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Design, Synthesis and Evaluation of New Pyridopyrimidine Derivatives as Anti Cancer Agents

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ABSTRACT:

A series of new pyrido[2,3-d]pyrimidine derivatives were synthesized adopting click chemistry approach. The obtained compounds were characterized by spectral data analysis and evaluated for their anticancer activity. All the derivatives were subjected to *in vitro* MTT cytotoxicity screening assay against a panel of four different human cancer cell lines, colon carcinoma (HT-29), tongue squamous cell carcinoma (CAL27), human cervical cancer (HeLa) and breast (MCF-7). Compounds 8a, 8b, showed the highest cytotoxic activities with EC_{50} values of 25.21±0.6, 20.13±1.2, 23.21±0.8 and 26.10±1.0 mM. Finally, docking studies revealed that the synthesised compounds have similar binding modes against the prospective biological targets. This work introduces compound 8a as promising anti-cancer agent.

Key words: Pyridine, Pyrimidine, Cytotoxic Activity; Carcinoma

1. INTRODUCTION:

Cancer, which is the result of uncontrolled growth of the cells, remains one of the life-threatening diseases causing death worldwide [1]. According to WHO, Cancer is the world's leading cause of mortality, accounting for around 19.3 million new cases and nearly 10 million deaths in the year 2021. Lung cancer led the list with a projected 18% (1.8 million) death rate, followed by colorectal, liver, stomach, and female breast cancer fatalities of 9.4%, 8.3%, 7.7%, and 6.9%, respectively. In 2040, new cancer cases are expected to increase by 47% globally compared to 2021 and demonstrating the need for novel active therapeutic agents to fight the disease [2]. Several factors can cause cancer. The most well-known factors are hormonal disorders, genetic mutations, radiations, smoking tobacco, metals, polluted food, chemicals, infectious organisms, oral contraceptives, immuno suppressants [3-5]. Resistance against anticancer drugs is considered one of the most serious problems in cancer management. Due to the high residence of many cancer types, the discovery of new anticancer agents with high effect, less resistance, and fewer side effects is an urgent need [6]. The presence of nitrogen containing heterocylces in medicinally active substances is due to their functional ability and stability in human anatomy, as well as proof that N-atoms are stable. It is easily attached to DNA due to hydrogen bonding. The anti-cancer activity of nitrogen-based heterocyclic compounds is primarily due to their affinity for DNA via hydrogen bonding [7]. The majority of drugs that the FDA has approved contain pyrimidines pyridine nucleus related substituents. Additionally, pyridine and its derivatives are promising anticancer drugs in the field of cancer research. Genetic materials contain pyridine and pyrimidine, which are organic and natural substances. Due to its efficacy in treating a number of fatal diseases like breast cancer, myeloid leukaemia, and idiopathic arthritis, pyridine and its derivatives (Fig. 1) have drawn significant interest in recent medical research. pulmonary fibrosis [8-9].

The majority of traditional anticancer drugs are cytotoxic agents with low selectivity for cancer cells, resulting in significant side effects (i.e., bone marrow suppression, alopecia, nausea and vomiting). Furthermore, the majority of the drugs are primarily active against proliferating cells but have no effect on cancer cells in the resting phase. As a result, novel drugs targeting various targets are constantly being developed to improve disease control and prevention. Later-generation anticancer drugs are designed to have higher potency and selectivity, better pharmacokinetics, and minimal toxicity. Pyridine and pyrimidine scaffolds are appealing for drug design and development. These privileged structures are present in many currently used anticancer drugs. These anticancer drugs were designed and developed to act on a variety of drug targets. DNA and RNA, for example, are pyrimidine and purine derivatives. Many antimetabolite anticancer drugs have been developed based on mimicking natural substrates to competitively bind with the targets, such as receptors or enzymes. [10].

2. RATIONALE OF MOLECULAR DESIGN

Ahmed et al. (2018) synthesised and tested 4-((2-(2,7-diphenyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl)hydrazono)methyl) phenol **(I)** for EGFRWT and EGFRT790M inhibitory activities. Compound I inhibited the two EGFR types with high apoptotic activity and stopped the cell cycle at the G2/M phase. **Fayed et al. (2019)** designed and synthesised a novel N-(3-cyano-4-(4-methoxyphenyl)-6-(2-oxo-2H-chromen-3-yl)pyridin-2-yl)acetamide **(II)** showed the most potent growth inhibitory activities against MCF-7 cell line, with IC_{50} values ranging from 1.1 to 2.4 M. **Nasser et al. (2020)** developed and synthesised a 2-(butylthio)-4-phenyl-6-(pyridin-2-ylamino)pyrimidine-5-carbonitrile **(III)** evaluated as EGFR and HER2 tyrosine kinase inhibitors. Furthermore, it induced apoptosis in HCT-116, HepG-2, and MCF-7 cells by arresting the cell cycle at the G2/M phase. One hetero-aromatic ring (pyrimidine) is present in this compound to occupy the adenine binding pocket55. **Zhang et al. (2019)** reported the new HDAC and PI3K dual inhibitor that is N-hydroxy-2-(6-(6-methoxypyridin-3-yl)-4-

methylquinazolin-8-yl)oxy)methyl)(methyl)amino)pyrimidine-5-carboxamide **(IV)** in which a zinc binding functional group was introduced as the hydroxamic acid moiety to a quinazoline-dependent PI3K pharmacophore via a suitable linker. Furthermore, compound was tested in HGC-27 and HCT116 xenograft models and demonstrated significant in vivo anti-tumor activity, with cancer growth retardations of 62.6% and 45.8%, respectively.

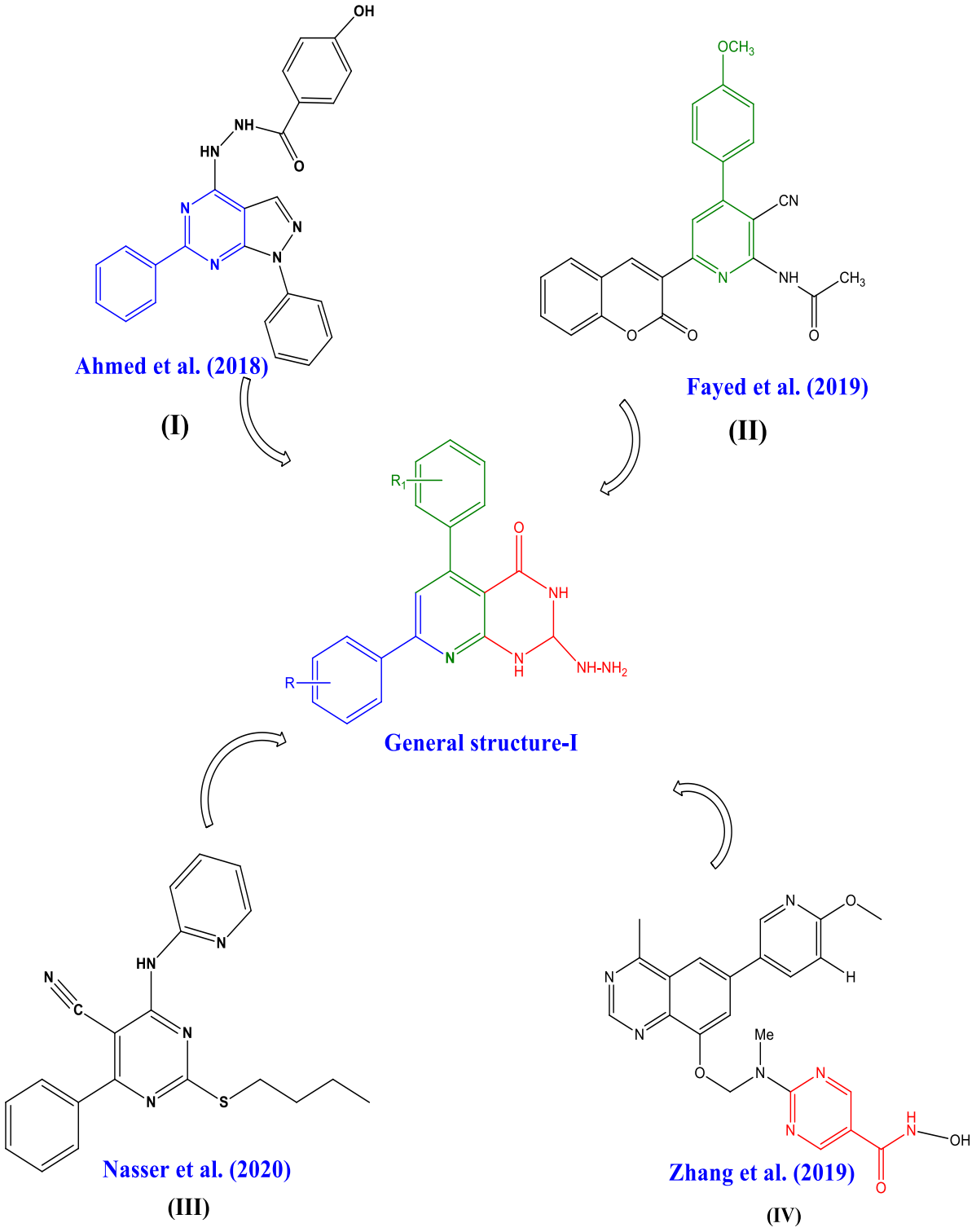


Figure-1: Design the pyridopyrimidine analogues

In the present work, we used the previously reported active candidates (**I, II, III and IV**) as lead compounds, Hence, it is proposed to synthesize Pyrido-pyrimidine conjugates with aromatic ring as shown in the **Figure 1** and evaluate the compounds for their anti-cancer activity.

3. MATERIALS AND METHODS:

3.1 Chemistry:

All chemicals, including standard drugs and solvents, were obtained from Sigma-Aldrich, HiMedia, Bangalore, India, and others and used without further purification, with the exception of liquid aldehydes, which were purified prior to use using standard procedures. The melting points of all compounds were measured in open capillary tubes using the VEEGO VMP-D Digital melting point apparatus. The FTIR spectra were obtained using KBr pellets on a JASCO FTIR 4100 series and are reported in cm-1. TMS was used as an internal standard to measure signals from ^{1}H NMR and ^{13}C NMR spectra on a BRUKER-II 400 (400 MHz NMR, ^{13}C NMR 100 MHz) spectrophotometer. To test the purity of the compounds, pre-coated TLC plates were used, and spots were visualised using iodine vapours and ultraviolet rays. Elemental analyses were performed using a CHN-VarioElico Micro elemental analyzer. The anti-cancer activity studies were estimated using commercially available test kits (Sigma-Aldrich).

3.2 Experimental Procedure:

3.2.1. General procedure for the synthesis of 2-Thioxopyrimidine(3):

A mixture of urea (**1**; 0.12 mol), ethyl 2-cyanoacetate (**2**; 0.1 mol), were refluxed in ethanol for 10 hours with stirring in the presence of sodium ethanoate. The reaction mixture was kept at room temperature for overnight. The crude precipitate resulted in was filtered, washed with cold ethanol and dried to produce the desired product **(3)**.

3.2.2. General procedure for the synthesis of chalcone (6a-p):

Synthesis of Chalcones was carried out by using Aldol condensation. A mixture of Acetophenone (**4**; 0.1mol; 5ml) and Benzaldehyde (**5**; 0.1mol; 5ml) were taken in round bottom flask with stirring in the presence of base NaOH as a catalyst. The reaction mixture was kept at 0-20℃ temperature for 12 hours. The progress of the reaction was monitored byTLC. The reactant mixture was acidified with HCl in an ice bath and the solid was then filtered and crystallized by ethanol.

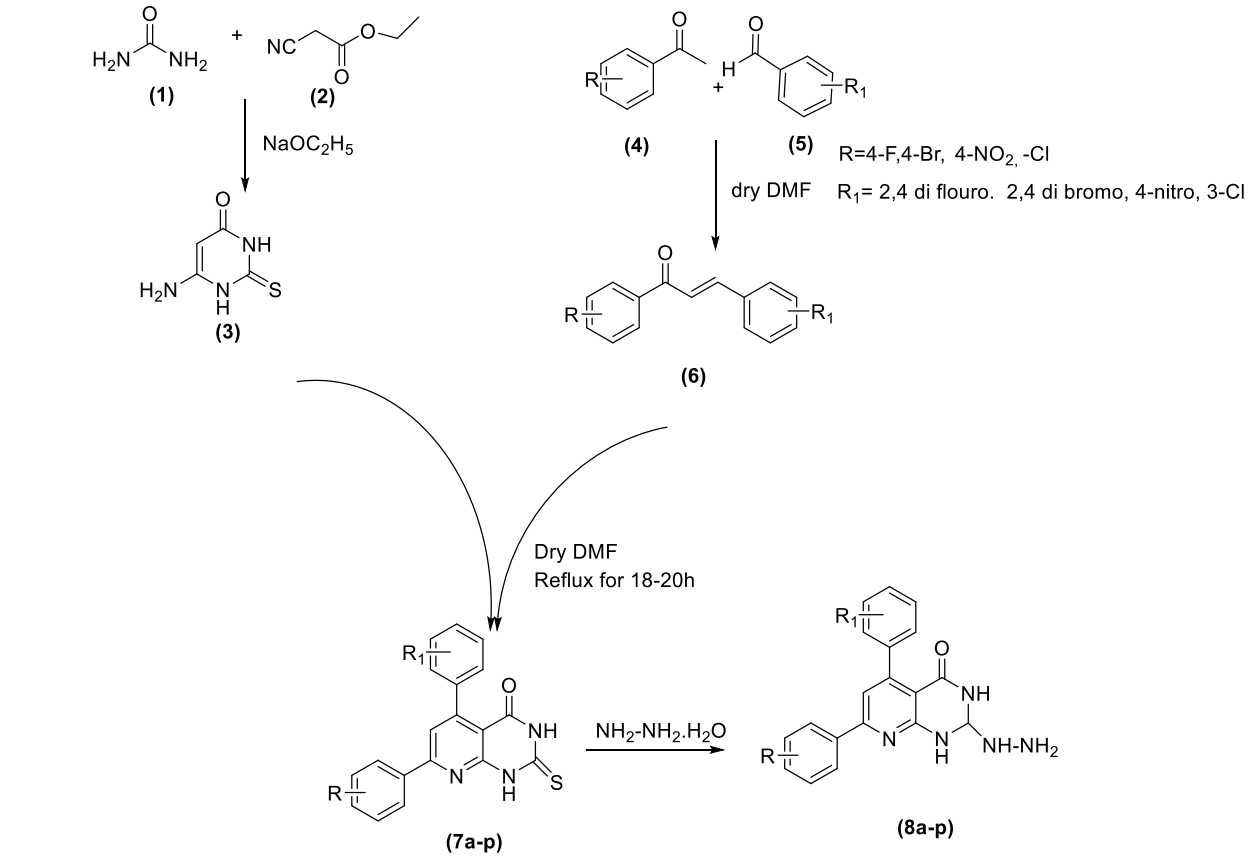
3.2.3. General procedure for the synthesis of 2,3-dihydro-2-thioxo-5,7- diarylpyrido[2,3-d]pyrimidin-4(1H)-one (7a-p):

2-Thioxopyrimidine **3** (1.43 g, 0.01 mol) and of ά-β-unsaturated ketones (**6,** 0.01 mol) in dry DMF solution (20 ml) was refluxed for 18–20 h and progress of the reaction was monitored by TLC. The solid mass created on cooling was filtered and crystallized from (DMF) to give the 2,3-dihydro-2-thioxo-5,7- diarylpyrido[2,3-d]pyrimidin-4(1H)-one (7).

3.2.4. General procedure for the synthesis of 2-hydrazinyl -5,7- diarylpyrido[2,3-d]pyrimidin-4(3H)-one (8a–p):

2,3-dihydro-2-thioxo-5,7- diarylpyrido[2,3-d]pyrimidin-4(1H)-ones derivatives (7a-p; 0.004 mol) and hydrazine reagent (3 ml, 0.006 mol) was refluxed in absolute ethyl alcohol (20 ml) for 10–15 h. On cooling, the residue created was filtered and purified from (DMF).

1

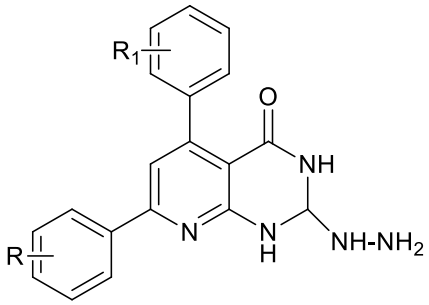


7a=R=4-F; R_1=2,4 diflouro
7b=R=4-F; R_1=2,4 dibromo
7c=R=4-F; R_1=4-nitro
7d=R=4-F; R_1=3-Cl
7e=R=4-Br; R_1=2,4 diflouro
7f=R=4-Br R_1=2,4 dibromo
7g=R=4-Br; R_1=4-nitro
7h=R=4-Br; R_1=3-Cl
7i=R=4-NO_2; R_1=2,4 diflouro
7j=R=4-NO_2; R_1=2,4 dibromo
7k=R=4-NO_2; R_1=4-nitro
7l=R=4-NO_2; R_1=3-Cl
7m=R=3-Cl; R_1=2,4 diflouro
7n=R=3-Cl R_1=2,4 dibromo
7o=R=3-Cl; R_1=4-nitro
7p=R=3-Cl; R_1=3-Cl

8a=R=4-F; R_1=2,4 diflouro
8b=R=4-F; R_1=2,4 dibromo
8c=R=4-F; R_1=4-nitro
8d=R=4-F; R_1=3-Cl
8e=R=4-Br; R_1=2,4 diflouro
8f=R=4-Br R_1=2,4 dibromo
8g=R=4-Br; R_1=4-nitro
8h=R=4-Br; R_1=3-Cl
8i=R=4-NO_2; R_1=2,4 diflouro
8j=R=4-NO_2; R_1=2,4 dibromo
8k=R=4-NO_2; R_1=4-nitro
8l=R=4-NO_2; R_1=3-Cl
8m=R=3-Cl; R_1=2,4 diflouro
8n=R=3-Cl R_1=2,4 dibromo
8o=R=3-Cl; R_1=4-nitro
8p=R=3-Cl; R_1=3-Cl

Scheme 1. General procedure for the synthesis of the 2-hydrazinyl -5,7- diarylpyrido[2,3-d]pyrimidin-4(3H)-one (8a-p).

Table-1. Physical data of new 2-hydrazinyl -5,7- diarylpyrido[2,3-d]pyrimidin-4(3H)-one (8a-p)



Comp.	R	R_1	M. Form	M.Wt	M.P°C	Rf*	% Yield
8a	4-F	2,4 di flouro	$C_{19}H_{14}F_3N_5O$	383	160-162.	0.6	87
8b	4-F	2,4 di bromo	$C_{19}H_{14}Br_2FN_5O$	507	154-156	0.5	80

8c	4-F	4-NO_2	$C_{19}H_{15}FN_6O_3$	394	150-152	0.6	68
8d	4-F	3-Cl	$C_{19}H_{15}ClFN_5O$	383	160-162	0.4	60
8e	4-Br	2,4 di flouro	$C_{19}H_{14}BrF_2N_5O$	446	168-170	0.5	74
8f	4-Br	2,4 di bromo	$C_{19}H_{14}Br_3N_5O$	568	180-182	0.8	54
8g	4-Br	4-NO_2	$C_{19}H_{15}BrN_6O_3$	455	186-188	0.5	70
8h	4-Br	3-Cl	$C_{19}H_{15}BrClN_5O$	444	158-160	0.7	38
8i	4-NO_2	2,4 di flouro	$C_{19}H_{14}F_2N_6O_3$	412	162-164	0.5	64
8j	4-NO_2	2,4 di bromo	$C_{19}H_{14}Br_2N_6O_3$	531	158-160	0.7	87
8k	4-NO_2	4-NO_2	$C_{19}H_{15}N_7O_5$	421	158-160	0.5	60
8l	4-NO_2	3-Cl	$C_{19}H_{15}ClN_6O_3$	410	148-150	0.6	60
8m	3-Cl	2,4 di flouro	$C_{19}H_{14}ClF_2N_5O$	401	190-192	0.6	54
8n	3-Cl	2,4 di bromo	$C_{19}H_{14}Br_2ClN_5O$	520	196-198	0.5	58
8o	3-Cl	4-NO_2	$C_{19}H_{15}ClN_6O_3$	410	150-152	0.6	60
8p	3-Cl	3-Cl	$C_{19}H_{15}Cl_2N_5O$	400	162-164	0.5	50

3.3. Characterization of compounds:

3.3.1. 5-(2,4-difluorophenyl)-7-(4-fluorophenyl)-2-hydrazinyl-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (8a): Compound **8a** obtained as yellowish orange solid (yield 87%), m.p. 160-162℃. ^{1}H NMR (400MHz CDCl3, δ ppm): 8.51 (s, 1H, amide), 7.95-8.00 (d, 1H, *J*=4.0 Hz, Ar-H) 7.89-7.91 (d, 1H, *J*=4.0 Hz, Ar-H), 7.77-7.81 (m, 2H, Ar-H),7.63-7.68 (m, 2H, Ar-H), 7.40-7.47 (m,2H, Ar-H), 2.63 (s, 3H, hydrazinyl); ^{13}C NMR (100MHz, DMSO): 168.65, 161.15, 142.08, 132.54, 131.08, 129.35, 128.56, 128.14 126.88, 125.27, 124.48, 114.52. MASS spectrum m/z: 385 $[M+2]^+$, 387 $[M+4]^+$, 389 $[M+6]^+$ Calc. for $C_{19}H_{14}F_3N_5O$ CHN: C, 59.22; H, 3.66; N, 18.17; Found C, 59.20; H, 3.60; N, 18.18; IR (KBr, cm^{-1}):3443.59 (NH, amide) 3103.33 (C-H, Aromatic), 2922.68 (C-H, Aliphatic), 1716.96 (C=O), 1590.56 (C=C, Aromatic), 1184.54 (C-O),724.62. (C-F).

3.3.2. 5-(2,4-dibromophenyl)-7-(4-fluorophenyl)-2-hydrazinyl-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (8b): Compound **8b** obtained as yellowish solid (yield 80%), m.p. 154-156℃. ^{1}H NMR (400MHz CDCl3, δ ppm): 8.54 (s, 1H, amide), 7.92-7.96 (d, 1H, *J*=4.0 Hz, Ar-H) 7.82-7.84 (d, 1H, *J*=4.0 Hz, Ar-H), 7.70-7.75 (m, 2H, Ar-H),7.62-7.68 (m, 2H, Ar-H), 7.38-7.42 (m,2H, Ar-H), 2.68 (s, 3H, hydrazinyl); ^{13}C NMR (100MHz, DMSO): 169.61, 168.15, 165.25, 150.25, 142.08, 132.54, 131.08, 129.35, 128.56, 128.14 126.88, 125.27, 120.48, 117.52. MASS spectrum m/z: 509$[M+2]^+$, 511 $[M+4]^+$, 513 $[M+6]^+$ Calc. for $C_{19}H_{14}Br_2FN_5O$ CHN: C, 45.00; H, 2.78; N, 13.81 Found C, 45.05; H, 2.70; N, 13.85; IR (KBr, cm^{-1}): 3451.21 (NH, amide), 3065.20 (C-H, Aromatic), 2952.10 (C-H, Aliphatic), 1725.96 (C=O), 1525.01 (C=C, Aromatic), 1260.51 (C-O).728.63. (C-F).

3.2.3. -(4-fluorophenyl)-2-hydrazinyl-5-(4-nitrophenyl)-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (8c): Compound **8c** obtained as white solid (yield 68%), m.p. 150-152℃. ^{1}H NMR (400MHz CDCl3, δ ppm): 8.56 (s, 1H, amide), 7.93-7.95 (d, 1H, *J*=4.0 Hz, Ar-H) 7.80-7.82 (d, 1H, *J*=4.0 Hz, Ar-H), 7.74-7.79 (m, 3H, Ar-H),7.62-7.68 (m, 2H, Ar-H),

7.38-7.44 (m,2H, Ar-H), 2.69 (s, 3H, hydrazinyl); ^{13}C NMR (100MHz, DMSO): 169.01, 167.10, 165.25, 150.25, 142.08, 132.54, 131.08, 129.35, 128.56, 128.14 126.88, 125.27, 120.48, 118.25, 117.52. MASS spectrum m/z: 394[M]$^+$, 396 [M+2]$^+$; Calc. for $C_{19}H_{15}FN_6O_3$ CHN: C, 57.87; H, 3.83; N, 21.31; Found 57.80; H, 3.80; N, 21.35; IR (KBr, cm^{-1}): 3451.21 (NH, amide), 3083.33 (C-H, Aromatic), 2958.15 (C-H, Aliphatic), 1721.28 (C=O**),** 1598.56 (C=C, Aromatic), 1180.54 (C-O). 720.63. (C-F).

3.2.4. 5-(3-chlorophenyl)-7-(4-fluorophenyl)-2-hydrazinyl-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one: Compound **8d** obtained as cream white solid (yield 60%), m.p. 160-162°C. ^{1}H NMR (400MHz CDCl3, δ ppm): 8.58 (s, 1H, amide), 7.92-7.94 (d, 1H, *J*=4.0 Hz, Ar-H) 7.82-7.84 (d, 1H, *J*=4.0 Hz, Ar-H), 7.75-7.79 (m, 3H, Ar-H),7.62-7.68 (m, 2H, Ar-H), 7.38-7.44 (m,2H, Ar-H), 2.65 (s, 3H, hydrazinyl); ^{13}C NMR (100MHz, DMSO): 168.25, 165.10, 163.25, 158.25, 148.08, 138.54, 135.08, 129.35, 128.56, 128.14 125.88, 122.27, 120.48, 119.25, 117.52. MASS spectrum m/z: 383[M]$^+$, 385 [M+2]$^+$, 387 [M+4]$^+$; Calc. for $C_{19}H_{15}ClFN_5O$ CHN: C, 59.46; H, 3.94; N, 18.25; Found C, 59.42; H, 3.90; N, 18.20;; IR (KBr, cm^{-1}): 3401.28 (NH, amide), 3091.35 (C-H, Aromatic), 2968.15 (C-H, Aliphatic), 1728.28 (C=O**),** 1590.56 (C=C, Aromatic), 1180.54 (C-O). 760.63. (C-Cl).

3.2.5. 7-(4-bromophenyl)-5-(2,4-difluorophenyl)-2-hydrazinyl-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (8e): Compound **8e** obtained as orange solid (yield 74%), m.p. 178-180°C. ^{1}H NMR (400MHz CDCl3, δ ppm): 8.59 (s, 1H, amide), 7.92-7.94 (d, 1H, *J*=4.0 Hz, Ar-H) 7.85-7.87 (d, 1H, *J*=4.0 Hz, Ar-H), 7.73-7.78 (m, 2H, Ar-H),7.62-7.68 (m, 2H, Ar-H), 7.40-7.45 (m,2H, Ar-H), 2.60 (s, 3H, hydrazinyl); ^{13}C NMR (100MHz, DMSO): 167.60, 161.18, 158.09, 138.14, 137.06, 128.30, 127.56, 126.88, 125.27, 124.48, 114.52. MASS spectrum m/z: 448 [M+2]$^+$, 450 [M+4]$^+$, 452 [M+6]$^+$ Calc. for $C_{19}H_{14}BrF_2N_5O$ CHN: C, 51.14; H, 3.16; N, 15.69; Found C, 51.10; H, 3.18; N, 15.70; IR (KBr, cm^{-1}):3440.19 (NH, amide) 3068.33 (C-H, Aromatic), 2924.68 (C-H, Aliphatic), 1726.96 (C=O), 1592.51 (C=C, Aromatic), 1124.54 (C-O),72862 (C-F).

3.3.2. 7-(4-bromophenyl)-5-(2,4-dibromophenyl)-2-hydrazinyl-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (8f): Compound **8f** obtained as yellowish solid (yield 54%), m.p. 180-182°C. ^{1}H NMR (400MHz CDCl3, δ ppm): 8.59 (s, 1H, amide), 7.96-7.98 (d, 1H, *J*=4.0 Hz, Ar-H) 7.86-7.88 (d, 1H, *J*=4.0 Hz, Ar-H), 7.75-7.79 (m, 2H, Ar-H),7.66-7.69 (m, 2H, Ar-H), 7.40-7.44 (m,2H, Ar-H), 2.62 (s, 3H, hydrazinyl); ^{13}C NMR (100MHz, DMSO): 165.60, 162.18, 160.20, 158.28, 149.38, 138.50, 138.08, 130.35, 128.56, 128.14 126.88, 125.27, 120.48, 117.52. MASS spectrum m/z: 570[M+2]$^+$, 572 [M+4]$^+$, 574 [M+6]$^+$ Calc. for $C_{19}H_{14}Br_3N_5O$ CHN: C, 40.17; H, 2.48; N, 12.33; Found C, 40.15; H, 2.40; N, 12.30; IR (KBr, cm^{-1}): 3401.21 (NH, amide), 3060.25(C-H, Aromatic), 2952.10 (C-H, Aliphatic), 1720.96 (C=O), 1520.01 (C=C, Aromatic), 1260.51 (C-O).628.63. (C-Br).

3.2.3. 7-(4-bromophenyl)-2-hydrazinyl-5-(4-nitrophenyl)-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (8g): Compound **8g** obtained as creamish white solid (yield 70%), m.p. 186-188°C. ^{1}H NMR (400MHz CDCl3, δ ppm): 8.54 (s, 1H, amide), 7.98-8.01 (d, 1H, *J*=4.0 Hz, Ar-H) 7.84-7.86 (d, 1H, *J*=4.0 Hz, Ar-H), 7.78-7.81 (m, 3H, Ar-H),7.60-7.65 (m, 2H, Ar-H), 7.40-7.44 (m,2H, Ar-H), 2.65 (s, 3H, hydrazinyl); ^{13}C NMR (100MHz, DMSO): 168.01, 165.10, 163.25, 158.25, 156.08, 132.54, 131.08, 129.35, 128.56, 128.14 126.88, 125.27, 120.48, 118.25, 117.52. MASS spectrum m/z: 455[M]$^+$, 457 [M+2]$^+$; Calc. for $C_{19}H_{15}BrN_6O_3$ CHN: C, 50.13; H, 3.32; N, 18.46; Found C, 50.13; H, 3.32; N, 18.46; IR (KBr, cm^{-1}): 3401.21 (NH, amide), 3080.30 (C-H, Aromatic), 2950.18 (C-H, Aliphatic), 1726.28 (C=O**),** 1590.56 (C=C, Aromatic), 1188.54 (C-O). 728.63. (C-F).

3.2.4. 7-(4-bromophenyl)-5-(3-chlorophenyl)-2-hydrazinyl-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (8h): Compound **8h** obtained as cream colour solid (yield 40%), m.p. 158-160℃. ^{1}H NMR (400MHz CDCl3, δ ppm): 8.59 (s, 1H, amide), 7.94-7.96 (d, 1H, *J*=4.0 Hz, Ar-H) 7.84-7.86 (d, 1H, *J*=4.0 Hz, Ar-H), 7.71-7.75 (m, 3H, Ar-H),7.60-7.65 (m, 2H, Ar-H), 7.30-7.32 (m, 2H, Ar-H), 2.69 (s, 3H, hydrazinyl); ^{13}C NMR (100MHz, DMSO): 165.20, 163.20, 158.25, 148.18, 139.51, 136.18, 128.31, 126.56, 125.14 124.88, 122.27, 120.48, 119.25, 117.52. MASS spectrum m/z: 444[M]$^{+}$, 446 [M+2]$^{+}$, 448 [M+4]$^{+}$; Calc. for $C_{19}H_{15}BrClN_5O$ CHN: C, 51.31; H, 3.40, N, 15.75; Found C, 51.30; H, 3.45, N, 15.15; IR (KBr, cm^{-1}): 3410.28 (NH, amide), 3095.30 (C-H, Aromatic), 2960.15 (C-H, Aliphatic), 1720.28 (C=O**),** 1595.56 (C=C, Aromatic), 1181.54 (C-O). 764.63. (C-Cl).

3.2.5. 5-(2,4-difluorophenyl)-2-hydrazinyl-7-(4-nitrophenyl)-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (8i): Compound **8i** obtained as orange solid (yield 64%), m.p. 162-164℃. ^{1}H NMR (400MHz CDCl3, δ ppm): 8.57 (s, 1H, amide), 7.98-8.00 (d, 1H, *J*=4.0 Hz, Ar-H) 7.92-7.94 (d, 1H, *J*=4.0 Hz, Ar-H), 7.78-7.84 (m, 2H, Ar-H),7.65-7.69 (m, 2H, Ar-H), 7.42-7.48 (m,2H, Ar-H), 2.68 (s, 3H, hydrazinyl); ^{13}C NMR (100MHz, DMSO): 167.15, 165.15, 152.08, 142.54, 138.15, 131.08, 129.35, 128.56, 128.14 126.88, 120.27, 117.48, 113.52. MASS spectrum m/z: 414 [M+2]$^{+}$, 416 [M+4]$^{+}$, 418 [M+6]$^{+}$ Calc. for $C_{19}H_{14}F_2N_6O_3$ CHN: C, 55.34; H, 3.42; N, 20.38; Found C, 55.30; H, 3.40; N, 20.382; IR (KBr, cm^{-1}):3413.50 (NH, amide) 3108.35 (C-H, Aromatic), 2920.60 (C-H, Aliphatic), 1716.86 (C=O), 1585.56 (C=C, Aromatic), 1180.54 (C-O),720.62. (C-F).

3.3.2. 5-(2,4-dibromophenyl)-2-hydrazinyl-7-(4-nitrophenyl)-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (8j): Compound **8j** obtained as white solid (yield 87%), m.p. 158-160℃. ^{1}H NMR (400MHz CDCl3, δ ppm): 8.59 (s, 1H, amide), 7.90-7.92 (d, 1H, *J*=4.0 Hz, Ar-H) 7.84-7.86 (d, 1H, *J*=4.0 Hz, Ar-H), 7.74-7.76 (m, 2H, Ar-H),7.62-7.68 (m, 2H, Ar-H), 7.38-7.42 (m,2H, Ar-H), 2.62 (s, 3H, hydrazinyl); ^{13}C NMR (100MHz, DMSO): 168.61, 167.15, 165.25, 150.25, 142.08, 132.54, 130.08, 129.35, 128.56, 128.14 126.88, 125.27, 120.48, 117.52. MASS spectrum m/z: 531[M+]$^{+}$, 533 [M+2]$^{+}$, 535 [M+2]$^{+}$. Calc. for $C_{19}H_{14}Br_2N_6O_3$ CHN: C, 42.72; H, 2.64; N, 15.73; Found C, 42.70; H, 2.60; N, 15.74; IR (KBr, cm^{-1}): 3421.20 (NH, amide), 3061.20 (C-H, Aromatic), 2958.10 (C-H, Aliphatic), 1715.96 (C=O), 1520.01 (C=C, Aromatic), 1265.51 (C-O).726.63. (C-F).

3.2.3. 2-hydrazinyl-5,7-bis(4-nitrophenyl)-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (8k): Compound **8k** obtained as yellowish solid (yield 60%), m.p. 158-160℃. ^{1}H NMR (400MHz CDCl3, δ ppm): 8.59 (s, 1H, amide), 7.95-7.97 (d, 1H, *J*=4.0 Hz, Ar-H) 7.86-7.88 (d, 1H, *J*=4.0 Hz, Ar-H), 7.75-7.79 (m, 3H, Ar-H), 7.65-7.68 (m, 2H, Ar-H), 7.40-7.44 (m,2H, Ar-H), 2.65 (s, 3H, hydrazinyl); ^{13}C NMR (100MHz, DMSO): 168.01, 166.10, 164.25, 150.25, 142.08, 132.54, 131.08, 129.35, 128.56, 128.14 126.88, 125.27, 120.48, 119.25, 118.52. MASS spectrum m/z: 421[M]$^{+}$, 422 [M+1]$^{+}$; Calc. for $C_{19}H_{15}N_7O_5$ CHN: C, 54.16; H, 3.59; N, 23.27; Found C, 54.10; H, 3.54; N, 23.25; IR (KBr, cm^{-1}): 3421.21 (NH, amide), 3080.30 (C-H, Aromatic), 2958.10 (C-H, Aliphatic), 1728.28 (C=O**),** 1590.56 (C=C, Aromatic), 1184.54 (C-O).

3.2.4. 5-(3-chlorophenyl)-2-hydrazinyl-7-(4-nitrophenyl)-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (8l): Compound **8l** obtained as cream white solid (yield 60%), m.p. 148-150℃. ^{1}H NMR (400MHz CDCl3, δ ppm): 7.91(s, 1H, amide), 7.86-7.89 (m, 2H, *J*=4.0 Hz, Ar-H) 7.47-7.55 (m, 2H, *J*=4.0 Hz, Ar-H), 7.31-7.42 (m, 2H, Ar-H), 6.50 (s, 1H, Ar-H), 6.35-6.37 (m,2H, Ar-H), 2.65 (s, 3H, hydrazinyl); ^{13}C NMR (100MHz, DMSO): 190.51, 158.96, 152.72, 150.03, 143.90, 143.19, 137.00, 135.53, 134.92, 131.29, 128.89, 128.50,

126.73, 124.41, 123.96, 120.92, 119.82, 117.52, 117.13. MASS spectrum m/z: 410 $[M]^+$, 412 $[M+2]^+$, Calc. for $C_{19}H_{15}ClN_6O_3$ CHN: C, 55.55; H, 3.68; N, 20.46; Found C, 55.50; H, 3.62; N, 20.40; IR (KBr, cm^{-1}): 3421.28 (NH, amide), 3095.35 (C-H, Aromatic), 2968.15 (C-H, Aliphatic), 1728.28 (C=O**),** 1590.56 (C=C, Aromatic), 1180.54 (C-O). 754.63. (C-Cl).

3.2.5. 7-(3-chlorophenyl)-5-(2,4-difluorophenyl)-2-hydrazinyl-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (8m): Compound **8m** obtained as orange solid (yield 54%), m.p. 190-192℃. ^{1}H NMR (400MHz CDCl3, δ ppm): 8.65 (s, 1H, amide), 7.90-7.92 (d, 1H, *J*=4.0 Hz, Ar-H) 7.86-7.88 (d, 1H, *J*=4.0 Hz, Ar-H), 7.73-7.78 (m, 2H, Ar-H),7.62-7.68 (m, 2H, Ar-H), 7.40-7.45 (m,2H, Ar-H), 2.62 (s, 3H, hydrazinyl); ^{13}C NMR (100MHz, DMSO): 168.60, 165.18, 158.09, 138.14, 137.06, 128.30, 127.56, 126.88, 125.27, 124.48, 117.25, 114.52. MASS spectrum m/z: 403 $[M+2]^+$, 405 $[M+4]^+$, 407 $[M+6]^+$ Calc. for $C_{19}H_{14}ClF_2N_5O$ CHN: C, 56.80; H, 3.51; N, 17.43; Found C, 56.82; H, 3.55; N, 17.40; IR (KBr, cm^{-1}):3420.15 (NH, amide) 3061.53 (C-H, Aromatic), 2929.60 (C-H, Aliphatic), 1726.96 (C=O), 1592.51 (C=C, Aromatic), 1124.54 (C-O),72862 (C-F).

3.3.2. 7-(3-chlorophenyl)-5-(2,4-dibromophenyl)-2-hydrazinyl-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (8n): Compound **8n** obtained as yellowish solid (yield 58%), m.p. 196-198℃. ^{1}H NMR (400MHz CDCl3, δ ppm): 8.65 (s, 1H, amide), 7.92-7.94 (d, 1H, *J*=4.0 Hz, Ar-H) 7.86-7.88 (d, 1H, *J*=4.0 Hz, Ar-H), 7.75-7.79 (m, 2H, Ar-H),7.68-7.71 (m, 2H, Ar-H), 7.41-7.45 (m,2H, Ar-H), 2.68 (s, 3H, hydrazinyl); ^{13}C NMR (100MHz, DMSO): 168.60, 164.18, 160.20, 158.28, 149.38, 138.50, 138.08, 130.35, 128.56, 128.14 126.88, 125.27, 120.48, 117.52. MASS spectrum m/z: 522$[M+2]^+$, 524 $[M+4]^+$, 526 $[M+6]^+$ Calc. for $C_{19}H_{14}Br_2ClN_5O$ CHN: C, 43.58; H, 2.69; N, 13.38; Found C, 43.50; H, 2.65; N, 13.30; IR (KBr, cm^{-1}): 3428.21 (NH, amide), 3050.21(C-H, Aromatic), 2952.10 (C-H, Aliphatic), 1726.96 (C=O), 1520.01 (C=C, Aromatic), 1260.51 (C-O).628.63. (C-Br).

3.2.3. 7-(3-chlorophenyl)-2-hydrazinyl-5-(4-nitrophenyl)-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (8o): Compound **8o** obtained as cream solid (yield 60%), m.p. 150-152℃. ^{1}H NMR (400MHz CDCl3, δ ppm): 8.59 (s, 1H, amide), 7.90-7.92 (d, 1H, *J*=4.0 Hz, Ar-H) 7.86-7.88 (d, 1H, *J*=4.0 Hz, Ar-H), 7.78-7.81 (m, 3H, Ar-H),7.60-7.65 (m, 2H, Ar-H), 7.40-7.44 (m,2H, Ar-H), 2.60 (s, 3H, hydrazinyl); ^{13}C NMR (100MHz, DMSO): 168.01, 165.10, 163.25, 158.25, 156.08, 132.54, 131.08, 129.35, 128.56, 128.14 126.88, 125.27, 120.48, 118.25, 117.52. MASS spectrum m/z: 410$[M]^+$, 412 $[M+2]^+$; Calc. for $C_{19}H_{15}ClN_6O_3$ CHN: C, 55.55; H, 3.68; N, 20.46; Found C, 55.55; H, 3.68; N, 20.46; IR (KBr, cm^{-1}): 3401.21 (NH, amide), 3080.30 (C-H, Aromatic), 2950.18 (C-H, Aliphatic), 1726.28 (C=O**),** 1590.56 (C=C, Aromatic), 1188.54 (C-O), 628.63. (C-Cl).

3.2.4. 5,7-bis(3-chlorophenyl)-2-hydrazinyl-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one (8p): Compound **8p** obtained as cream colour solid (yield 50%), m.p. 162-164℃. ^{1}H NMR (400MHz CDCl3, δ ppm): 8.61 (s, 1H, amide), 7.96-7.98 (d, 1H, *J*=4.0 Hz, Ar-H) 7.80-7.84 (d, 1H, *J*=4.0 Hz, Ar-H), 7.71-7.75 (m, 3H, Ar-H),7.60-7.65 (m, 2H, Ar-H), 7.32-7.34 (m, 2H, Ar-H), 2.71 (s, 3H, hydrazinyl); ^{13}C NMR (100MHz, DMSO): 165.20, 163.20, 158.25, 148.18, 139.51, 136.18, 128.31, 126.56, 125.14 124.88, 122.27, 120.48, 119.25, 117.52. MASS spectrum m/z: 400$[M]^+$, 402 $[M+2]^+$; Calc. for $C_{19}H_{15}Cl_2N_5O$ CHN: C, 57.01; H, 3.78; N, 17.50; Found C, 51.30; H, 3.45, N, 15.15; IR (KBr, cm^{-1}): 3410.28 (NH, amide), 3095.30 (C-H, Aromatic), 2960.15 (C-H, Aliphatic), 1720.28 (C=O**),** 1595.56 (C=C, Aromatic), 1181.54 (C-O). 764.63. (C-Cl).

4. BIOLOGICAL ASSAY :

4.1. Cell Cultures and Treatments

The following human tumor cell lines were utilized in the present study: MCF-7, HeLa, colon carcinoma HT-29, tongue squamous cell carcinoma CAL27 all of which obtained from the American Type Culture Collection (Hyderabad). In addition, human dermal fibroblast primary cultures (HF) used as control of non-transformed cells, had been previously established in our laboratory The cells were grown at 37 °C and 5% CO2 in Dulbecco's modified Eagle's medium or RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine and 50 U/mL penicillin-streptomycin (Sigma-Aldrich). All compounds were solubilized in dimethylsulfoxide (DMSO) (Sigma) for a 10 Mm stock solution and utilized to final concentrations from 100 nM to 60 μM for 24 and 48 h. Control cells were treated with equivalent amounts of DMSO in every experiment [11].

4.2. Cytotoxicity Assay:

A colorimetric sulforhodamine B assay was used to determine cytotoxicity. Cells (1.5 104) were plated in a 96-well plate, grown for 24 hours, and then treated for 24 and 48 hours at 37 °C with various concentrations of each compound. The cells were then fixed for 1 hour at 4 °C in 50% trichloroacetic acid and stained for 30 minutes at room temperature in 0.4% sulforhodamine B in 1% acetic acid. Washing four times with 1% acetic acid removed the excess dye. The protein-bound dye was dissolved in 10 mM Tris (pH 10), and the optical density at 510 nm was determined using a microplate reader. Every experiment was repeated three times, and the media was calculated each time.

4.3. In-Vitro Proliferation Assay:

The effects of these new synthesized compounds on the proliferation of four human tumour cell lines of different histotypes were used to assess their potential antitumoral activity. As a result, the effects of each single compound treatment on cell viability and replication were assessed. The human tumour cell lines MCF-7, HeLa, colon carcinoma HT-29, and tongue squamous cell carcinoma CAL27 were treated for 24 and 48 hours with 10, 20, 30, 40, 50, and 60 M of each single compound. When the compound inhibited cell proliferation significantly, other cell replication tests were performed at lower concentrations (100 nM, 1 M, and 5 M) [13]. When a compound showed significant effects on cell replication inhibition, the proliferation assay was established on human dermal fibroblasts (HF) at the same concentrations shown to be effective on tumour cells, and the CC50 at 48 h was calculated and reported in order to evaluate potential cytotoxicity in somatic, non-transformed cells. When a compound failed to exhibit significant anti-proliferative activity at acceptable concentrations (greater than 30 M), the proliferation assay on HF was abandoned as ineffective. The median values from three different experiments of proliferation assays of all compounds for each cell line were evaluated, and the EC50 at 24 and 48 h were calculated and reported. Table 2 summarizes anti-proliferation activities (compounds 8a-p).

4.4. Statistical Analysis

All results were analyzed using one-way analysis of variance, and significance was evaluated using Tukey's honest significant difference post-hoc test.

S.No	Compound	EC_{50} (μM)			
		MCF-7	HeLa	HT-29	CAL27

1	8a	25.21 ±0.6 (24h) 10.65±0.6 (48h)	20.13±1.2 (24hr) 8.24±1.0 (48h)	23.21 ±0.8 (24h) 12.65±0.7 (48h)	26.10±1.0 (24hr) 11.24±1.0 (48h)
2	8b	26.25 ±0.8 (24h) 13.17±0.2 (48h)	28.13±0.2 (24hr) 11.24±1.0 (48h)	25.21 ±0.8 (24h) 12.12±0.3 (48h)	28.10±1.0 (24hr) 11.34±0.8 (48h)
3	8c	50.21 ±0.4 (24h) 21.60±0.2 (48h)	54.13±1.2 (24hr) 26.24±1.2 (48h)	52.22 ±0.8 (24h) 28.65±0.7 (48h)	54.10±1.0 (24hr) 24.24±1.0 (48h)
4	8d	32.18 ±0.6 (24h) 18.65±0.2 (48h)	40.13±1.2 (24hr) 18.20±1.0 (48h)	44.21 ±0.8 (24h) 20.65±0.7 (48h)	42.10±1.0 (24hr) 18.24±1.0 (48h)
5	8e	54.20 ±0.8 (24h) 20.60±0.4 (48h)	55.13±0.2 (24hr) 22.24±1.0 (48h)	53.21 ±0.8 (24h) 22.65±0.7 (48h)	56.10±1.0 (24hr) 23.14±1.0 (48h)
6	8f	55.21 ±0.6 (24h) 30.16±0.8 (48h)	54.13±1.2 (24hr) 28.24±1.0 (48h)	54.21 ±0.8 (24h) 32.65±0.7 (48h)	56.10±1.0 (24hr) 29.24±1.0 (48h)
7	8g	60.14 ±0.6 (24h) 30.65±0.6 (48h)	58.13±1.2 (24hr) 28.24±1.0 (48h)	53.21 ±0.8 (24h) 22.60±0.7 (48h)	56.10±1.0 (24hr) 23.14±1.0 (48h)
8	8h	45.21 ±0.6 (24h) 22.65±0.6 (48h)	40.13±1.2 (24hr) 18.24±1.0 (48h)	43.21 ±0.8 (24h) 19.65±0.7 (48h)	46.10±1.0 (24hr) 21.24±1.0 (48h)
9	8i	45.21 ±0.6 (24h) 21.60±0.6 (48h)	48.13±1.2 (24hr) 25.24±1.0 (48h)	50.21 ±0.8 (24h) 27.65±0.7 (48h)	52.10±1.0 (24hr) 26.24±1.0 (48h)
10	8j	38.29 ±0.6 (24h) 19.65±0.6 (48h)	40.13±1.2 (24hr) 20.24±1.0 (48h)	41.21 ±0.8 (24h) 21.65±0.7 (48h)	39.10±1.0 (24hr) 26.24±1.0 (48h)
11	8k	65.21 ±0.4 (24h) 30.65±0.6 (48h)	62.13±1.2 (24hr) 32.24±1.0 (48h)	63.21 ±0.8 (24h) 32.65±0.7 (48h)	68.10±1.0 (24hr) 31.24±1.0 (48h)
12	8l	52.20 ±0.5 (24h) 21.65±0.8 (48h)	50.13±1.2 (24hr) 28.21±1.0 (48h)	53.21 ±0.8 (24h) 22.65±0.7 (48h)	56.10±1.0 (24hr) 31.24±1.0 (48h)
13	8m	46.28 ±0.5 (24h) 20.65±0.7 (48h)	38.13±1.2 (24hr) 28.28±1.0 (48h)	43.21 ±0.8 (24h) 22.65±0.7 (48h)	48.10±1.0 (24hr)

					23.24±1.0 (48h)
14	8n	38.21 ±0.7 (24h) 18.65±0.8 (48h)	40.13±1.2 (24hr) 21.20±1.0 (48h)	43.21 ±0.8 (24h) 12.65±0.7 (48h)	46.10±1.0 (24hr) 11.24±1.0 (48h)
15	8o	55.21 ±0.6 (24h) 38.65±0.6 (48h)	60.13±1.2 (24hr) 24.24±1.0 (48h)	53.21 ±0.8 (24h) 22.65±0.7 (48h)	56.10±1.0 (24hr) 31.24±1.0 (48h)
16	8p	45.20 ±0.5 (24h) 20.65±0.6 (48h)	40.13±1.2 (24hr) 18.24±1.0 (48h)	43.26 ±0.8 (24h) 22.65±0.7 (48h)	46.10±1.0 (24hr) 21.24±1.0 (48h)
17	Doxorubicin	24.20 ±0.6 (24h) 8.62±0.6 (48h)	19.18±1.2 (24hr) 7.21±1.0 (48h)	20.25 ±0.8 (24h) 10.61±0.7 (48h)	24.10±1.0 (24hr) 9.20±1.0 (48h)

5. RESULTS AND DISCUSSIONS:

In general, derivatives of present scheme caused significant negative regulation of cell proliferation for all four tumors, showing higher potencies with a slight cytotoxic activity for normal fibroblasts, anyway completely not significant when considered the concentrations required for antitumor activity. Indeed, among the newly synthesized compounds the most potent derivative proved to be 8a that reported EC_{50s} = 20–26 μM at 24 h of treatment and 8–12 μM at 48 h of treatment. In particular, **8a, 8b** compounds proved to be active at concentrations lower than 20 μM against all the tested cell lines at 48 h of treatment, compared to standard doxorubicin. Compound **8a** was active at 20 μM on CAL27 at 48h of treatment. Furthermore, the 2,4 di halo phenyl moiety and 4-Flouro substitution on phenyl ring proved to be generally endowed with higher potencies in respect to the hit compound. As regards 8b compound was reported EC_{50} values ranging from 25 and 28 μM at 48 h of treatment without exerting cytotoxic activity on normal somatic cells up to 60 μM. Notably, compound 8a, 8b showed higher potencies in respect to the hit against the tested cell lines.

Among the compounds the compound **8a, 8b, 8c 8d** substituted derivatives were characterized by different substituents in para ortho position of the phenyl ring whose activity decreases in the following order: F > br > Cl>NO2. In particular, compound **8k** proved to be completely poor activity against all the tested cell. These results indicate the importance of an electron withdrawing substituent in para position of the phenyl residue. Differently, as regards 2,4 di flouro and 2,4 dibromo substituted derivatives (**8a, 8b**) led to the best acting compound 1e, while the p-F,p-Br disubstitution led to promising potent active compound. The compound **8k** ($R=NO_2$; $R_1=N0_2$) led to a less effective decrease in the cell viability of tumor cells. These results indicate that, for compounds bearing a m-Cl substituent on the Phenyl residue, the activity
decreasing.

6. MOLECULAR DOCKING :

Molecular docking studies of **Scheme-1** were carried out using Schrödinger software (Schrödinger, Version 2019-1,) installed on Intel Xenon W 3565 processor and Ubuntu enterprise (version 14.04) as an operating system. The ligands were drawn by using ChemDraw 18.0. With the help of XP Visualiser (Schrödinger , Version 2019-1). The results were analyzed.

Schrodinger software (Version 2019-1; Schrodinger) (Glide module). The ligands used as inputs for docking were sketched by using ChemDraw software. Ligands were prepared using OPLS3e force field in Ligprep (Dizdaroglu et al. 2020) (Version 2019-1, Schrodinger) was used to carry out, the docking studies This minimization helps to assign bond orders, Addition of the hydrogens to the ligands. The generated output file containing the best conformations of the ligands was used for docking studies. Protein was prepared by using the protein preparation wizard (Dizdaroglu et al. 2020) (Version 2019-1, Schrodinger). Charges were assigned to the protein after addition of hydrogen atoms Generated Het states using epik at pH 7.2. The protein was pre-processed refined, modified by analyzing workspace. Atoms which are non-significant were excluded from the crystal structure. Finally, the protein was optimized by using OPLS3e force filed. A receptor grid was generated around the cocrystal ligand (X-ray pose of the ligand in the protein). Ligand centroid was selected to generate grid box, and Vander Waal radius of receptor atoms was scaled to 1.00 Å having a partial atomic charge of 0.25. From the output, The best-docked structure was determined using Glide docking score. Poses of the generated output of ligands after docking was analyzed by the help of XP Visualizer (Version 2019-1, Schrodinger). The results are presented in **Tables-3** and **Figures-1** and **2.**

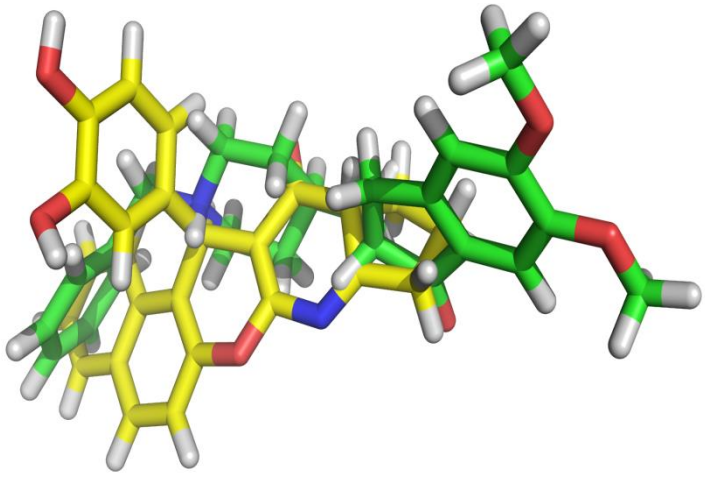


Figure 7: Superimposed pose of **8a** (yellow) with co-crystal ligand (green).

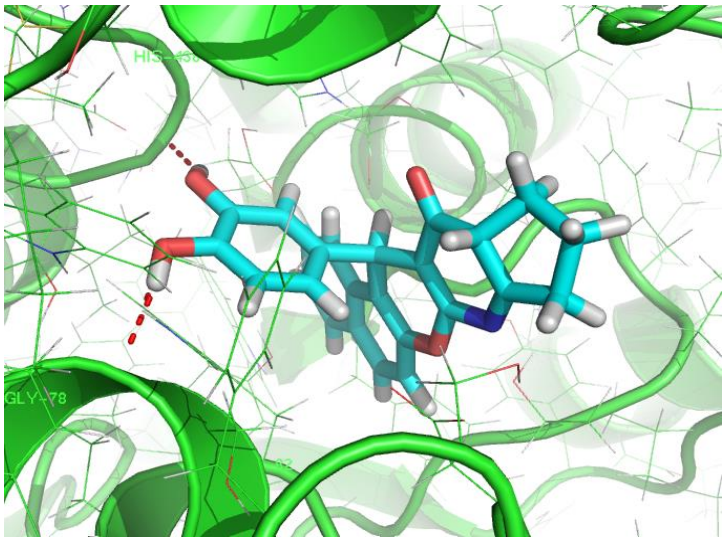


Figure-2: Docking pose of **8a** (cyan) with MCF-7 (green) hydrogen bonds are shown in red dotted line

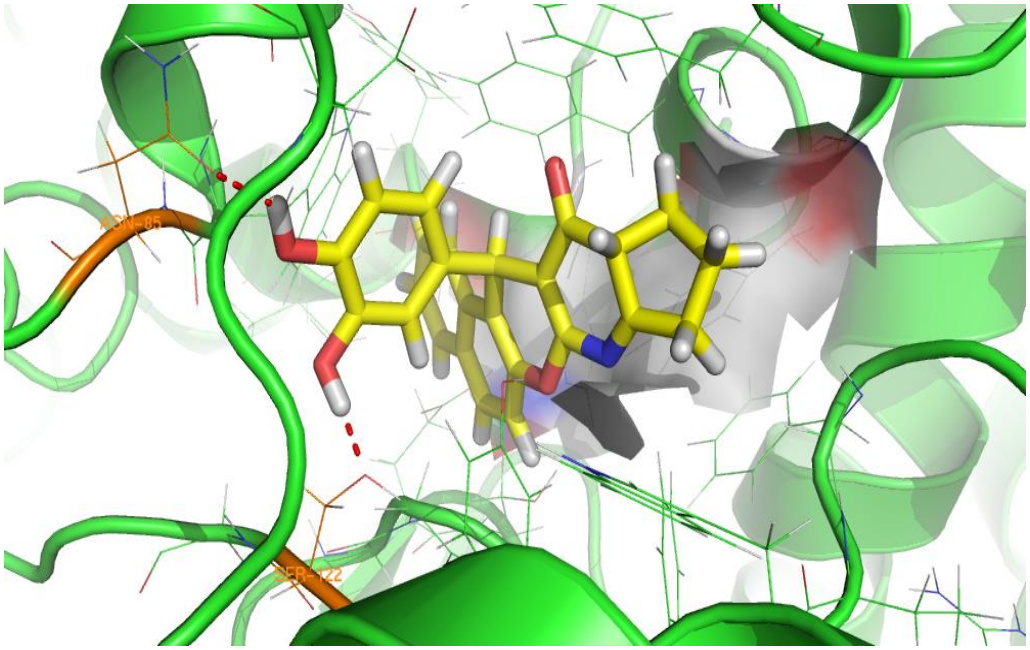


Figure-3: Docking pose of **8a** (cyan) with Hela (green) hydrogen bonds are shown in red dotted line

Table 4:

S.No	Compound	Docking score of MCF-7 (3HY3)	Docking score of HELA (6I2I)	Docking score of Carcinoma HT-29 (4IJ0)	Docking score of tongue carcinoma Cal 27 (6M2O)
1	**8a**	**-11.91**	**-10.82**	**-12.71**	**-10.78**
2	**8b**	**-10.70**	**-9.723**	**-11.78**	**-9.73**
3	8c	-2.05	-5.41	-6.05	-5.98
4	8d	-5.14	-3.71	-5.14	-3.71
5	8e	-6.86	-5.39	**-6.25**	**-7.12**
6	8f	7.40	-6.71	**7.40**	**-6.71**
7	8g	-5.38	-4.48	6.12	-6.48
8	8h	-4.71	-3.48	-5.12	-4.89
9	8i	-3.942	-3.52	-5.942	-6. 26
10	8j	-4.38	-3.47	-4.38	-3.47
11	8k	-2.38	-3.241	-5.38	-4.24
12	8l	-6.934	-5.24	-6.23	-5.45
13	8m	-5.70	-4.22	-5.70	-4.52
14	8n	-4.32	-4.21	-5.52	-4.21
15	8o	4.401	-4.711	4.401	-4.711
16	8p	-6.38	-5.45	6.15	-5.25
17	Cocrystal Ligand	-5.787	-9.068	-4. 871	-9.060

Table 4: Docking energies of new pyrido-pyrimidine (8a-p) ; Binding Energies (Kcal/mol), No. of HBs and Binding Sites

In-vitro studies of synthesized compounds showed the potential anti-cancer activity and among the compounds **8a** showed promising anti-cancer activity. These result encouraged us to perform docking studies to get the insight in to the binding mode of synthesized compounds within binding pocket of MCF-7, HeLa carcinoma Cal 27 and Carcinoma HT-29 All structures of ligands were built using maestro and further prepared using LigPrep form Schrodinger package. Protein structures were obtained from the Protein Data Bank (PDB ID: MCF-7-3HY3 and HeLa -6I2I, HT-29-4IJ0, Cal 27-6M2O) and necessary correction to the protein structure were done using Protein Preparation Wizard in Schrodinger package. Docking studies were performed using Glide docking software and docking protocol was validated by docking the cocrystal ligand which resulted with RMSD of docked conformation and cocrystal ligand pose was found to be 0.6. The binding interactions of compounds with MCF-7-3HY3 and HeLa -6I2I, HT-29-4IJ0, Cal 27-6M2O have been listed in **Table- 1, Figure- 1 and 2 and 3.**

Docking study was performed on binding poses of synthesized compounds with MCF-7 have shown that these molecules bind well within binding pocket of enzyme. Among the all synthesized molecules, compound with potent cancer activity **8a,** has shown the highest binding score(-11.91, -10.82, 12.71 and 10.78 against MCF-7, HeLa, HT-29 and Cal 27). In superimposed pose of **8a,** with cocrystal ligand, isatin ring was coinciding with skeleton of cocrystal ligand as depicted in **Figure-1**

7. CONCLUSION:

New sixteen pyrido[2,3-d]pyrimidin-4(3H)-one derivatives have been designed and synthesised as anti cancer agents. These compounds were evaluated for antiproliferative activities against A- MCF-7, HeLa, HT-29 and Cal 27 cell lines. Compounds **8a, 8 b** exhibited the highest activities. Compound 8a showed promising activities against MCF-7, HeLa, HT-29 and Cal 27 cell lines with IC50 values of 25.21±0.6, 20.13±1.2, 23.21±0.8 and 26.10±1.0 mM, respectively. Structure-activity relationship studies revealed that pyrido [20,30:4,5]pyrimido[2,1-b]quinazoline- 5,7(12H)-dione derivatives 8a–d have the preferred impact on the anticancer activity. In addition, the existence of an electron- donating (NO_2) group at 4-position of compounds 8i-l showed less potent activity. Docking studies revealed that the synthesised compounds have similar binding modes against the prospective biological targets. This work introduces compounds 8a as a potential promising anti-cancer activity.

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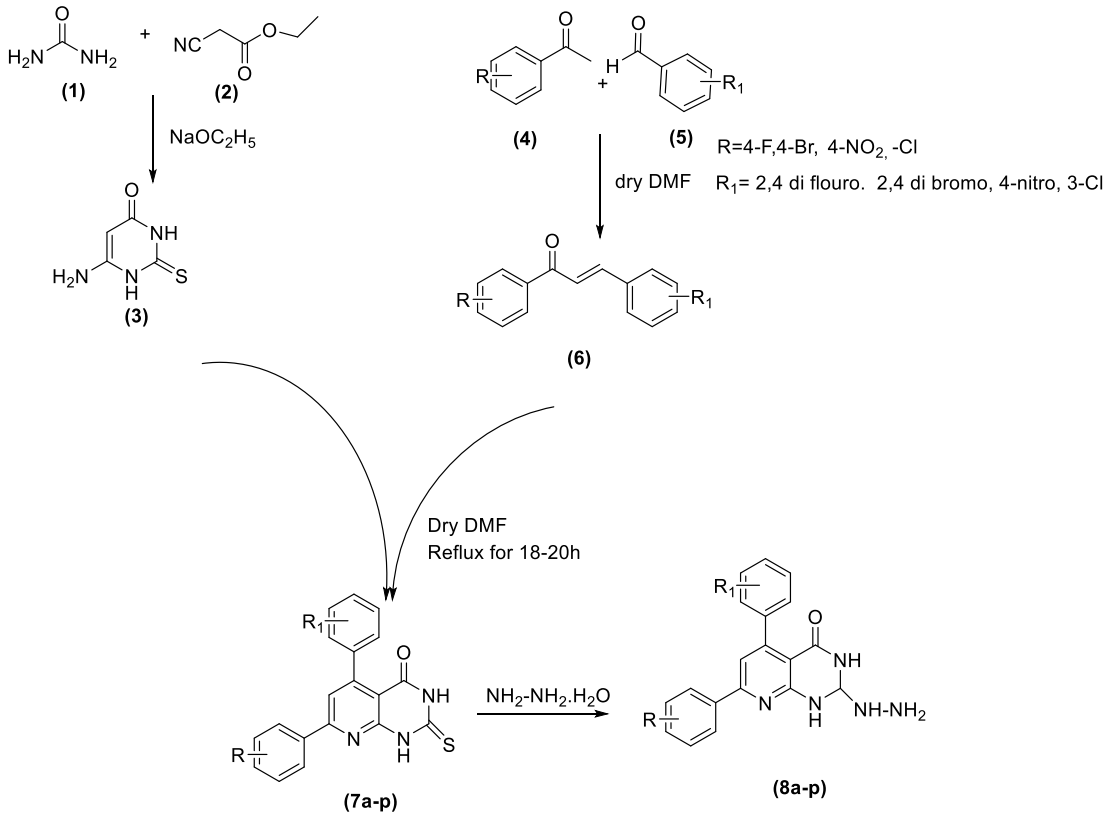

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7b=R=4-F; R_1=2,4 dibromo
7c=R=4-F; R_1=4-nitro
7d=R=4-F; R_1=3-Cl
7e=R=4-Br; R_1=2,4 diflouro
7f=R=4-Br R_1=2,4 dibromo
7g=R=4-Br; R_1=4-nitro
7h=R=4-Br; R_1=3-Cl
7i=R=4-NO_2; R_1=2,4 diflouro
7j=R=4-NO_2; R_1=2,4 dibromo
7k=R=4-NO_2; R_1=4-nitro
7l=R=4-NO_2; R_1=3-Cl
7m=R=3-Cl; R_1=2,4 diflouro
7n=R=3-Cl R_1=2,4 dibromo
7o=R=3-Cl; R_1=4-nitro
7p=R=3-Cl; R_1=3-Cl

8a=R=4-F; R_1=2,4 diflouro
8b=R=4-F; R_1=2,4 dibromo
8c=R=4-F; R_1=4-nitro
8d=R=4-F; R_1=3-Cl
8e=R=4-Br; R_1=2,4 diflouro
8f=R=4-Br R_1=2,4 dibromo
8g=R=4-Br; R_1=4-nitro
8h=R=4-Br; R_1=3-Cl
8i=R=4-NO_2; R_1=2,4 diflouro
8j=R=4-NO_2; R_1=2,4 dibromo
8k=R=4-NO_2; R_1=4-nitro
8l=R=4-NO_2; R_1=3-Cl
8m=R=3-Cl; R_1=2,4 diflouro
8n=R=3-Cl R_1=2,4 dibromo
8o=R=3-Cl; R_1=4-nitro
8p=R=3-Cl; R_1=3-Cl