [4] anti-hypnotic, [5] anticancer [2] and anti-inflammatory, we aimed to suitably combine these two molecular entities through N-formulation of 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 % control on cell proliferation their over 2 cell line panel of breast cancer.

Barbiturates have been in the center of attention of researchers over many years because of the high practical value of these compounds. In the first place, barbiturates are very important class of compounds, for their high reactivity in synthesis, as key starting materials to form various classes of biologically and pharmacologically active candidates. The diverse biological activity and coverage of a broad chemical space make barbituric acid and thiobarbituric acid derivatives excellent target compounds for organic and medicinal chemists. Owing to their ready availability and various functionalization possibilities, the parent barbituric acid and thiobarbituric acid are convenient starting materials for the preparation of different fused heterocyclic and literature survey also ascribes that 5-substituted derivatives are pharmacologically active compounds [6]. The medicinal importance of pyrimidine derivatives such as barbituric acid and thiobarbituric acid play vital role among various heterocyclic compounds due to their anti-neoplastic [7] antiviral [8], antibiotic [9], and anti-inflammatory [10] activity. The pyrimidine ring system is present in various natural compounds such as nucleic acids, vitamins, coenzymes, uric acid, purines, and some marine microorganisms (e.g., sponge) [11]. In view of these facts and in continuation of our interest in the synthesis of a variety of heterocyclic of biological importance, we report here an efficient and convenient method for the synthesis of novel nucleon based barbiturates derivatives attached to pyrimidine moiety.

2. Result and discussion

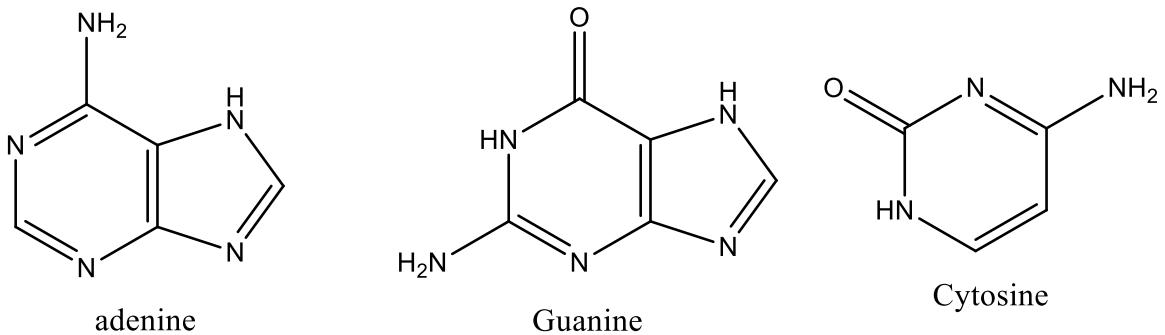
2.1 Chemistry

The required barbiturates were synthesized by Hantzch Reaction according to literature procedure [12]. In this study, a series of 5-subtituted barbiturates has been synthesized by one pot three component reaction using condensation reaction of various Nucleobases 1, Formic acid 2 and Barbiturates 3 in aqueous medium gives moderate to good yields (58-88) Scheme 1. In accordance with the mechanism suggested in literature (Scheme 2 and Scheme 3) [13]. The first step of this process may involve the *N-Formulation* of Nucleobases with formic acid to form corresponding N-Form amide analogues. The second step involves Knoevenagel condensation reaction of N-form amide with barbiturates to form *>C=CH-NH-* of the adducts under the same condition.

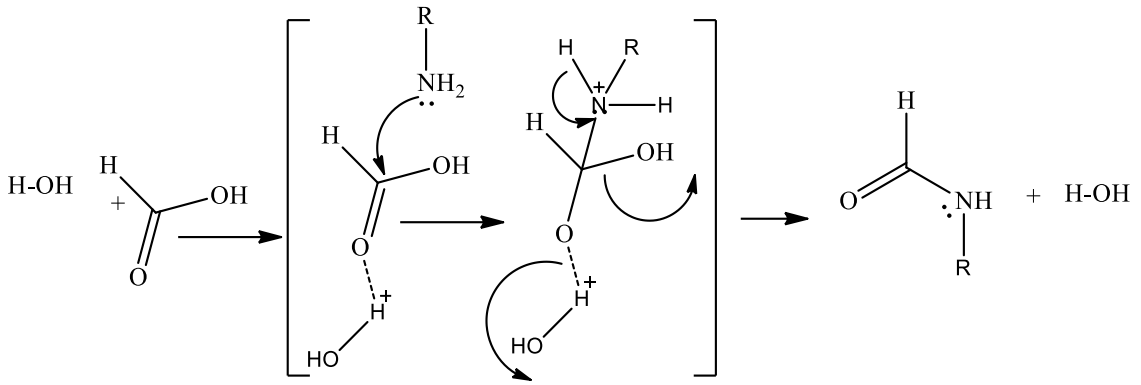


Where X= O or S and R1 & R2= -H or -CH3

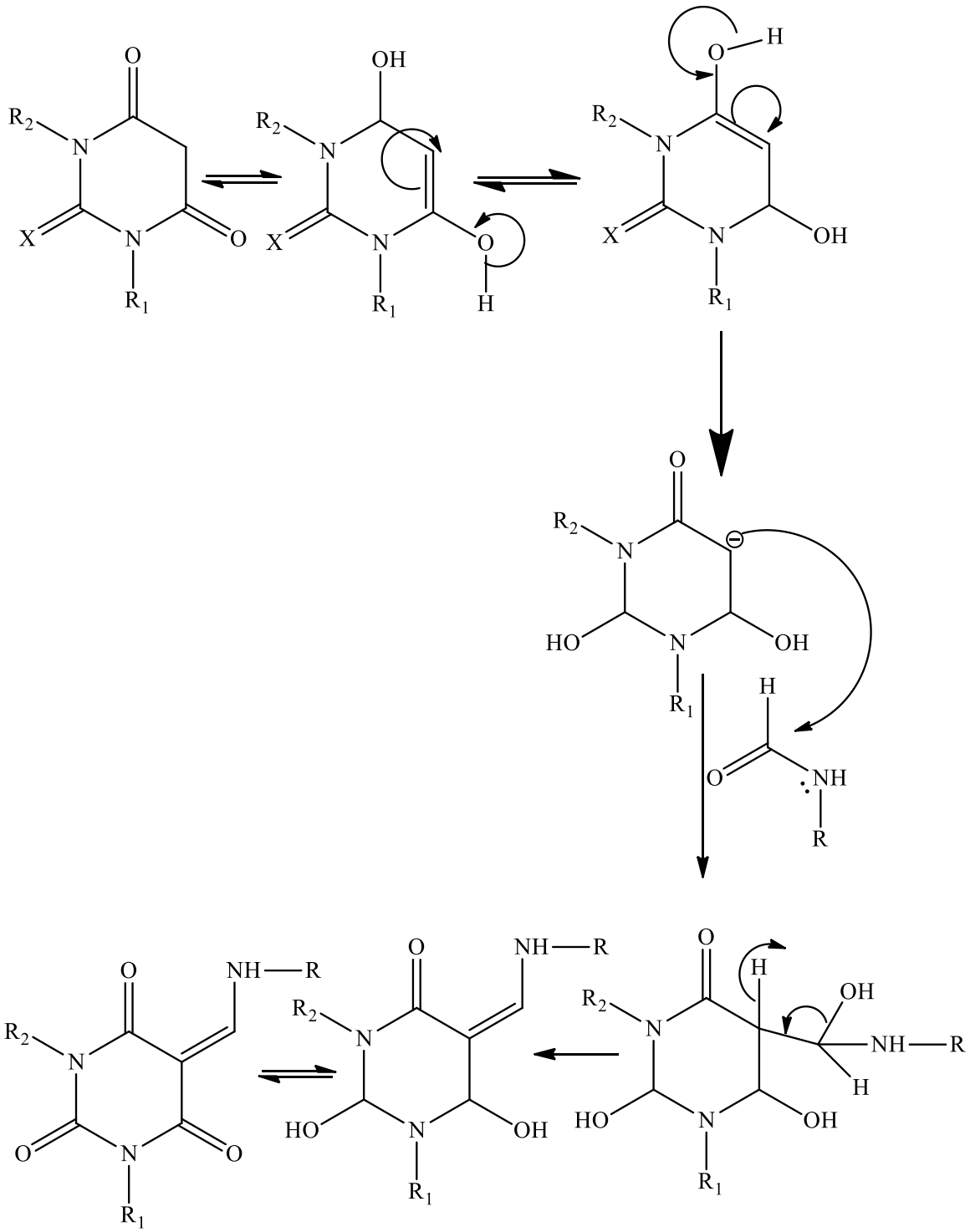
Where R are different Nucleo-base Amines



Scheme 1: Synthesis nucleosides of barbiturates



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



Scheme 3: Plausible reaction mechanism pathway for Knoevenagel condensation in water

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	1,3-BG	$-CH_3$	$-CH_3$	-O	Guanine
8	1,3-TBG	$-CH_3$	$-CH_3$	-S	Guanine
9	BC	-H	-H	-O	Cytosine
10	TBC	-H	-H	-S	Cytosine
11	1,3-BC	$-CH_3$	$-CH_3$	-O	Cytosine
12	1,3-TBC	$-CH_3$	$-CH_3$	-S	Cytosine

The structures of all the new synthesized compounds were established by FT-IR, ^{1}H NMR, ^{13}C-NMR, HMBC, Elemental Analysis and molecular weight confirmed by mass spectrometry.

The important infrared spectral bands and their tentative assignments for were recorded on a Perkin Elmer - Spectrum RX-IFTIR using KBR disks. IR spectrum of the synthesized compounds showed a characteristic bands between 1691 cm^{-1} and 1708 cm^{-1} confirming the presence of C=O groups.

^{1}H NMR spectra were recorded on an Avance-II (Bruker) model using DMSO (d_6) as a solvent and TMS as internal standard with ^{1}H resonant frequency of 400 MHz. ^{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.

^{13}C NMR spectra were recorded on an Avance-II (Bruker) model using DMSO (d_6) as a solvent and TMS as internal standard with ^{13}C resonant frequency of 400 MHz. From ^{13}C NMR spectra, exocyclic CH signal was observed between 144.5 to 158.25 δ ppm.

U.V. Spectra were recorded on Maya pro 2000 (Ocean Optics USA) using DMSO as a solvent with 10^{-5} M solution. 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 π→ π* and n→ π* transitions as well as presence of chromophoric exocyclic CH of Pyrimidine ring in their UV spectra [14].

2.2 Cell proliferation assay

2.3 Structure Activity Relationship (MCF-7)

Case (1) Testing in 5% fetal bovine Serum (FBS) RPMI Media

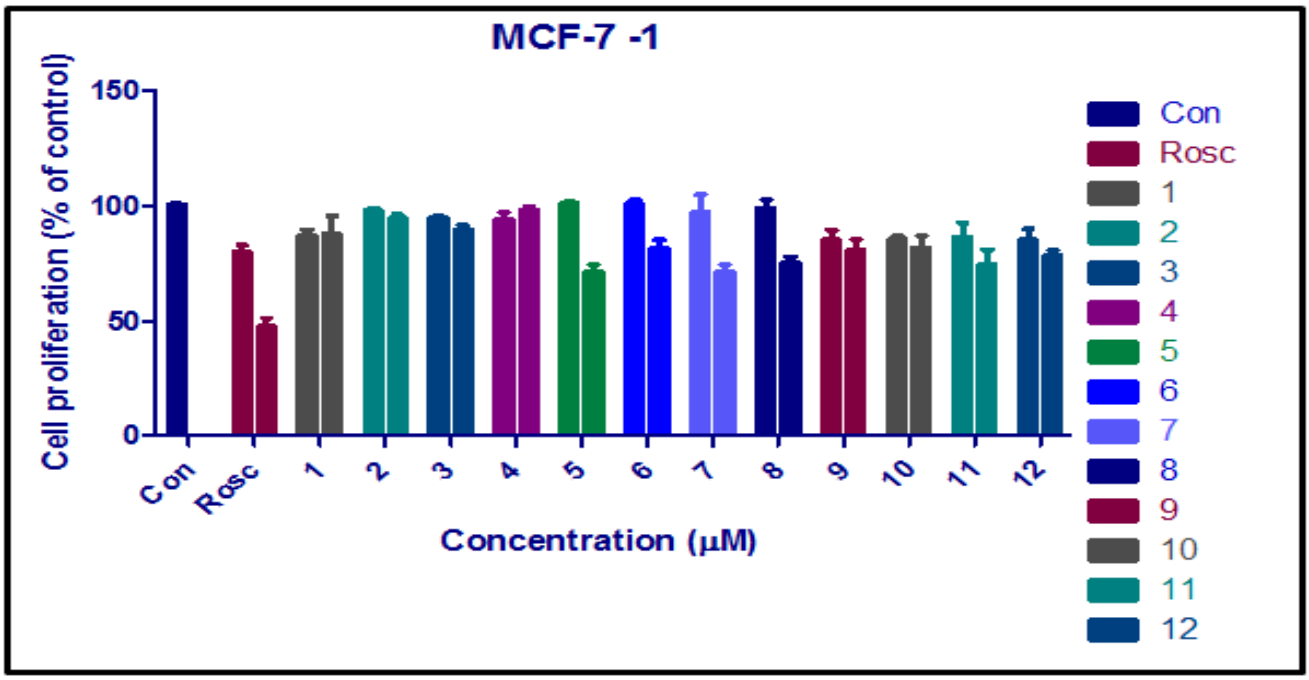


Fig 1: % FBS RPMI media 1µ molar and 10 µ molar Concentration of the drugs

Compound (BA) having (R= adenine as a DNA base), (R_1 & R_2=-H) and (X=O), shows good % cell proliferation control with respect to standard drug (R-Roscovitine) against breast cancer cell-line (MCF-07), While Compound (TBA) (R= adenine as a DNA base), (R_1 & R_2=-H) and (X=S), is more potent towards than (BA) % cell proliferation control with respect to (BA) due to presence of more electro negative (Oxo) than (thioxo) group. Compound (1, 3-BA) having (R= adenine as a DNA base), (R_1 & R_2=-Me) and (X=O), shows more in % cell proliferation control with respect to compound (BA) but less potent than compound (TBA). Here more with respect to (BA) due to presence of electron releasing group ($-CH_3$) group and Less with respect to (TBA) due to (thioxo) group. Compound (1, 3-TBA) having (R= adenine as a DNA base), (R_1 & R_2=-Me) and (X=S) is shows comparable % cell proliferation control with respect to (1, 3-BA) and more with respect to (BA and TBA) due to presence of both (X=S and R_1 & R_2=-Me) groups. Compound (BC) having (R= Cytosine as a DNA base), (R_1 & R_2=-H) and (X=O), shows more % cell proliferation control with respect to standard drug (R-Roscovitine) against breast cancer cell-line (MCF-07), While Compound (TBC) (R= Cytosine as a DNA base), (R_1 & R_2=-H) and (X=S), shows more % cell proliferation control with respect to (BC) due to presence of more electro negative (-Oxo) than (thioxo) group. Compound (1, 3-BC) having (R=

Cytosine as a DNA base), (R_1 & R_2=-CH_3) and (X=O), shows more in % cell proliferation control with respect to compound (BC) but less potent than compound (TBC). Here more with respect to (BC) due to presence of electron releasing group (-CH_3) group and Less with respect to (TBC) due to (thioxo) group. Compound (1, 3-TBC) having (R= Cytosine as a DNA base), (R_1 & R_2=- CH_3) and (X=S) is shows comparable % cell proliferation control with respect to (1, 3-BC) and more with respect to (BC and TBC) due to presence of both (X=S and R_1 & R_2=- CH_3) groups. Compound (BG) having (R= Guanine as a DNA base), (R_1 & R_2=-H) and (X=O), shows good % cell proliferation control with respect to standard drug (R-Roscovitine) against breast cancer cell-line (MCF-07), While Compound (TBG) (R= Guanine as a DNA base), (R_1 & R_2= H) and (X=S), shows comparable % cell proliferation with respect to (BG). Compound (1, 3-BG) having (R=Guanine as a DNA base), (R_1 & R_2=-CH_3) and (X=O), shows comparable % cell proliferation control with respect to compound (BG) and (TBG). Here more with respect to (BG) due to presence of electron releasing group (-CH_3) group and less with respect to (TBG) due to (thioxo) group. Compound (1, 3-TBG) having (R=Guanine as a DNA base), (R_1 & R_2= -CH_3) and (X=S) is shows comparable % cell proliferation control with respect to (BG), (TBG) and (1, 3-BG) due to presence of (-OH) group in Guanine and electron releasing –CH_3 group.

Case (2) Testing in 5% dextran coated charcoal-striped Serum (DCC)

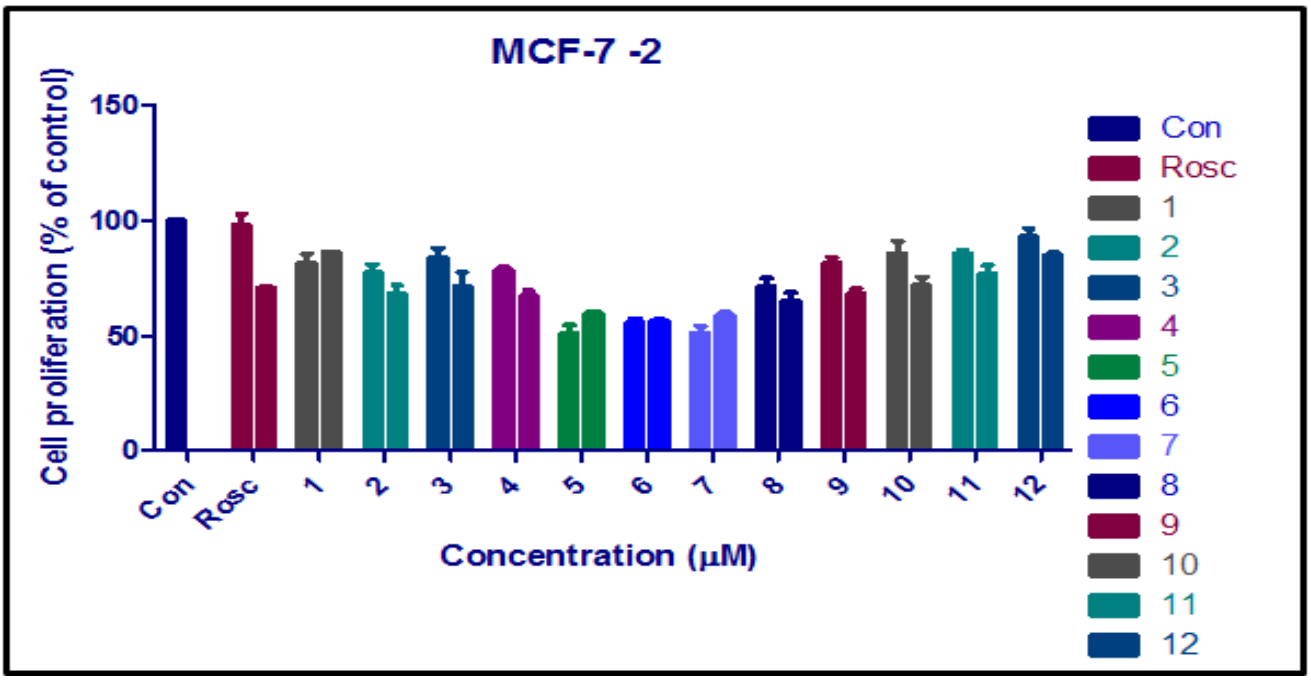


Fig 2.5: % FBS RPMI media 1µ molar and 10 µ molar Concentration of the drugs

Compound (BA) having (R= adenine as a DNA base), (R_1 & R_2=-H) and (X=O), shows less % cell proliferation control with respect to standard drug (R-Roscovitine) against breast cancer cell-line (MCF-07), While Compound (TBA) (R= adenine as a DNA base), (R1 & R2=-H) and (X=S), shows % cell proliferation control than (BA) and standard drugs due to presence of less electro negative (-Oxo) than (thioxo) group. Compound (1, 3-BA) having (R= adenine as a DNA base), (R_1 & R_2=- CH_3) and (X=O), shows comparable in % cell proliferation control with respect to compound (BA) but more potent than compound (TBA). Here more with respect to (TBA) due to presence of electron releasing (-CH_3) group and more with respect to (TBA) due to (thioxo) group. Compound (1, 3-TBA) having (R= adenine as a DNA base), (R_1 & R_2=- CH_3) and (X=S) is shows comparable % cell proliferation control with respect to (TBA) and less with respect to (BA and 1, 3-BA) due to presence of both (X= -O and R_1 & R_2=- CH_3) groups.

Compound (BC) having (R= Cytosine as a DNA base), (R_1 & R_2=-H) and (X=O), shows less % cell proliferation control with respect to standard drug (R-Roscovitine) against breast cancer cell-line (MCF-07), While Compound (TBC) (R= Cytosine as a DNA base), (R_1 & R_2=-H) and (X=S), shows % cell proliferation control with respect to (BC) and standard drugs due to presence of more electro negative (Oxo) than (thioxo) group. Compound (1, 3-BC) having (R= Cytosine as a DNA base), (R_1 & R_2=-Me) and (X=O), shows more in % cell proliferation control with respect to compound (TBC) and comparable with respect to compound (BC). Here more with respect to (TBC) due to presence of electron releasing group (Me) group. Compound (1, 3-TBC) having (R= Cytosine as a DNA base), (R_1 & R_2=-CH_3) and (X=S) is shows more % cell proliferation control with respect to (BC, TBC & 1, 3-BC) due to presence of both (X=S and R_1 & R_2=- CH_3) groups.

Compound (BG) having (R= Guanine as a DNA base), (R1 & R2=-H) and (X=O), shows lower % cell proliferation control with respect to standard drug (R-Roscovitine) against breast cancer cell-line (MCF-07), While Compound (TBG) (R= Guanine as a DNA base), (R_1 & R_2=-H) and (X=S), shows more % cell proliferation with respect to (BG) due to presence of more electro-negative. Compound (1, 3-BG) having (R=Guanine as a DNA base), (R_1 & R_2=-CH_3) and (X=O), shows comparable % cell proliferation control with respect to compound (BG) and less with respect (TBG). Here less with respect to (TBG) due to presence of electron releasing (-CH_3) group and Less with respect to (TBG) due to (thioxo) group. Compound (1, 3-TBG) having

(R= Guanine as a DNA base), (R1 & R2=- CH_3) and (X=S) is shows more % cell proliferation control with respect to (BG), (TBG) and (1, 3-TBG) due to presence of (-OH) group in Guanine.

Structure Activity Relationship in case of breast cancer (ZR-75) Cell lines

Case (1) Testing in 5% fetal bovine Serum (FBS)

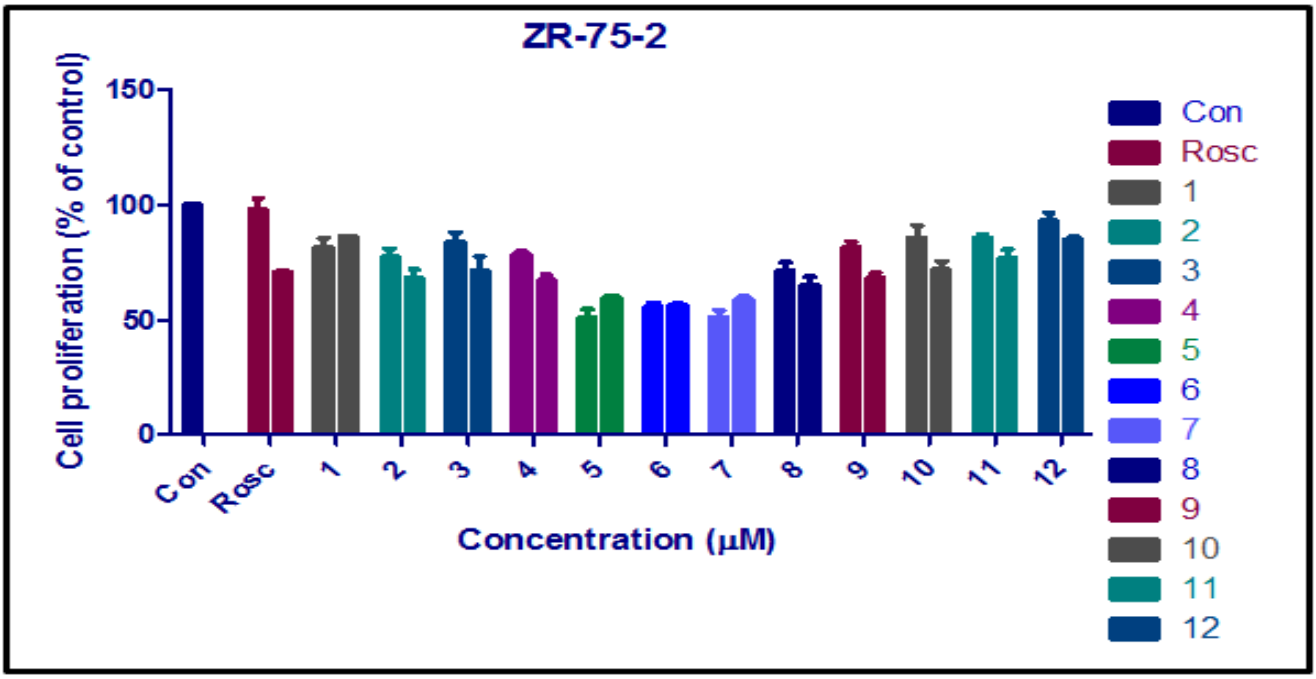


Fig 3.5: % FBS RPMI media 1µ molar and 10 µ molar Concentration of the drugs

Compound (BA) having (R= adenine as a DNA base), (R_1 & R_2=-H) and (X=O), shows higher % cell proliferation control with respect to standard drug (R-Roscovitine) against breast cancer cell-line (ZR-75), While Compound (TBA) (R= adenine as a DNA base), (R_1 & R_2=-H) and (X=S), shows lower % cell proliferation control with respect to (BA) due to presence of more electro negative (-Oxo) than (thioxo) group. Compound (1, 3-BA) having (R= adenine as a DNA base), (R_1 & R_2=-CH_3) and (X=O), shows lower in % cell proliferation control with respect to compound (BA & TBA). Here less with respect to (BA & TBA) due to presence of electron releasing group (-CH_3) and (thioxo) group. Compound (1, 3-TBA) having (R= adenine as a DNA base), (R_1 & R_2=- CH_3) and (X=S) lower % cell proliferation control with respect to (BA, TBA & 1, 3-BA) due to presence of both (X=S and R_1 & R_2=- CH_3) groups. Compound (BC) having (R= Cytosine as a DNA base), (R_1 & R_2=-H) and (X=O), shows more % cell proliferation control with respect to standard drug (R-Roscovitine) against breast cancer cell-line (ZR-75), While Compound (TBC) (R= Cytosine as a DNA base), (R_1 & R_2=-H) and (X=S), shows comparable % cell proliferation control with respect to (TBC) due to presence of more electro negative (Oxo) than (thioxo) group. Compound (1, 3-BC) having (R= Cytosine as a DNA base), (R_1 & R_2=- CH_3) and (X=O), shows more in % cell proliferation control with respect to compound (BC) and comparable with the compound (TBC). Here more with respect to (BC) due to presence of electron releasing group (-CH_3) group and comparable with respect to (TBC) due to (thioxo) group. Compound (1, 3-TBC) having (R= Cytosine as a DNA base), (R_1 & R_2=-CH_3) and (X=S) is shows more % cell proliferation control with respect to (BC) and less with respect to (TBC and 1, 3-BC) due to presence of both (X=S and R_1 & R_2=-CH_3) groups. Compound (BG) having (R= Guanine as a DNA base), (R_1 & R_2=-H) and (X=O), shows higher % cell proliferation control with respect to standard drug (R-Roscovitine) against breast cancer cell-line (ZR-75), While Compound (TBG) (R= Guanine as a DNA base), (R_1 & R_2=-H) and (X=S), shows comparable % cell proliferation with respect to (BG) more with respect to standard drug. Compound (1, 3-BG) having (R=Guanine as a DNA base), (R_1 & R_2=-CH_3) and (X=O), shows higher % cell proliferation control with respect to compound (BG) and (TBG). Here more with respect to (BG & TBG) due to presence of electron releasing group (CH_3) group. Compound (1, 3-TBG) having (R= Guanine as a DNA base), (R_1 & R_2=- CH_3) and (X=S) is shows comparable % cell proliferation control with respect to (1, 3-BG) due to presence of (-OH) group in Guanine and higher with respect to (BG & TBG).

Case (2) Testing in 5% dextran coated charcoal-striped Serum (DCC).

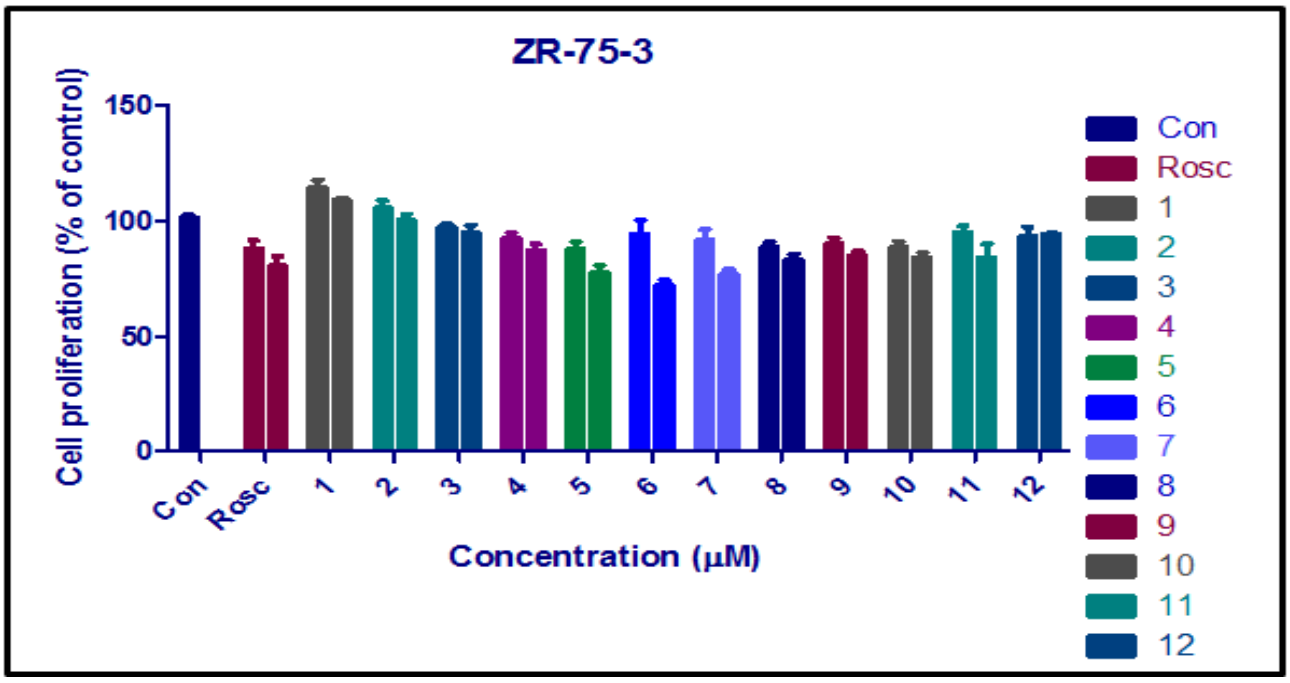


Fig 4. 5: % FBS RPMI media 1µ molar and 10 µ molar Concentration of the drugs

Compound (BA) having (R= adenine as a DNA base), (R_1 & R_2=-H) and (X=O), shows lower % cell proliferation control with respect to standard drug (R-Roscovitine) against breast cancer cell-line (ZR-75), While Compound (TBA) (R= adenine as a DNA base), (R_1 & R_2=-H) and (X=S), shows less % cell proliferation control than (BA) due to presence of more electro negative (-Oxo) than (-thioxo)group. Compound (1, 3-BA) having (R= adenine as a DNA base), (R1 & R2=-CH_3) and (X=O), shows comparable in % cell proliferation control with respect to compound (BA) but more than compound (TBA). Here more with respect to (TBA) due to presence of electron releasing nature of (-CH_3 group). Compound (1, 3-TBA) having (R= adenine as a DNA base), (R_1 & R_2=-CH_3) and (X=S) is shows comparable % cell proliferation control with respect to (TBA) and more with respect to (BA and 1, 3-BA) due to presence of both (X=S and R_1 & R_2=-CH_3) groups. Compound (BC) having (R= Cytosine as a DNA base), (R_1 & R_2=-H) and (X=O), shows more % cell proliferation control with respect to standard drug (R-Roscovitine) against breast cancer cell-line (ZR-75), While Compound (TBC) (R= Cytosine as a DNA base), (R_1 & R_2=-H) and (X=S), shows comparable % cell proliferation control with respect to (BC) due to presence of more electro negative (-Oxo) than (-thioxo) group. Compound (1, 3-BC) having (R= Cytosine as a DNA base), (R_1 & R_2=-CH_3) and (X=O), shows more in % cell proliferation control with respect to compound (BC and TBC). Compound (1, 3-TBC) having (R= Cytosine as a DNA base), (R_1 & R_2=- CH_3) and (X=-S) is shows more % cell proliferation control with respect to (BC, TBC, 1, 3-BC) due to presence of both (X=S and R_1 & R_2=- CH_3) groups. Compound (BG) having (R= Guanine as a DNA base), (R_1 & R_2=-H) and (X=O), shows lower % cell proliferation control with respect to standard drug (R-Roscovitine) against breast cancer cell-line (ZR-75), While Compound (TBG) (R= Guanine as a DNA base), (R_1 & R_2=-H) and (X=S), shows more % cell proliferation with respect to (BG). Compound (1, 3-BG) having (R=Guanine as a DNA base), (R_1 & R_2=- CH_3) and (X=O), shows comparable % cell proliferation control with respect to compound (TBG) and more with respect to (BG). Here more with respect to (BG) due to presence of electron releasing group (-CH_3) group and comparable with respect to (TBG) due to (-thioxo) group, Compound (1,3-TBG) having (R= Guanine as a DNA base), (R_1 & R_2=-CH_3) and (X=S) is shows more % cell proliferation control with respect to (BG), (TBG) and (1,3-TBG) due to(-thioxo) presence of (-OH) group in Guanine.

3. Experimental

The barbituric acid was synthesized by using diethyl maleate and urea using the standard procedure [12]. Melting points were determined in open capillaries on a Veego electronic apparatus VMP-D (Veego Instrument Corporation, Mumbai, India). IR spectra (4000-400 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 x 7.5 cm) coated with silica gel-G and spots were visualized under UV irradiation. ^{1}H 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 ^{1}H resonant frequency of 400 MHz and ^{13}C resonant frequency of 100 MHz. The ^{1}H 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; m, multiplet. U.V. Spectra were recorded on Maya pro 2000 (Ocean Optics USA) using DMSO as a solvent with 10^{-5} M solution.

3.1 Cell Proliferation

Cell proliferation rates were measured in 96-well plate by using traditional MTT assay. Breast cancer cells ZR-75 or MCF-7 (2×10^3 cells per well) were seeded in RPMI medium containing 10% fetal bovine serum or 5% DCC treated serum. After 24 hours of incubation, cells were treated with varying concentrations of various compounds for 72 hours. Then the cells were further incubated in 37^0C by adding 25 µl of working MTT reagent (5 mg/mL in PBS). Cells were

then lysed in 100 µl of DMSO per well and measured at 490 nM with Bio-Rad micro plate reader, model 550. Cell proliferation rates were expressed in % of control. [15-17]

3.2 General procedure of preparation Nucleosides of 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 hrs. 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 hrs. 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 [13].

3.3 Characterization data of synthesized compounds

3.3.1 5–[(9H-purin-6-ylamino)-methylene]-pyrimidine-2, 4, 6-trione (BA)

Yellow powder, Yield 77%; M.P. >250°C; ^{1}H 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, end cyclic –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 stretching). λ max: 303.22 nm; (€: 1.10 x 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.

3.3.2 5–[(9H-purin-6-ylamino)-methylene]-2-thioxo-dihydro-pyrimidine-4,6-dione (TBA)

Yellow powder, Yield 58%; M.P. >250°C; ^{1}H 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), 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 stretching). λ max: 299 nm; (€: 1.03 x 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.

3.3.3.1 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<u>H$_3$</u> group of pyrimidine ring), 5.67 δ ppm (1H, DD, exocyclic N<u>H</u> of purine ring, *j*=14.08 Hz), 6.85 δ ppm (2H, s,-C<u>H</u> of purine ring), 8.09 δ ppm (1H, DD, exocyclic C<u>H</u> of pyrimidine ring, , *j*=24.94 Hz), 8.16 δ ppm (1H, DD, N<u>H</u> 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$_3$ stretching), 2816 (exocyclic CH), 3070(m, CH-NH stretching). λ max: 303.22 nm; (€: 1.00 x 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.

3.3.4 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 C<u>H$_3$</u> group of pyrimidine ring), 4.03 δ ppm (1H, DD, exocyclic N<u>H</u> of purine ring, *j*=15.28 Hz), 6.90 δ ppm (2H, s,-C<u>H</u> of purine ring), 8.14 δ ppm (1H, DD, exocyclic C<u>H</u> 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 x 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$_3$ stretching), 2812 (exocyclic CH), 3081(m, CH-NH stretching). 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.

3.3.5 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 N<u>H</u> of purine ring, *j*=15.68 Hz), 6.97 δ ppm (1H, s,-C<u>H</u> of purine ring), 8.06 δ ppm (1H, DD, exocyclic C<u>H</u> of pyrimidine ring, *j*=24.76 Hz), 8.15 δ ppm (1H, DD, N<u>H</u> of purine ring), 11.15 δ ppm (1H, s, N<u>H</u> of pyrimidine ring), 11.27 δ ppm (1H, s, N<u>H</u> of pyrimidine ring), 12.30 δ ppm (1H, s, O<u>H</u> 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 stretching) 3345 (, C-OH). λ max: 276 nm; (€: 0.95 x 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.

3.3.6 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 N<u>H</u> of purine ring, *j*=14.98 Hz), 6.93 δ ppm (2H, s,-C<u>H</u> of purine ring), 8.03 δ ppm (1H, DD, exocyclic C<u>H</u> of pyrimidine ring, *j*=23.26 Hz), 10.97 δ ppm (1H, DD, N<u>H</u> of purine ring), 11.17 δ ppm (1H, s, N<u>H</u> of pyrimidine ring), 11.26 δ ppm (1H, s, N<u>H</u> of pyrimidine ring), 12.36 δ ppm (1H, s, O<u>H</u> 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 stretching), 3342 (b, C-OH). λ max: 291.03 nm; (€: 0.95^{x} 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.

3.3.7 1,3-Dimethyl-5–[(2-Hydroxy-9H-purin-6-ylamino)-methylene]-pyrimidine-2, 4, 6-trione (1, 3-BG)

Dark brown Powder, Yield 86%; M.P. >250◦C; ^{1}H NMR (400 MHz, DMSO), 2.69 (6H, s, two CH_3 group of pyrimidine ring), 4.07 δ ppm (1H, DD, exocyclic NH of purine ring, *j*=14.24 Hz), 6.95 δ ppm (2H, s,-CH of purine ring), 8.05 δ ppm (1H, DD, exocyclic CH of pyrimidine ring, *j*=24.12 Hz), 8.16 δ ppm (1H, DD, NH of purine ring) 12.32 δ ppm (1H, s, OH of purine ring). ^{13}C NMR (400 MHz, DMSO), 76.37 (C-5), 113.67 (C-9), 113.87 (C-8), 122.92 (C-13), 126.43 (C-14, C-15), 129.63 (C-11, C-12), 131.45 (C-10) 157.12 (C-7), 162.27 (C-4, C-6), 168.67 (C-2) δ ppm. FTIR (KBr) υ max cm-1: 1219 (m,-O-C stretching), 1358 (C-NH, aromatic amine), 1626 (=C-NH aliphatic amine), 1695 (C=O aromatic and α, β-unsaturated ketone), 2811 (N-CH3 stretching), 3065 (m, CH-NH stretching), 3342 (C-OH, aromatic stretching). λ max: 368.09 nm; (€: 1.16^{x} 10^5 L mol^{-1} cm^{-1}); M.W. 317.26, ESIMS: m/z 318.23 (M+1); Anal. Calcd. For $C_{12}H_{11}N_7O_4$ (%):C 45.43, H 3.49, N 30.90, Found (%): C 45.41, H 3.52, N 30.87.

3.3.8. 1,3-Dimethyl-5–[(2-Hydroxy-9H-purin-6-ylamino)-methylene]-2-thioxo-dihydropyrimidine- 4, 6-Dione (1, 3-TBG)

Brown Powder, Yield 88%; M.P. >250◦C; ^{1}H NMR (400 MHz, DMSO), 2.75 (6H, s, two CH_3 group of pyrimidine ring), 4.06 δ ppm (1H, DD, exocyclic NH of purine ring, *j*=14.68 Hz), 6.99 δ ppm (2H, s,-CH of purine ring), 8.04 δ ppm (1H, DD, exocyclic CH of pyrimidine ring, *j*=23.96 Hz), 8.14 δ ppm (1H, DD, NH of purine ring) 12.27 δ ppm (1H, s, OH of purine ring). 13C 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: 1226 (-O-C stretching), 1364 (C-NH, aromatic amine), 1635 (s, =C-NH aliphatic amine), 1705 (C=O aromatic and α, β-unsaturated ketone), 2793 (N-CH3 stretching), 3084 (CH-NH stretching), 3339 (C-OH, aromatic stretching). λ max: 300.41 nm; (€: 0.89^{x} 10^5 L mol^{-1} cm^{-1}); M.W. 333.33, ESIMS: m/z 335.27 (M+2); Anal. Calcd. $C_{12}H_{11}N_7O_3S$ (%): C 43.24, H 3.33, N 29.41, Found (%): C 43.21, H 3.30, N 29.38.

3.3.9 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 N<u>H</u> of purine ring, *j*=15.68 Hz), 6.98 δ ppm (2H, s,-C<u>H</u> of cytosine ring), 8.11 δ ppm (1H, DD, exocyclic C<u>H</u> of pyrimidine ring, *j*=23.60 Hz), 8.16 δ ppm (1H, DD, N<u>H</u> of cytosine ring), 11.18 δ ppm (1H, s, N<u>H</u> of pyrimidine ring), 11.26 δ ppm (1H, s, N<u>H</u> 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^{x} 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.

3.3.10 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 N<u>H</u> of purine ring, *j*=13.8 Hz), 6.95 δ ppm (2H, s,-C<u>H</u> of cytosine ring), 8.07 δ ppm (1H, DD, exocyclic C<u>H</u> of pyrimidine ring, *j*=23.66 Hz), 8.18 δ ppm (1H, DD, N<u>H</u> of cytosine ring), 11.12 δ ppm (1H, s, N<u>H</u> of pyrimidine ring), 11.19 δ ppm (1H, s, N<u>H</u> 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^{x} 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.

3.3.11 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 C<u>H</u>$_3$ group of pyrimidine ring), 4.07 δ ppm (1H, DD, exocyclic N<u>H</u> of purine ring, *j*=14.52 Hz), 6.92 δ ppm (2H, s,-C<u>H</u> of cytosine ring), 8.13 δ ppm (1H, DD, exocyclic C<u>H</u> 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^{x} 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.

3.3.12 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 C<u>H</u>$_3$ group of pyrimidine ring), 4.04 δ ppm (1H, DD, exocyclic N<u>H</u> of purine ring, *j*=16.28 Hz), 6.89 δ ppm (2H, s,-C<u>H</u> of cytosine ring), 8.17 δ ppm (1H, DD, exocyclic C<u>H</u> 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^{x} 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.

4. Conclusion

In summary, we have successfully developed presented a synthetic novel an operationally simple, efficient and eco-friendly protocol for the preparation of nucleosides of barbiturates which is very important in biological and pharmaceutical sciences. From the CDK inhibition assay the compounds showed highest activity under steroid free conditions (5% DCC serum) than the (10% serum conditions). Most of them seem to have better activity than Roscovitine. In future, Nucleobases based barbiturates probes will be used for the further development of new biologically active leads.

5. Acknowledgement

We are very much thankful to the Department of Applied Chemistry, SVNIT, Surat for providing laboratory facilities, financial Support from Gujarat Council on Science and Technology (Project No. Gujcost/MRP/202042/12-13/04). For MTT assay analysis we are we are thankful to Dr. Ratan K. Vadlamudi and his teams from *Obstetrics & Gynecology Department, School of Medicine, San Antonia, Taxas, U.S.A.* For characterization, we are thankful to Mr. D. M. Thumar (Vice President) from Amoli Organic Pvt, Ltd, Baroda, Gujarat and Avtarsingh & Manish Kumar from SAIF, Punjab University, Chandigarh, India.

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