

Novel Pyridopyrimidine Derivatives As Anticancer Leads: Rational Design, Synthesis, Structure–Activity Relationship, And Biological Evaluation

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ABSTRACT

Structurally diverse series of novel pyrido[2,3-*d*]pyrimidine derivatives was designed and synthesized using a click-chemistry-based approach to identify promising anticancer candidates. The structures and purity of the synthesized compounds were established through comprehensive spectral characterization. Their antiproliferative potential was investigated by an *in vitro* MTT assay against a panel of human cancer cell lines. Most derivatives exhibited measurable cytotoxic activity, with compounds **11m** and **11e** emerging as the most promising candidates. Compound **11m**, bearing an 8-bis(4-fluorophenyl)-4-oxo-2-phenyl-substituted pyrido[2,3-*d*]pyrimidine framework, demonstrated the strongest activity against MDA-MB-231, HeLa, and MCF-7 cells, with IC₅₀ values of **1.29 ± 0.18**, **1.34 ± 0.02**, and **1.57 ± 0.12 μM**, respectively. Compound **11e**, containing 5-amino, 3-chlorophenyl, 4-fluorophenyl, and 4-oxo-2-phenyl pharmacophoric features, also displayed pronounced cytotoxicity, producing IC₅₀ values of **1.42 ± 0.12**, **1.54 ± 0.13**, and **1.85 ± 0.23 μM** against the corresponding cell lines. Molecular-docking investigations revealed favourable accommodation of the active derivatives within the binding pockets of the selected biological targets and identified stabilizing interactions consistent with their observed cytotoxic effects. The collective structure–activity and molecular-modelling findings indicate that appropriately substituted pyrido[2,3-*d*]pyrimidines represent a valuable scaffold for developing potent anticancer agents. In particular, compounds **11m** and **11e** warrant further mechanistic, selectivity, and *in vivo* investigations as promising lead candidates for anticancer drug development.

Keywords: Pyrido[2,3-*d*]pyrimidines; click chemistry; anticancer activity; cytotoxicity; MTT assay; molecular docking; structure–activity relationship.

INTRODUCTION

Human health is one of the most significant global economic growth factors. So, investigating diseases and their possible treatments is an important research topic. According to the World Health Organization (WHO), there are now 18 million peoples who have been diagnosed with cancer. Additionally, in the year 2018, more than 9 million people lost their lives due to cancer. As a result of the absence of efficient and targeted treatments for cancer, these figures continue to rise. Compounds derived from the pyrimidine scaffold have a wide range of pharmacological effects, including anti-inflammatory properties (Kaur H et al., 2015), antidiabetic (Barakat A et al., 2015) antimicrobial (Su L et al., 2017), and anticancer (Zimmermann J et al., 2009 and Kaldrikyan MA et al., 2000) anti-HIV activity (Desai NC et al., 2015). The

most commonly recognized drugs derived from pyrimidine analogs include antibacterial agents (such as sulfadiazine and trimethoprim), antiviral agents (like trifluridine and idoxuridine), anti-malarial therapies (such as sulfadoxine), anti-HIV medications (such as Retrovir or zidovudine and stavudine), anti-tuberculosis treatments (like viomycin), and anticancer agents (such as 5-fluorouracil). The findings also indicate that the addition of a substituted aniline and phenyl and alkyl moieties increases the effectiveness of drugs in fighting cancer (Tylinska B et al., 2018, Jasztołd-Howorko R et al., 2013 and Nguyen, CH et al., 1999). Based on these facts, and with the aim of developing new lead structures with potential chemotherapeutic activities, it was selected to design, synthesize, and evaluate the antitumor properties of a number of new compounds containing the pyridino[3,4-*d*]pyrimidine as the primary

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framework. In order to achieve this, certain modifications were made to the original lead compound.

2.0. Design of 5-amino-4-oxo-N-2,8- substituted triphenyl-3,4,5,6,7,8-hexahydropyrido[2,3-d]-pyrimidine-6-carboxamide (11a-o):

Kisliuk et al., 1996 reported the 2,4-Diamino-6-[N-(2',5'-dimethoxybenzyl)-N-methylamino]pyrido[2,3-d]pyrimidine (**I**) was the most potent anti-cancer agent against tgDHFR (IC₅₀) 6.3 nM) and was the second most selective analogue for tgDHFR (compared to rIDHFR) in the nonclassical series. Lierman E, et al., 2007 reported the Sorafenib (**II**) (BAY43-9006, Nexavar®) is a potent B-RAF inhibitor that effectively treats renal cell carcinoma. It also exhibits significant inhibitory effects on receptor tyrosine kinases belonging to the PDGFR and VEGFR families. Kelly, et al. 2012 developed Tandutinib (**III**) (CT53518A) approximately 30% of people with acute myelogenous leukemia (AML) have an activating internal tandem duplication (ITD)

in the juxtamembrane domain of the FLT3 receptor. This indicates that the FLT3 receptor might potentially be targeted for treatment with kinase inhibitors. We have created, a powerful antagonist that effectively blocks FLT3, platelet-derived growth factor receptor (PDGFR), and c-Kit (with an IC₅₀ of 200 nM). Campos JF et al., 2022 reported Trametinib (**IV**) is a drug that inhibits the activity of kinases and is specifically prescribed for certain forms of melanoma. The FDA has specifically authorized this medication, in conjunction with other compounds like Mekinist (trametinib), for the treatment of degenerative thyroid cancer. From the *insilico* screening results, the Series of compounds designed based on the structure of the ligand 5-amino-4-oxo-N-2,8- substituted triphenyl-3,4,5,6,7,8-hexahydropyrido[2,3-d]-pyrimidine-6-carboxamide that belongs to pyrido[2,3-d]-pyrimidine- scaffold. In the present Scheme, a series of 5-amino-4-oxo-N-2,8- substituted triphenyl-3,4,5,6,7,8-hexahydropyrido[2,3-d]-pyrimidine-6-carboxamide derivatives were designed as shown below in **General Structure-I**.

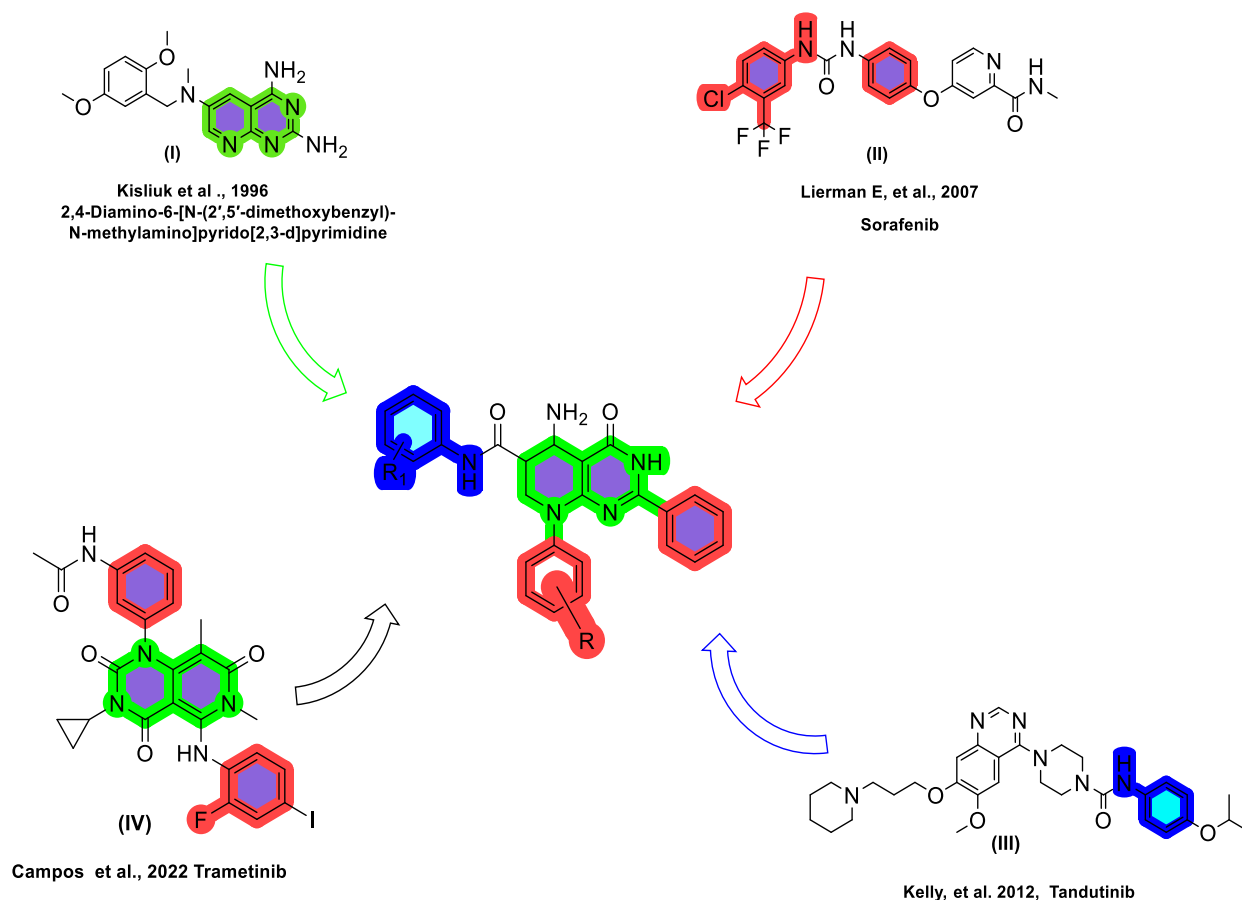
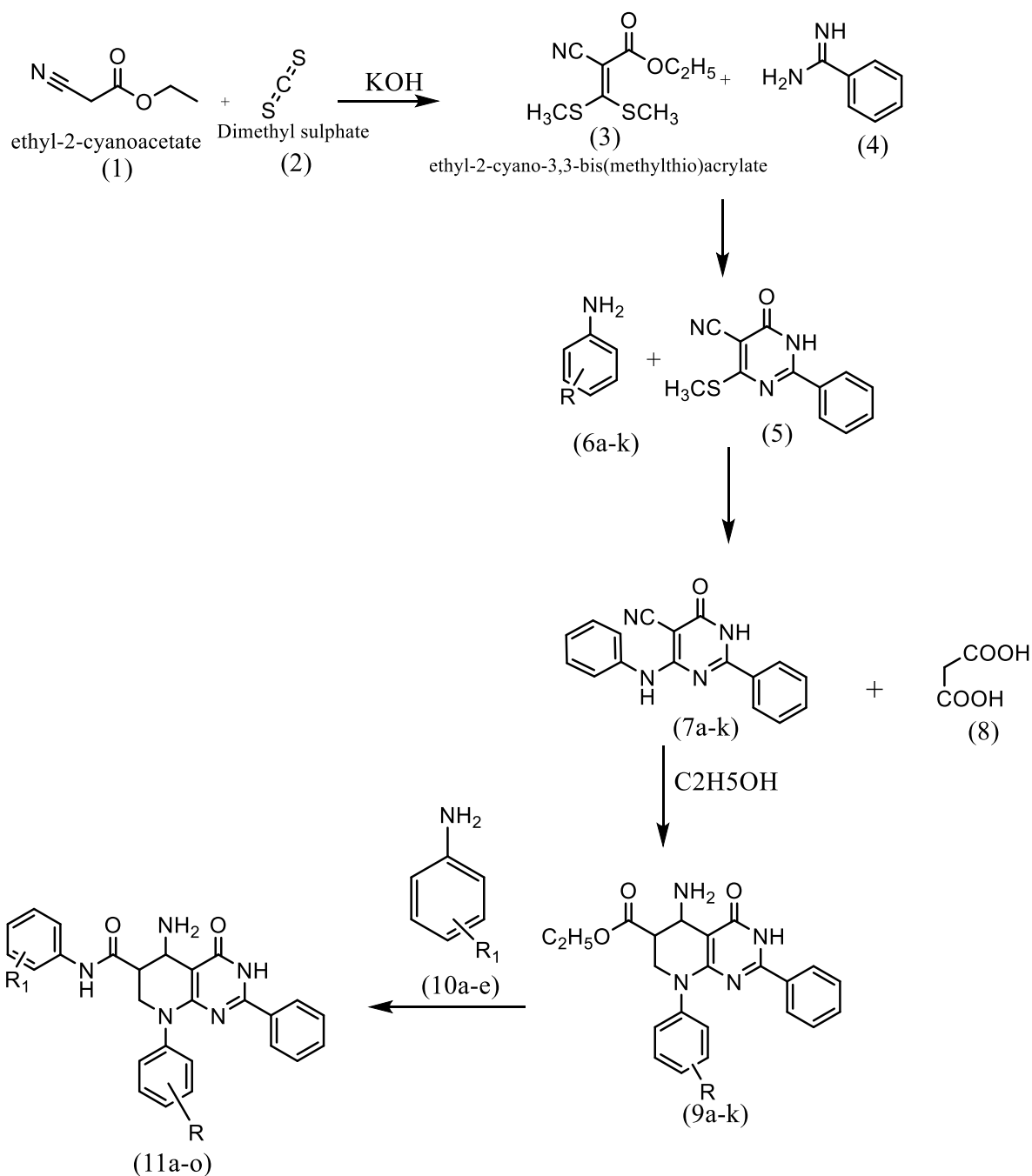


Fig:1: Design of 5-amino-4-oxo-N-2,8- substituted triphenyl-3,4,5,6,7,8-hexahydropyrido[2,3-d]-pyrimidine-6-carboxamide (11a-o).



Scheme-1: 5-amino-4-oxo-N-2,8- substituted triphenyl-3,4,5,6,7,8-hexahydropyrido[2,3-d]-pyrimidine-6-carboxamide (11a-o).

3.0. Experimental Procedure:

3.1. Preparation of Ethyl 3,3 bis (methylthio) -2-cyanoacrylate (3):

30 ml of dimethyl formamide, 0.1 mol of ethylcyano acetate (1), and 0.1 mol of carbon disulfide (2) were added to an ice-cold solution of 0.2 mol of potassium hydroxide. The mixture was continuously cooled and stirred. The mixture was stirred for a duration of one hour at the ambient temperature, then cooled, and then

subjected to the gradual addition of dimethyl sulphate (0.2 mol) while keeping the temperature at 20°C. The reaction mixture was kept at room temperature for 12 hours and then transferred into 500 ml cold water. The solid was filtered, rinsed with cold water and recrystallization using n-hexane then dried.

3.2. Preparation of 2-substituted-4-(methylthio)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (5): A mixture of ethyl 2- cyano-3,3-bis(methylthio) acrylates (3; 0.02 mol) and aromatic amidines (4; 0.02

mol) in 30 ml of ethanol were refluxed for three hours. The reaction mixture was allowed to stand at room temperature for 12 hours. The solid obtained was filtered, washed with cold ethanol, recrystallized with n-hexane and benzene mixture and dried.

3.3. Step 3: Preparation of 4- (substituted phenyl amino) -2-substituted-6-oxo-1,6-dihydro pyrimidine-5-carbonitrile (7a-k): A mixture of 2-substituted-4-(methylthio)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (5; 0.02 mol) and substituted anilines (6a-k; 0.01mol) in 30 ml of ethanol were refluxed for one hour. The reaction mixture was allowed to stand at room temperature for 24 hours. The solid obtained was filtered, washed with cold ethanol, recrystallized from n-hexane and dried.

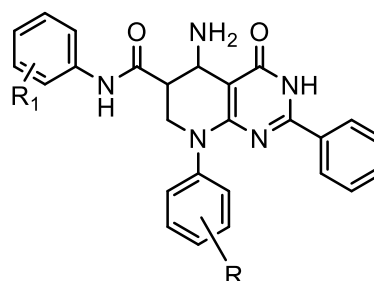
3.4. Step 4: Preparation of Ethyl-5-amino-8- (substituted phenyl) -2-substituted-4, 7-dioxo-3,4,5,6,7,8-hexa hydro pyrido (2,3-d) pyrimidine -6- carboxylate (9a-k):

A mixture of 4-(4-halo phenyl amino) -2-substituted-6-oxo-1,6-dihydro pyrimidine-5-carbonitrile (7a-k; 0.01mol) and malonic acid (8; 0.02mol) and 30 ml of ethanol were refluxed for one hour. The reaction mixture was allowed to stand at room temperature for 24 hours. The solid obtained was filtered, washed with

cold ethanol, recrystallized from benzene-hexane mixture and dried.

3.5. Step 5: Preparation of 5-amino-4-oxo-N-substituted,2,8-triphenyl-3,4,5,6,7,8-hexahydropyrido[2,3-d]pyrimidine-6-carboxamide (11a-o):

A mixture of Ethyl-5-amino-8- (substituted phenyl) -2-substituted-4, 7-dioxo- 3,4,5,6,7,8-hexa hydro pyrido (2,3-d) pyrimidine -6- carboxylate (9a-k; 0.02 mol) and substituted anilines (10a-e; 0.01mol) in 30 ml of ethanol were refluxed for one hour. The reaction mixture was allowed to stand at room temperature for 24 hours. The solid obtained was filtered, washed with cold ethanol, recrystallized from n-hexane and dried to get title compounds.



General structure-I

Comp.	R	R ₁	M. Form	M.Wt	M.P°C	Rf*	% Yield
11a	H	4-F	C ₂₆ H ₂₂ FN ₅ O ₂	471	106-108	0.6	56
11b	H	4-Cl	C ₂₆ H ₂₂ ClN ₅ O ₂	507	102-104	0.5	70
11c	H	4-Br	C ₂₆ H ₂₂ BrN ₅ O ₂	394	106-108	0.6	65
11d	H	4-NO ₂	C ₂₆ H ₂₂ N ₆ O ₄	482	130-132	0.4	75
11e	3-Cl	4-F	C ₂₆ H ₂₁ ClFN ₅ O ₂	489	168-170	0.5	74
11f	3-Cl	4-Cl	C ₁₉ H ₁₄ Br ₃ N ₅ O	506	180-182	0.8	54
11g	3-Cl	4-Br	C ₁₉ H ₁₅ BrN ₆ O ₃	455	158-160	0.5	70
11h	3-Cl	4-NO ₂	C ₁₉ H ₁₅ BrClN ₅ O	444	118-120	0.7	38
11i	4-Br	4-F	C ₁₉ H ₁₄ F ₂ N ₆ O ₃	533	122-124	0.5	64
11j	4-Br	4-Cl	C ₁₉ H ₁₄ Br ₂ N ₆ O ₃	531	150-152	0.7	80
11k	4-Br	4-Br	C ₁₉ H ₁₅ N ₇ O ₅	421	162-164	0.5	70
11l	4-Br	4-NO ₂	C ₁₉ H ₁₅ ClN ₆ O ₃	410	150-152	0.6	48

11m	4-F	4-F	C ₁₉ H ₁₄ ClF ₂ N ₅ O	401	190-192	0.6	62
11n	4-F	4-Cl	C ₁₉ H ₁₄ Br ₂ ClN ₅ O	520	196-198	0.5	60
11o	4-F	4-Br	C ₁₉ H ₁₅ ClN ₆ O ₃	410	160-162	0.6	54

Table-1. 5-amino-4-oxo-N- 2, 8- substituted triphenyl-3,4,5,6,7,8-hexahydropyrido[2,3-d]-pyrimidine-6-carboxamide (11a-o)

3.0. Spectral data:

3.1. 5-amino-N-(4-fluorophenyl)-4-oxo-2,8-diphenyl-3,4,5,6,7,8-hexahydropyrido[2,3-d]pyrimidine-6-carboxamide (11a): IR spectrum (KBr, cm⁻¹): 3408.28 (NH (str)), 3045.12 (C-H Aromatic (str)), 2980.10 (C-H Aliphatic (str)), 1590.18 (C=C Aromatic(str)), 1228.45(C=S (str)). ¹H NMR (400MHz CDCl₃, δ ppm): 8.012-8.120(s, 1H, aromatic CH), 7.967-8.010 (d, 1H, aromatic CH), 7.910-7.924 (m, 2H, aromatic CH), 7.817-7.823 (m, 2H, aromatic CH), 7.684-7.694 (d, 2H, aromatic CH), 5.054 (s, 1H, NH), 2.435 (s, 2H, aliphatic CH). 1.581 (s, 2H, aliphatic CH). ¹³C NMR (100MHz, CDCl₃): 161.10, 158.18, 156.90, 149.09, 140.57, 138.14, 134.25, 130.80, 129.13, 128.48, 128.95, 120.80, 115.75, 58.12, 37.25. MASS spectrum m/z: 395 [M+2]⁺, 397 [M+4]⁺; Calc. for C₂₆H₂₂FN₅O₂; CHN: C, 68.56; H, 4.87; N, 15.38; Found C, 68.50; H, 4.81; N, 15.30.

3.2. 5-amino-N-(4-chlorophenyl)-4-oxo-2,8-diphenyl-3,4,5,6,7,8-hexahydropyrido[2,3-d]pyrimidine-6-carboxamide (11b): IR spectrum (KBr, cm⁻¹): 3340.99 (NH (str)), 3058.56 (C-H Aromatic (str)), 2976.58(C-H Aliphatic (str)), 1591.58 (C=C Aromatic (str)), 1222.35(C=S(str)). ¹H NMR (400MHz CDCl₃, δ ppm): 7.975-8.000 (d, 1H, aromatic CH), 7.775-7.795 (d, 1H, aromatic CH), 7.672-7.679 (s, 1H, aromatic CH), 7.634-7.656 (s, 1H, aromatic CH), 7.419-7.471 (d, 1H, aromatic CH), 7.381-7.398 (d, 1H, aromatic CH), 6.808-7.285 (d, 1H, aromatic CH), 6.267 (s, 1H, NH), 4.297 (s, 1H, aliphatic), 4.209 (s, 2H, aliphatic), 2.433 (s, 1H, aliphatic CH). 1.149 (s, 1H, aliphatic CH). ¹³C NMR (100MHz, CDCl₃): 157.81, 152.64, 142.08, 132.54, 131.08, 129.35, 129.09, 128.56, 128.14, 125.58, 125.27, 124.48, 114.02, 101.28, 57.20, 42.08. MASS spectrum m/z: 348 [M+H]⁺, 350 [M+2]⁺, 352 [M+4]⁺, Calc. for C₂₆H₂₂ClN₅O₂; CHN: C, 66.17; H, 4.70; N, 14.84; Found C, 66.01; H, 4.74; N, 14.80.

3.3. 5-amino-N-(4-bromophenyl)-4-oxo-2,8-diphenyl-3,4,5,6,7,8-hexahydropyrido[2,3-d]pyrimidine-6-carboxamide (11c): IR spectrum (KBr, cm⁻¹): 3408.28 (NH (str)), 3045.12 (C-H Aromatic (str)), 2980.10 (C-H Aliphatic (str)), 1590.18 (C=C Aromatic(str)), 1228.45(C=S (str)). ¹H NMR (400MHz CDCl₃, δ ppm): 8.012-8.120(s, 1H, aromatic CH), 7.967-8.010 (d, 1H, aromatic CH), 7.910-7.924 (m, 2H, aromatic CH), 7.817-7.823 (m, 2H, aromatic CH), 7.684-7.694 (d, 2H, aromatic CH), 5.054 (s, 1H, NH), 2.435 (s, 2H, aliphatic CH). 1.581 (s, 2H, aliphatic CH). 1.256 (s, 1H, NH). ¹³C NMR (100MHz, CDCl₃): 161.10, 158.18, 156.90, 149.09, 140.57, 138.14, 134.25, 130.80, 129.13, 128.48, 128.95, 120.80, 115.75, 58.12, 37.25. MASS spectrum m/z: 395 [M+2]⁺, 397 [M+4]⁺; Calc. for C₂₆H₂₂BrN₅O₂; C, 48.86; H, 3.59; N, 14.25; S, 8.15; Found C, 48.86; H, 3.50; N, 14.24; S, 8.10.

3.4. 5-amino-N-(4-nitrophenyl)-4-oxo-2,8-diphenyl-3,4,5,6,7,8-hexahydropyrido[2,3-d]-pyrimidine-6-carboxamide (11d): IR spectrum (KBr, cm⁻¹): 3481.20 (NH (str)), 3062.10 (C-H Aromatic (str)), 2960.10 (C-H Aliphatic (str)), 1570.04(C=C Aromatic (str)), 1254.18(C=S, (str)). ¹H NMR (400MHz CDCl₃, δ ppm): 8.134-8.139 (d, 2H, aromatic CH), 7.960-7.978 (s, 1H, aromatic CH), 7.820-7.824 (s, 2H, aromatic CH), 7.650-7.675 (m, 2H, aromatic CH), 7.640-7.648 (s, 1H, aromatic CH), 5.059 (s, 1H, NH), 4.502 (s, 2H, aliphatic CH), 1.581 (s, 1H, aliphatic CH). 1.270 (s, 2H, aliphatic CH). ¹³C NMR (100MHz, CDCl₃): 168.96, 148.15, 146.50, 138.50, 135.08, 130.31, 128.10, 126.10, 124.88, 122.27, 121.38, 114.32, 101.28, 57.28, 42.15, 28.45. MASS spectrum m/z: 332 [M+H]⁺, 334 [M+2]⁺, 336 [M+2]⁺. Calc. for C₂₆H₂₂N₆O₄; CHN: C, 64.72; H, 4.60; N, 17.42; Found C, 64.70; H, 4.65; N, 17.40.

3.5. 5-amino-8-(3-chlorophenyl)-N-(4-fluorophenyl)-4-oxo-2-phenyl-3,4,5,6,7,8-hexahydropyrido[2,3-d]pyrimidine-6-

carboxamide (11e): IR spectrum (KBr, cm^{-1}): 3551.56 (NH (str)), 3080.32 (C-H Aromatic (str)), 2931.73 (C-H Aliphatic (str)), 1585.20 (C=C Aromatic(str)), 1266.18(C=S (str)). **^1H NMR (400MHz CDCl_3 , δ ppm):** 7.901-7.923 (d, 1H, aromatic CH), 7.550-7.810 (m, 1H, aromatic CH), 7.654-7.681 (d, 1H, aromatic CH), 7.553-7.556 (d, 1H, aromatic CH), 7.494-7.539 (d, 1H, aromatic CH), 7.313-7.363 (m, 1H, aromatic CH), 7.093-7.130 (m, 1H, aromatic CH), 6.887-6.911 (d, 1H, aromatic CH), 5.063 (s, 1H, NH), 5.057 (s, 1H, aliphatic CH), 3.780 (s, 2H, aliphatic CH). 2.675 (s, 1H, aliphatic CH). 1.625 (s, 2H, aliphatic CH). **^{13}C NMR (100MHz, CDCl_3):** 153.78, 152.34, 148.50, 143.63, 142.44, 139.59, 138.74, 136.03, 129.96, 129.40, 127.96, 127.65, 126.73, 125.01, 119.69, 48.15, 38.66. **MASS spectrum m/z:** 385 $[\text{M}+2]^+$, 387 $[\text{M}+4]^+$, 389 $[\text{M}+6]^+$. Calc. for $\text{C}_{26}\text{H}_{21}\text{ClFN}_5\text{O}_2$; C, 63.74; H, 4.32; N, 14.29; Found C, 63.70; H, 4.30; N, 14.21.

3.6. 5-amino-8-(3-chlorophenyl)-N-(4-chlorophenyl)-4-oxo-2-phenyl-3,4,5,6,7,8-hexahydropyrido[2,3-d]pyrimidine-6-carboxamide (11f): IR spectrum (KBr, cm^{-1}): 3551.56 (NH (str)), 3080.32 (C-H Aromatic (str)), 2931.73 (C-H Aliphatic (str)), 1585.20 (C=C Aromatic(str)), 1266.18(C=S (str)). **^1H NMR (400MHz CDCl_3 , δ ppm):** 7.901-7.923 (d, 1H, aromatic CH), 7.550-7.810 (m, 1H, aromatic CH), 7.654-7.681 (d, 1H, aromatic CH), 7.553-7.556 (d, 1H, aromatic CH), 7.494-7.539 (d, 1H, aromatic CH), 7.313-7.363 (m, 1H, aromatic CH), 7.093-7.130 (m, 1H, aromatic CH), 6.887-6.911 (d, 1H, aromatic CH), 5.063 (s, 1H, NH), 5.057 (s, 1H, aliphatic CH), 3.780 (s, 2H, aliphatic CH). 2.675 (s, 1H, aliphatic CH). 1.625 (s, 2H, aliphatic CH). **^{13}C NMR (100MHz, CDCl_3):** 153.78, 152.34, 148.50, 143.63, 142.44, 139.59, 138.74, 136.03, 129.96, 129.40, 127.96, 127.65, 126.73, 125.01, 119.69, 48.15, 38.66. **MASS spectrum m/z:** 385 $[\text{M}+2]^+$, 387 $[\text{M}+4]^+$, 389 $[\text{M}+6]^+$. Calc. for $\text{C}_{26}\text{H}_{21}\text{Cl}_2\text{N}_5\text{O}_2$; C, 61.67; H, 4.18; N, 13.83; Found C, 61.60; H, 4.11; N, 13.80.

3.7. 5-amino-N-(4-bromophenyl)-8-(3-chlorophenyl)-4-oxo-2-phenyl-3,4,5,6,7,8-hexahydropyrido-[2,3-d]pyrimidine-6-carboxamide (11g): IR spectrum (KBr, cm^{-1}): 3420.10 (NH (str)), 3061.10 (C-H Aromatic (str)), 2968.18 (C-H Aliphatic (str)), 1578.04 (C=C Aromatic (str)), 1258.10(C=S, (str)). **^1H NMR**

(400MHz CDCl_3 , δ ppm): 8.812-8.816 (d, 1H, aromatic CH), 8.428-8.432(d, 2H, aromatic CH), 7.960-7.978 (m, 2H, aromatic CH), 7.824-7.828 (m, 2H, aromatic CH), 7.652-7.674 (d, 1H, aromatic CH), 5.052 (s, 1H, NH), 4.508 (s, 1H, OH), 1.581 (s, 2H, aliphatic CH). 1.270 (s, 2H, aliphatic CH). **^{13}C NMR (100MHz, CDCl_3):** 160.96, 156.10, 154.10, 148.50, 140.08, 138.31, 128.10, 127.10, 124.88, 122.27, 121.38, 114.32, 101.28, 57.28, 42.15, 28.45. **MASS spectrum m/z:** 365 $[\text{M}+H]^+$, 367 $[\text{M}+2]^+$, 369 $[\text{M}+4]^+$. Calc. for $\text{C}_{26}\text{H}_{21}\text{BrClN}_5\text{O}_2$; CHN: C, 56.69; H, 3.84; Br, 14.51; N, 12.71; Found C, 56.64; H, 3.80; Br, 14.56; N, 12.70.

3.8. 5-amino-8-(3-chlorophenyl)-N-(4-nitrophenyl)-4-oxo-2-phenyl-3,4,5,6,7,8-hexahydropyrido[2,3-d]pyrimidine-6-carboxamide (11h): IR spectrum (KBr, cm^{-1}): 3420.10 (NH (str)), 3052.01 (C-H Aromatic (str)), 2980.10 (C-H Aliphatic (str)), 1580.10 (C=C Aromatic(str)), 1265.40(C=S (str)). **^1H NMR (400MHz CDCl_3 , δ ppm):** 8.812-8.810 (m, 3H, aromatic CH), 8.768-8.772 (d, 1H, aromatic CH), 8.240-8.245 (d, 1H, aromatic CH), 7.980-8.052 (m, 2H, aromatic CH), 7.968-7.970 (m, 2H, aromatic CH), 5.040 (s, 1H, NH), 2.410 (s, 2H, aliphatic CH). 1.541 (s, 2H, aliphatic CH). 1.250 (s, 1H, aliphatic CH). **^{13}C NMR (100MHz, CDCl_3):** 158.10, 158.10, 158.10, 150.10, 148.50, 146.10, 134.20, 132.80, 129.13, 128.40, 127.95, 126.18, 121.18, 50.12, 48.20. **MASS spectrum m/z:** 361 $[\text{M}+2]^+$. Calc. for $\text{C}_{26}\text{H}_{21}\text{ClN}_6\text{O}_4$; C, 60.41; H, 4.09; N, 16.26; Found C, 60.40; H, 4.15; N, 16.20.

3.9. 5-amino-8-(4-bromophenyl)-N-(4-fluorophenyl)-4-oxo-2-phenyl-3,4,5,6,7,8-hexahydropyrido[2,3-d]pyrimidine-6-carboxamide (11i): IR spectrum (KBr, cm^{-1}): 3551.56 (NH (str)), 3080.32 (C-H Aromatic (str)), 2931.73 (C-H Aliphatic (str)), 1585.20 (C=C Aromatic(str)), 1266.18(C=S (str)). **^1H NMR (400MHz CDCl_3 , δ ppm):** 7.901-7.923 (d, 1H, aromatic CH), 7.550-7.810 (m, 1H, aromatic CH), 7.654-7.681 (d, 1H, aromatic CH), 7.553-7.556 (d, 1H, aromatic CH), 7.494-7.539 (d, 1H, aromatic CH), 7.313-7.363 (m, 1H, aromatic CH), 7.093-7.130 (m, 1H, aromatic CH), 6.887-6.911 (d, 1H, aromatic CH), 5.063 (s, 1H, NH), 5.057 (s, 1H, aliphatic CH), 3.780 (s, 2H, aliphatic CH). 2.675 (s, 1H, aliphatic CH). 1.625 (s, 2H, aliphatic CH). **^{13}C NMR**

(100MHz, CDCl₃): 153.78, 152.34, 148.50, 143.63, 142.44, 139.59, 138.74, 136.03, 129.96, 129.40, 127.96, 127.65, 126.73, 125.01, 119.69, 48.15, 38.66. **MASS spectrum m/z**: 385 [M+2]⁺, 387 [M+4]⁺, 389 [M+6]⁺. Calc. for C₂₆H₂₁BrFN₅O₂; C, 58.44; H, 3.96; N, 13.11; Found C, 58.40; H, 3.90; N, 13.16.

3.10. 5-amino-8-(4-bromophenyl)-N-(4-chlorophenyl)-4-oxo-2-phenyl-3,4,5,6,7,8-hexahydropyrido[2,3-d]pyrimidine-6-carboxamide (11j): IR spectrum (KBr, cm⁻¹): 3420.10 (NH (str)), 3061.10 (C-H Aromatic (str)), 2968.18 (C-H Aliphatic (str)), 1578.04 (C=C Aromatic (str)), 1258.10(C=S, (str)). **¹H NMR (400MHz CDCl₃, δ ppm):** 8.812-8.816 (d, 1H, aromatic CH), 8.428-8.432(d, 2H, aromatic CH), 7.960-7.978 (m, 2H, aromatic CH), 7.824-7.828 (m, 2H, aromatic CH), 7.652-7.674 (d, 1H, aromatic CH), 5.052 (s, 1H, NH), 4.508 (s, 1H, OH), 1.581 (s, 2H, aliphatic CH). 1.270 (s, 2H, aliphatic CH). **¹³C NMR (100MHz, CDCl₃):** 160.96, 156.10, 154.10, 148.50, 140.08, 138.31, 128.10, 127.10, 124.88, 122.27, 121.38, 114.32, 101.28, 57.28, 42.15, 28.45. **MASS spectrum m/z**: 365 [M+H]⁺, 367 [M+2]⁺, 369 [M+4]⁺ Calc. for C₂₆H₂₁BrClN₅O₂; CHN: C, 56.69; H, 3.84; N, 12.71; Found C, 56.60; H, 3.80; N, 12.70.

3.11. 5-amino-N, 8-bis(4-bromophenyl)-4-oxo-2-phenyl-3,4,5,6,7,8-hexahydropyrido[2,3-d]pyrimidine-6-carboxamide (11k): IR spectrum (KBr, cm⁻¹): 3431.10 (NH (str)), 3082.18 (C-H Aromatic (str)), 2950.61 (C-H Aliphatic (str)), 1585.10 (C=C Aromatic (str)), 1255.75 (C=S (str)). **¹H NMR (400MHz CDCl₃, δ ppm):** 8.088-8.134 (m, 2H, aromatic CH), 7.901-7.923 (d, 1H, aromatic CH), 7.750-7.810 (m, 1H, aromatic CH), 7.654-7.681(d, 1H, aromatic CH), 7.494-7.556 (d, 1H, aromatic CH), 7.284-7.363 (d, 1H, aromatic CH), 6.887-6.911 (s, 1H, aromatic CH), 5.057 (s, 1H, NH), 2.675 (s, 2H, aliphatic CH), 1.625 (m, 2H, aliphatic CH). 1.279 (s, 1H, NH). **¹³C NMR (100MHz, CDCl₃):** 161.65, 160.15, 142.08, 132.54, 131.08, 129.05, 128.56, 128.14, 126.88, 125.27, 124.48, 114.02, 42.48, 28.56. **MASS spectrum m/z**: 409 [M+H]⁺, 411 [M+2]⁺, 413 [M+4]⁺; Calc. for C₁₆H₁₄BrClN₄S; CHN: C, 46.90; H, 3.44; N, 13.67; S, 7.83; Found C, 46.91; H, 3.43; N, 13.63; S, 7.82.

3.12. 5-amino-8-(4-bromophenyl)-N-(4-nitrophenyl)-4-oxo-2-phenyl-3,4,5,6,7,8-hexahydropyrido[2,3-d]pyrimidine-6-carboxamide (11l): IR spectrum (KBr, cm⁻¹): 3414.01 (NH (str)), 3061.10 (C-H Aromatic (str)), 2960.18 (C-H Aliphatic (str)), 1570.03(C=C Aromatic (str)), 1226.10(C=S (str)). **¹H NMR (400MHz CDCl₃, δ ppm):** 8.812-8.815 (d, 2H, aromatic CH), 8.428-8.432(d, 2H, aromatic CH), 7.960-7.978 (d, 1H, aromatic CH), 7.835-7.842 (d, 1H, aromatic CH), 7.650-7.675 (d, 2H, aromatic CH), 5.059 (s, 1H, NH), 4.502 (s, 1H, NH), 1.581 (s, 2H, aliphatic CH). 1.270 (s, 2H, aliphatic CH). **¹³C NMR (100MHz, CDCl₃):** 160.96, 159.10, 148.50, 137.50, 135.08, 130.31, 128.10, 126.10, 124.88, 122.27, 121.38, 115.32, 105.28, 57.28, 41.10. **MASS spectrum m/z**: 348[M+H]⁺, 350[M+2]⁺, 352[M+4]⁺; Calc. for C₂₆H₂₁BrN₆O₄; CHN: C, 55.63; H, 3.77; N, 14.97; Found C, 55.60; H, 3.70; N, 14.90.

3.13. 5-amino-N, 8-bis(4-fluorophenyl)-4-oxo-2-phenyl-3,4,5,6,7,8-hexahydropyrido[2,3-d]pyrimidine-6-carboxamide (11m): IR spectrum (KBr, cm⁻¹): 3551.56 (NH (str)), 3080.32 (C-H Aromatic (str)), 2931.73 (C-H Aliphatic (str)), 1585.20 (C=C Aromatic(str)), 1266.18(C=S (str)). **¹H NMR (400MHz CDCl₃, δ ppm):** 7.901-7.923 (d, 1H, aromatic CH), 7.550-7.810 (m, 1H, aromatic CH), 7.654-7.681 (d, 1H, aromatic CH), 7.553-7.556 (d, 1H, aromatic CH), 7.494-7.539 (d, 1H, aromatic CH), 7.313-7.363 (m, 1H, aromatic CH), 7.093-7.130 (m, 1H, aromatic CH), 6.887-6.911 (d, 1H, aromatic CH), 5.063 (s, 1H, NH), 5.057 (s, 1H, aliphatic CH), 3.780 (s, 2H, aliphatic CH). 2.675 (s, 1H, aliphatic CH). 1.625 (s, 2H, aliphatic CH). **¹³C NMR (100MHz, CDCl₃):** 153.78, 152.34, 148.50, 143.63, 142.44, 139.59, 138.74, 136.03, 129.96, 129.40, 127.96, 127.65, 126.73, 125.01, 119.69, 48.15, 38.66. **MASS spectrum m/z**: 475 [M+2]⁺, 477[M+4]⁺. Calc. for C₂₆H₂₁F₂N₅O₂; C, 65.95; H, 4.47; N, 14.79; Found C, 65.94; H, 4.42; N, 14.75.

3.14. 5-amino-N-(4-chlorophenyl)-8-(4-fluorophenyl)-4-oxo-2-phenyl-3,4,5,6,7,8-hexahydropyrido[2,3-d]pyrimidine-6-carboxamide (11n): IR spectrum (KBr, cm⁻¹): 3414.01 (NH (str)), 3061.10 (C-H Aromatic (str)), 2960.18 (C-H Aliphatic (str)), 1570.03(C=C Aromatic (str)), 1226.10(C=S (str)). **¹H NMR (400MHz CDCl₃, δ ppm):** 8.812-8.815 (d, 2H,

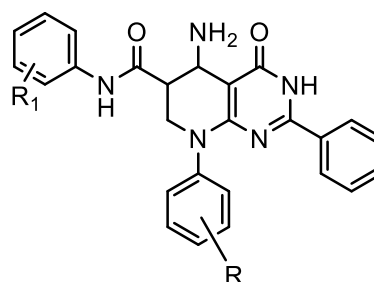
aromatic CH), 8.428-8.432(d, 2H, aromatic CH), 7.960-7.978 (d, 1H, aromatic CH), 7.835-7.842 (d, 1H, aromatic CH), 7.650-7.675 (d, 2H, aromatic CH), 5.059 (s, 1H, NH), 4.502 (s, 1H, NH), 1.581 (s, 2H, aliphatic CH). 1.270 (s, 2H, aliphatic CH). ¹³C NMR (100MHz, CDCl₃): 160.96, 159.10, 148.50, 137.50, 135.08, 130.31, 128.10, 126.10, 124.88, 122.27, 121.38, 115.32, 105.28, 57.28, 41.10. **MASS spectrum m/z:** 491[M+2]⁺, 493[M+4]⁺. Calc. for C₂₆H₂₁ClFN₅O₂; CHN: C, 63.74; H, 4.32; N, 14.29; Found C, 63.70; H, 4.30; N, 14.25.

3.15. 5-amino-N-(4-bromophenyl)-8-(4-fluorophenyl)-4-oxo-2-phenyl-3,4,5,6,7,8-hexahydropyrido[2,3-d]pyrimidine-6-carboxamide (11o): IR spectrum (KBr, cm⁻¹): 3551.56 (NH (str)), 3080.32 (C-H Aromatic (str)), 2931.73 (C-H Aliphatic (str)), 1585.20 (C=C Aromatic(str)), 1266.18(C=S (str)). ¹H NMR (400MHz CDCl₃, δ ppm): 7.901-7.923 (d, 1H, aromatic CH), 7.550-7.810 (m, 1H, aromatic CH), 7.654-7.681 (d, 1H, aromatic CH), 7.553-7.556 (d, 1H, aromatic CH), 7.494-7.539 (d, 1H, aromatic CH), 7.313-7.363 (m, 1H, aromatic CH), 7.093-7.130 (m, 1H, aromatic CH), 6.887-6.911 (d, 1H, aromatic CH), 5.063 (s, 1H, NH), 5.057 (s, 1H, aliphatic CH), 3.780 (s, 2H, aliphatic CH). 2.675 (s, 1H, aliphatic CH). 1.625 (s, 2H, aliphatic CH). ¹³C NMR (100MHz, CDCl₃): 153.78, 152.34, 148.50, 143.63, 142.44, 139.59, 138.74, 136.03, 129.96, 129.40, 127.96, 127.65, 126.73, 125.01, 119.69, 48.15, 38.66. **MASS spectrum m/z:** 385 [M+2]⁺, 387 [M+4]⁺, 389 [M+6]⁺. Calc. for C₂₆H₂₁BrFN₅O₂; C, 61.67; H, 4.18; N, 13.83; Found C, 61.60; H, 4.11; N, 13.80.

4.0. Molecular docking studies:

Molecular docking studies of **5-amino-4-oxo-N-2,8-substituted triphenyl-3,4,5,6,7,8-hexahydropyrido[2,3-d]pyrimidine-6-carboxamide (11a-o)** were carried out using Schrödinger software (Schrödinger, Version 2023-4) 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 Visualizers (Schrödinger, Version 2023-4). The results were analyzed.

The program being referred to is Schrodinger software, namely Version 2023-4, developed by Schrodinger. It includes the Glide module. The ligands used for docking were drawn using ChemDraw software. The ligands were synthesized using the OPLS3e force field in Ligprep (Dizdaroglu et al., 2020). Docking experiments were performed using Version 2023-3 of Schrodinger. This minimizing process facilitates the assignment of bond ordering and the addition of hydrogen atoms to the ligands. The output file obtained, which contains the most optimal conformations of the ligands, was used for conducting docking experiments. The protein was synthesized using the protein preparation wizard developed by Dizdaroglu et al. in 2020, specifically Version 2023-3 from Schrodinger. The protein was charged with the addition of hydrogen atoms. Epik was used to generate the Het states at a pH of 7.2. The protein undergoes pre-processing, refinement, and modification by analysis of the workspace. Atoms that were not relevant were omitted from the crystal structure. Ultimately, the protein was enhanced by the use of the OPLS3e force field. A receptor grid was created around the cocrystal ligand, which represents the X-ray posture of the ligand inside the protein. The ligand centroid was chosen as the basis for creating a grid box, and the Van der Waals radius of the receptor atoms was adjusted to 1.00 Å, with a partial atomic charge of 0.25. The optimal docking structure was chosen based on the Glide docking score obtained from the output. The poses of the ligands obtained after docking were studied using XP Visualizer (Version 2023-3, Schrodinger). The findings are shown in Tables-2 and Figures-1 and 2.



General structure-I

S.No	Compound	Docking score of MCF-7 (6ENV)	Docking score of HELA (7ACF)	Docking score of MDA-MB-231 (6VJ3)
1	11a	-6.971	-5.782	-6.102
2	11b	-5.707	-4.723	-5.124
3	11c	-7.861	-8.395	-7.125
4	11d	-5.146	-4.714	-5.254
5	11e	-11.057	-10.415	-10.015
6	11f	-6.181	-6.284	-7.114
7	11g	-6.381	-6.484	-6.214
8	11h	-5.712	-4.489	-4.105
9	11i	-4.942	-4.526	-4.142
10	11j	-5.386	-4.475	-4.434
11	11k	-6.386	-4.241	-4.547
12	11l	-5.934	-6.224	-6.021
13	11m	12.401	-11.711	-10.711
14	11n	-5.352	-6.149	-6.341
15	11o	-9.352	-9.721	-9.141
16	Cocrystal Ligand	6.787	-8.068	-10.068

Table 2: Binding Energies (Kcal/mol), No. of HBs and Binding Sites

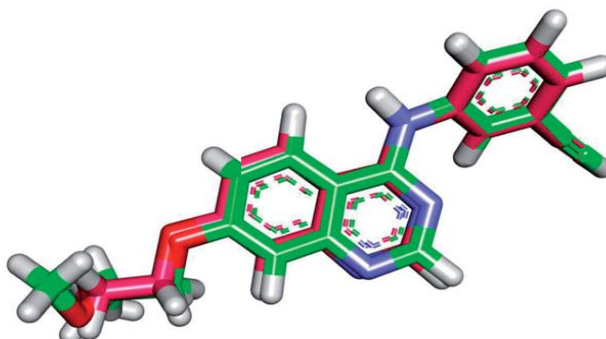


Figure 2. 3D superimposition of the docked ligand (erlotinib; pink) and the original ligand (green) with RMSD value of 0.88 Å.

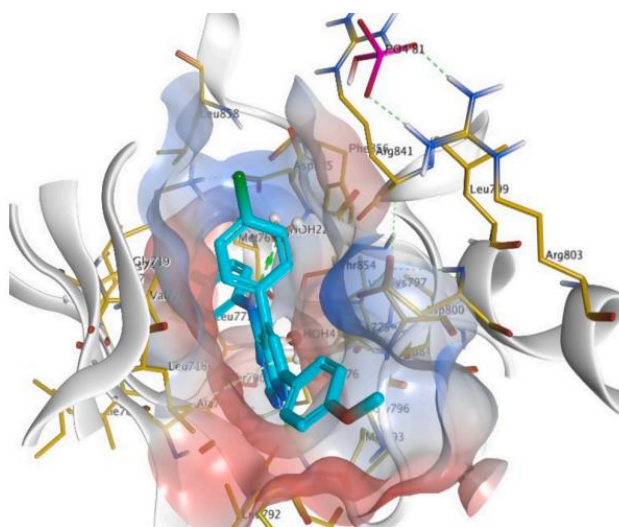


Fig.3. The compound 11e is positioned in the active site of HELA (7ACF) in a docking posture. The binding mode of the compound reveals the major hydrogen bonds with Met793 and a water-mediated hydrogen bond with Asp855.

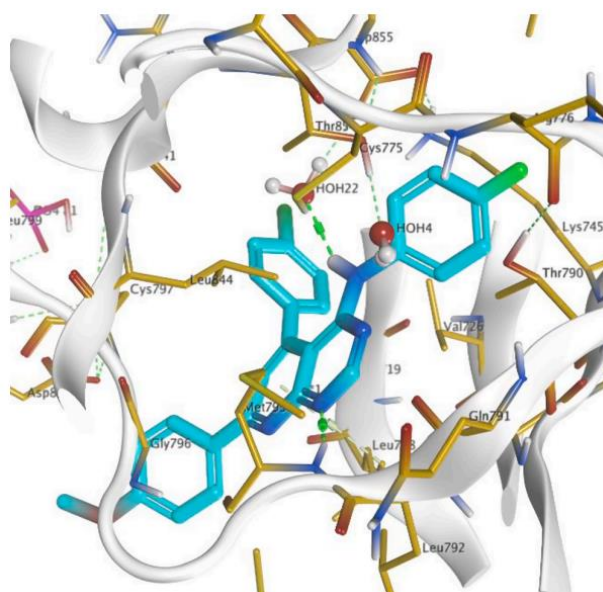


Fig. 4. The docking position of compound 11m in the active site of MDA-MB-231 (6VJ3) is shown the binding mode shows two main hydrogen bonds with Met-793 and a water-mediated hydrogen bond with Asp855.

5.0. RESULTS AND DISCUSSIONS:

5.1. Chemistry:

4.1. Results and discussions of molecular docking:

In-vitro studies of synthesized compounds **5-amino-4-oxo-N- 2, 8- substituted triphenyl-3,4,5,6,7,8-hexahydropyrido[2,3-d]-pyrimidine-6-carboxamide (11a-o)** showed the potential anti-cancer activity. Compound 11m exhibited significant anti-cancer action. We were encouraged by these

findings to conduct docking experiments in order to get a better understanding of the way the synthesized compounds participate inside the binding pocket of MCF-7 (6ENV), HeLa (7-ACF), and MDA-MB-231 (6VJ3). The ligand structures were constructed using the maestro software and then processed using LigPrep from the Schrodinger program. The protein structures of MCF-7 (6ENV), HeLa (7-ACF), and MDA-MB-231 (6VJ3) were acquired from the Protein Data Bank. The required adjustments to the protein structures were performed using the Protein

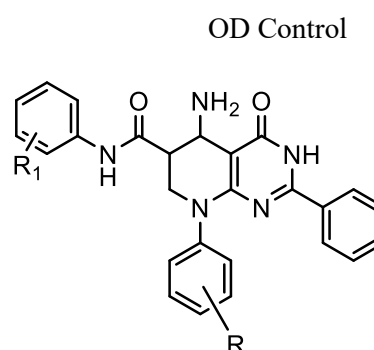
Preparation Wizard in the Schrodinger software. The docking investigations were conducted using the Glide docking program. The docking methodology was verified by docking the cocrystal ligand, which yielded a root mean square deviation (RMSD) of 0.6 between the docked conformation and the cocrystal ligand posture. The compound's binding interactions with MCF-7 (6ENV), HeLa (7-ACF), and MDA-MB-231 (6VJ3) are documented in Table-2, Figure-1, Figure-2, and Figure-3. A docking analysis was conducted to analyze the binding poses of the synthesized compounds with MCF-7 (6ENV), HeLa (7-ACF), and MDA-MB-231 (6VJ3). The results indicate that these molecules effectively bind inside the enzyme's binding pocket. Out of all the produced compounds, the compound **11m** and **11e** has shown the most powerful anti-proliferative activity, it has achieved the highest binding scores of -11.352, -11.721, and -10.015 and -11.057, -10.415 and -10.015 against MCF-7 (6ENV), HeLa (7-ACF), and MDA-MB-231 (6VJ3) respectively. In the superimposed configuration with a distance of 11 meters, the pyrido pyrimidine ring coincided with the skeleton of the cocrystal ligand, as shown in Figure-1.

5.0. Anti-cancer activity [17]:

The in-vitro MTT test is used to evaluate the anticancer activity of novel Pyrimidine derivatives, which have been identified for their broad spectrum of biological characteristics, including their anticancer properties. To test the biological effectiveness of the synthesized compounds, we conducted an in vitro evaluation of their anticancer activity against aware cell lines: MDA-MB-231 (breast cancer), HeLa (cervical carcinoma), and MCF-7 (breast cancer). The evaluation was conducted using the MTT (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide) test, using the

previously reported method. Initially, a concentration of 1,105 cells per milliliter was distributed on 96 well microtiter plates and incubated overnight in minimal needed medium supplemented with fetal bovine serum. The compounds were dissolved in dimethyl sulfoxide (DMSO) to get a final concentration of 0.1M. Subsequently, the samples were mixed with complete medium in a sequential manner to produce test concentrations of 0.001, 0.01, 0.1, 1.0, and 10 micromolar (uM). The MCF-7 breast cancer cells were cultured in a 96-well plate and treated with varying concentrations of the test compounds for a duration of 96 hours at a temperature of 37°C. The pH of the environment was regulated at a concentration of 5% CO₂. Afterwards, the cells were exposed to MTT reagent and kept in an incubator for an extra 4 hours. The solution above each well, including both medium and MTT, was carefully removed. The cells produced a formazan material with a deep blue color, which was then dissolved in 100 milliliters of DMSO. To evaluate the liveliness of cells, the measurement of absorbance at a specific wavelength of 570nm was performed using a 96-well plate reader. The % inhibitions were calculated using the provided methodology and plotted against the concentrations used to get the IC₅₀ values.

$$\% \text{ Inhibition} = \frac{\text{OD Control} - \text{OD treated} * 100}{\text{OD Control}}$$



S.No	Comp.	R	R1	MDA-MB-231 IC ₅₀ (μM)	HELa IC ₅₀ (μM)	MCF-7 IC ₅₀ (μM)
1	11a	H	4-F	4.12±0.03	4.20±0.12	5.15±0.12
2	11b	H	4-Cl	3.25±0.24	3.84±0.18	3.95±0.25
3	11c	H	4-Br	3.28±0.13	3.42±0.03	3.26±0.03
4	11d	H	4-NO ₂	5.14±0.42	5.62±0.12	6.45±0.03

5	11e	3-Cl	4-F	1.42±0.12	1.54±0.13	1.85±0.23
6	11f	3-Cl	4-Cl	2.36±0.03	2.96±0.03	2.62±0.02
7	11g	3-Cl	4-Br	3.65±0.02	3.85±0.03	5.95±0.03
8	11h	3-Cl	4-NO ₂	4.18±0.13	4.38±0.21	4.16±0.13
9	11i	4-Br	4-F	3.11±0.12	3.60±0.32	3.45±0.13
10	11j	4-Br	4-Cl	2.29±0.18	2.34±0.02	2.57±0.12
11	11k	4-Br	4-Br	3.84±0.14	3.86±0.22	3.35±0.28
12	11l	4-Br	4-NO ₂	4.86±0.13	4.02±0.10	4.08±0.24
13	11m	4-F	4-F	1.29±0.18	1.34±0.02	1.57±0.12
14	11n	4-F	4-Cl	1.82±0.05	1.91±0.12	1.98±0.20
15	11o	4-F	4-Br	1.92±0.25	2.05±0.03	2.10±0.23
16	Cisplatin		-	1.14±0.05	0.95±0.02	1.02±0.22

Table-3 : Anti-cancer activity of 5-amino-4-oxo-N- 2, 8- substituted triphenyl-3,4,5,6,7,8-hexahydropyrido[2,3-d]-pyrimidine-6-carboxamide (11a-o)

5.1. RESULTS AND DISCUSSIONS:

Pyrimidine is a highly effective framework for the development of anticancer drugs. Its derivatives have demonstrated strong efficacy through various mechanisms, such as regulating cell growth through apoptosis, disrupting cell migration, inhibiting angiogenesis, regulating nuclear receptor responsiveness, and inducing cell cycle arrest. The efficacy of pyrimidine derivatives has been shown in several cancer cell lines, including breast cancer, colon cancer, lung cancer, colorectal cancer, renal cancer, and others. The Effect of new synthesized 5-amino-4-oxo-N- 2, 8- substituted triphenyl-3,4,5,6,7,8-hexahydropyrido[2,3-d]-pyrimidine-6-carboxamide (11a-o) derivatives for the evaluation of anti-cancer activity. We evaluated our compounds for their anti-proliferative activity for breast cancer cell line (MDA-MB-231), cervical carcinoma cell line (HeLa), breast cancer cell line (MCF-7). The study finding that among all the compounds **11m** containing pyrido[2,3-d]-pyrimidine fused with 8-bis(4-fluorophenyl)-4-oxo-2-phenyl moiety showed potent cytotoxic activity at low concentration with IC₅₀ value 1.29±0.18µM, 1.34±0.02µM, 1.57±0.12µM and

compound **11e** containing pyrido[2,3-d]-pyrimidine fused with 5-amino-8-(3-chlorophenyl)-N-(4-fluorophenyl)-4-oxo-2-phenyl showed good anti-cancer activity 1.42±0.12µM, 1.54±0.13µM, 1.85±0.23µM against breast cancer cell line (MDA-MB-231), cervical carcinoma cell line (HeLa), breast cancer cell line (MCF-7). The Compound 5-amino-N-(4-nitrophenyl)-4-oxo-2,8-diphenyl fused with pyrido[2,3-d]-pyrimidine (**11d**) showed less potent anti-cancer activity with IC₅₀ value of 5.14±0.42 µM, 5.62±0.12 µM and 6.45±0.03µM against breast cancer cell line (MDA-MB-231), cervical carcinoma cell line (HeLa), breast cancer cell line (MCF-7). Our results also showed that some of the synthesized products exhibited a moderate to strong growth inhibition activity on the tested cell lines between 0.001 and 10 µM concentrations in compared with reference anticancer drug Cisplatin.

CONCLUSION

In conclusion, fifteen novel pyrido [2,3-*d*]pyrimidine derivatives (11a-o), were synthesized, spectroscopically confirmed and evaluated for their anti-cancer activity. The corresponding pyrido [2,3-d]pyrimidine derivatives (11a-o) were also

synthesized from starting materials. In the MTT assay, the synthesized compounds showed low to moderate reducing activity. However, the majority of the compounds presented potent anti-cancer activity, with the most potent being pyrimidine derivatives. The compounds **11m** containing pyrido[2,3-d]-pyrimidine fused with 8-bis(4-fluorophenyl)-4-oxo-2-phenyl moiety showed potent cytotoxic activity at low concentration with IC_{50} value $1.29 \pm 0.18 \mu M$, $1.34 \pm 0.02 \mu M$, $1.57 \pm 0.12 \mu M$ and compound **11e** containing pyrido[2,3-d]-pyrimidine fused with 5-amino-8-(3-chlorophenyl)-N-(4-fluorophenyl)-4-oxo-2-phenyl showed good anti-cancer activity $1.42 \pm 0.12 \mu M$, $1.54 \pm 0.13 \mu M$, $1.85 \pm 0.23 \mu M$ against breast cancer cell line (MDA-MB-231), cervical carcinoma cell line (HeLa), breast cancer cell line (MCF-7). Thus, they could be further investigated as multifunctional molecules.

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REFERENCES

- Barakat, A.; Soliman, S.M.; Al-Majid, A.M.; Lotfy, G.; Ghabbour, H.A.; Fun, H.K.; Yousuf, S.; Choudhary, M.I.; Wadood, A. Synthesis and structure investigation of novel pyrimidine-2,4,6-trione derivatives of highly potential biological activity as anti-diabetic agent. *J. Mol. Struct.* 2015, 1098, 365–376.
- Campos JF, Besson T, Berteina-Raboin S. Review on the Synthesis and Therapeutic Potential of Pyrido [2, 3-d],[3, 2-d],[3, 4-d] and [4, 3-d] pyrimidine Derivatives. *Pharmaceuticals*. 2022 Mar 14;15(3):352.
- Desai, N.C.; Kotadiya, G.M.; Trivedi, A.R. Studies on molecular properties prediction, antitubercular and antimicrobial activities of novel quinoline based pyrimidine motifs. *Bioorgan. Med. Chem. Lett.* 2014, 24, 3126–3130.
- E. Lierman, et al., The ability of sorafenib to inhibit oncogenic PDGFRb and FLT3 mutants and overcome resistance to other small molecule inhibitors, *Haematologica*, 2007, 92(1), 27–34.
- Gangjee, A.; Vasudevan, A.; Queener, S.F.; Kisliuk, R.L. 2,4-Diamino-5-deaza-6-substituted pyrido[2,3-d]pyrimidine antifolates as potent and selective nonclassical inhibitors of dihydrofolate reductases. *J. Med. Chem.* 1996, 39, 1438–1446.
- Jasztold-Howorko, R.; Tylińska, B.; Biaduń, B.; Gębarowski, T.; Gęsiorowski, K. New pyridocarbazole derivatives. Synthesis and their in vitro anticancer activity. *Acta Pol. Pharm.* 2013, 70, 823–832.
- Kaldrikyan, M.A.; Grigoryan, L.A.; Geboyanyan, V.A.; Arsenyan, F.G.; Stepanyan, G.M.; Garibdzhanyan, B.T. Synthesis and antitumor activity of some disubstituted 5-(3-methyl-4-alkoxybenzyl)pyrimidines. *Pharm. Chem. J.* 2000, 34, 521–524.
- Kaur, H.; Machado, M.; de Kock, C.; Smith, P.; Chibale, K.; Prudêncio, M.; Singh, K. Primaquine-pyrimidine hybrids: Synthesis and dual-stage antiparasitic activity. *Eur. J. Med. Chem.* 2015, 101, 266–273.
- L. M. Kelly, et al., CT53518, a novel selective FLT3 antagonist for the treatment of acute myelogenous leukemia (AML), *Cancer Cell*, 2002, 1(5), 421–432.
- Nguyen, C.H.; Bisagni, E.; Lhoste, J.M.; Lavelle, F.; Bissery, M.C. Synthesis and Antitumor Activity of 1-[[[(Dialkylamino)alkyl] amino]-4-methyl-5H-pyrido[4,3-b]benzo[e]- and -benzo[g]]indoles. A New Class of Antineoplastic Agents. *J. Med. Chem.* 1990, 33, 1519–1528.
- Piasny, J.; Wiatrak, B.; Dobosz, A.; Tylińska, B.; Gębarowski, T. Antitumor Activity of New Olivacine Derivatives. *Molecules* 2020, 25, 2512.
- Su, L.; Li, J.; Zhou, Z.; Huang, D.; Zhang, Y.; Pei, H.; Guo, W.; Wu, H.; Wang, X.; Liu, M.; et al. Corrigendum to “Design, synthesis and evaluation of hybrid of tetrahydrocarbazole with 2,4-diaminopyrimidine scaffold as antibacterial agents” [*Eur. J. Med. Chem.* 162 (162) (2019) 203–211]. *Eur. J. Med. Chem.* 2019, 168, 385.
- Tylińska, B.; Jasztold-Howorko, R.; Kowalczevska, K.; Szczauńska-Nowak, K.; Gębarowski, T.; Wietrzyk, J. Design, synthesis and analysis of anticancer activity of new SAR-based

S16020 derivatives. Acta Pol. Pharm. Drug Res. 2018.

13. Zimmermann, J. Pyrimidine Derivatives and Processes for the Preparation Thereof. U.S. Patent US5521184A, 28 May 1996. 6. Xie, F.; Zhao, H.; Zhao, L.; Lou, L.; Hu, Y. Synthesis and biological evaluation of novel 2,4,5-substituted pyrimidine derivatives for anticancer activity. Bioorgan. Med. Chem. Lett. 2009, 19, 275–278.

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