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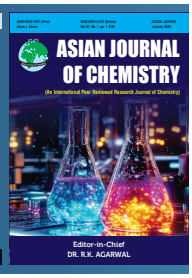

Novel Pyridopyrimidine-Based Compounds: Design, Synthesis and Cytotoxic Evaluation

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In this study, fifteen novel pyrido[2,3-*d*]pyrimidine derivatives (**11a-o**) were successfully designed, synthesized and characterized using spectroscopic techniques. The synthesized compounds were evaluated for their anticancer activity against three human cancer cell lines: MDA-MB-231 (breast cancer), HeLa (cervical cancer) and MCF-7 (breast cancer). The MTT assay results revealed that while some compounds exhibited low to moderate activity, the majority demonstrated potent anticancer effects, with pyrido[2,3-*d*]pyrimidine derivatives standing out as particularly effective. Among the synthesized compounds, compound **11m** featuring a pyrido[2,3-*d*]pyrimidine core fused with an 8-*bis*(4-fluorophenyl)-4-oxo-2-phenyl moiety, exhibited the most potent cytotoxic activity. It displayed IC_{50} values of 1.29 ± 0.18 μM, 1.34 ± 0.02 μM and 1.57 ± 0.12 μM against MDA-MB-231, HeLa and MCF-7 cell lines, respectively. Similarly, compound **11e** containing a pyrido[2,3-*d*]pyrimidine scaffold fused with a 5-amino-8-(3-chlorophenyl)-N-(4-fluorophenyl)-4-oxo-2-phenyl moiety, also demonstrated significant anticancer activity, with IC_{50} values of 1.42 ± 0.12 μM, 1.54 ± 0.13 μM and 1.85 ± 0.23 μM against the same cell lines. The potent activity of compounds **11m** and **11e** highlights the importance of pyrido[2,3-*d*]pyrimidine core and the strategic incorporation of electron-withdrawing substituents, such as fluoro and chloro groups, in enhancing anticancer efficacy. These findings suggest that these compounds could serve as promising lead candidates for further development as multifunctional anticancer agents. Future studies should focus on optimizing their structural features, evaluating their mechanisms of action and assessing their *in vivo* efficacy and safety profiles to advance their potential as therapeutic agents for cancer treatment.

Keywords: Pyrido[2,3-*d*]pyrimidine, Breast cancer, Cervical cancer, Cytotoxicity, Molecular docking.

INTRODUCTION

Cancer remains one of the most formidable challenges to global health, with profound socioeconomic implications. According to the World Health Organization (WHO), cancer is the second leading cause of mortality worldwide, accounting for approximately 9.6 million deaths in 2018, with 18 million new cases diagnosed annually [1]. Despite significant advancements in oncology, the lack of targeted and efficacious therapies continues to contribute to the rising burden of cancer-related morbidity and mortality. This underscores the urgent need for the discovery and development of novel chemotherapeutic agents with improved efficacy, selectivity and reduced toxicity.

Heterocyclic compounds, particularly those containing the pyrimidine scaffold, have garnered considerable attention in medicinal chemistry due to their diverse pharmacological properties. Pyrimidine derivatives exhibit a broad spectrum of biological activities, including anti-inflammatory [2], antidiabetic [3], antimicrobial [4] and anticancer effects [5,6] as well as anti-HIV activity [7]. Clinically, pyrimidine-based drugs have been successfully employed as antibacterial agents (*e.g.* sulfadiazine and trimethoprim), antiviral agents (*e.g.* trifluridine and idoxuridine), antimalarial therapies (*e.g.* sulfadoxine), anti-HIV drugs (*e.g.* zidovudine and stavudine), antituberculosis treatments (*e.g.* viomycin) and anticancer agents (*e.g.* 5-fluorouracil) [8]. These applications highlight the versatility and therapeutic potential of the pyrimidine nucleus.

Recent studies have demonstrated that structural modifications to the pyrimidine scaffold, particularly the incorporation of substituted aniline, phenyl and alkyl moieties, can significantly enhance anticancer activity [9-11]. These findings have inspired the design and synthesis of novel pyridopyrimidine derivatives, which combine the pyrimidine core with additional pharmacophoric elements to optimize biological activity. The pyridino[3,4-*d*]pyrimidine framework, in particular, has emerged as a promising scaffold for the development of anticancer

agents due to its structural similarity to purine bases, which are critical for DNA and RNA synthesis in rapidly proliferating cancer cells [12].

The design of the target compounds, 5-amino-4-oxo-N-2,8-substituted triphenyl-3,4,5,6,7,8-hexahydropyrido-[2,3-*d*]pyrimidine-6-carboxamide derivatives (**11a-o**), was inspired by the structural and pharmacological features of several well-established anticancer agents. These compounds were strategically designed to incorporate key pharmacophoric elements from known inhibitors, leveraging their mechanisms of action and structural motifs to enhance anticancer activity. Kisliuk *et al.* [8] reported that 2,4-diamino-6-[N-(2′,5′-dimethoxybenzyl)-N-methylamino]pyrido[2,3-*d*]pyrimidine (I) exhibited potent inhibitory activity against *tg*DHFR (IC_{50} = 6.3 nM) and demonstrated high selectivity for *tg*DHFR over *rl*DHFR, highlighting the therapeutic potential of pyrido[2,3-*d*]pyrimidine derivatives as non-classical antifolates [8]. Similarly, sorafenib (II) (BAY43-9006, Nexavar®), a multikinase inhibitor developed by Lierman *et al.* [13], has shown significant efficacy in treating renal cell carcinoma by targeting B-RAF, as well as receptor tyrosine kinases in the PDGFR and VEGFR families. These findings underscore the importance of kinase inhibition in cancer therapy.

Further, Kelly *et al.* [14] developed tandutinib (III) (CT-53518A), a potent FLT3 inhibitor, which has shown promise in treating acute myelogenous leukemia (AML). Approximately 30% of AML cases involve activating internal tandem duplications (ITDs) in the FLT3 receptor, making it a viable target for kinase inhibitors. Tandutinib effectively inhibits FLT3, PDGFR and *c*-Kit, with an IC_{50} of 200 nM, demonstrating the therapeutic potential of targeting multiple kinases. Campos *et al.* [12] also highlighted the clinical relevance of trametinib (IV), an FDA-approved MEK inhibitor used in combination therapies for melanoma and thyroid cancer, further emphasizing the importance of kinase-targeted therapies in oncology.

Based on these insights, the target compounds were designed to incorporate the pyrido[2,3-*d*]pyrimidine scaffold, a versatile pharmacophore known for its ability to interact with multiple biological targets. The core structure of 5-amino-4-oxo-*N*-2,8-substituted triphenyl-3,4,5,6,7,8-hexahydropyrido-[2,3-*d*]pyrimidine-6-carboxamide was selected due to its structural similarity to purine bases, which are critical for DNA and RNA synthesis in rapidly proliferating cancer cells. The substitution pattern at the 2,8-positions with triphenyl moieties was introduced to enhance binding affinity and selectivity, while the carboxamide group at the 6-position was incorporated to improve solubility and pharmacokinetic properties.

The designed compounds were further optimized through *in silico* screening to ensure favourable drug-like properties and target interactions. The general structure of the target compounds (Fig. 1), highlighted the strategic placement of substituents to maximize anticancer activity while minimizing off-target effects. This rational design approach aims to develop novel pyrido[2,3-*d*]pyrimidine derivatives with potent anticancer activity, leveraging the structural and mechanistic insights from established kinase inhibitors and antifolates.

In this study, we report the rational design, synthesis and evaluation of a series of novel pyridino[3,4-*d*]pyrimidine derivatives as potential anticancer agents. Structural modifications were introduced to the lead compound to enhance its pharmacological profile, including improved potency, selectivity and pharmacokinetic properties. The synthesized compounds were evaluated for their anticancer activity against a panel of human cancer cell lines, with the aim of identifying new lead structures for further development as chemotherapeutic agents. This work contributes to the ongoing efforts to discover innovative and effective treatments for cancer, addressing the unmet medical needs in oncology.

EXPERIMENTAL

The synthetic grade compounds and solvents employed in this investigation were all obtained from Sigma-Aldrich, Bangalore, India, AVRA Chemicals, Hyderabad, India and no additional purification steps were implemented. Merck pre-coated aluminium TLC plates coated with silica gel 60 F_{254} were employed for the purpose of reaction monitoring. The melting points were determined using Remi electronic melting point apparatus. The chemical shift values, which were reported in ppm, were relative to the internal standard, tetramethyl silane and were taken using a BRUKER DRX instrument to acquire the ^{1}H and ^{13}C NMR spectra. The MASS spectra were obtained by utilizing a Waters Xevo Q-Tof Mass spectrometer in their collection. The cell lines (ATCC) for the anticancer activity were obtained from HiMedia Laboratories Pvt. Ltd., India.

Synthesis of ethyl 3,3-*bis*(methylthio)-2-cyanoacrylate (3): To an ice-cold solution of KOH (0.2 mol) in 30 mL DMF, ethyl cyanoacetate (**1**, 0.1 mol) and CS_2 (**2**, 0.1 mol) were added dropwise under continuous stirring. The reaction mixture was maintained at a low temperature (0-5 ºC) during the addition. After the complete addition, the mixture was stirred for an additional hour at ambient temperature (25 ºC). The reaction mixture was then cooled again and 0.2 mol dimethyl sulfate was added gradually while maintaining at 20 ºC. The reaction was allowed to proceed at room temperature for 12 h. The resulting mixture was poured into 500 mL of cold water and the precipitated solid was filtered, washed thoroughly with cold water and recrystallized from *n*-hexane to yield ethyl 3,3-*bis*(methylthio)-2-cyanoacrylate (**3**) as a pure product. The product was dried under vacuum to remove residual solvents.

Synthesis of 2-substituted-4-(methylthio)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (5): A mixture of ethyl 3,3-*bis*(methylthio)-2-cyanoacrylate (**3**, 0.02 mol) and aromatic amidines (**4**, 0.02 mol) in 30 mL ethanol was refluxed for 3 h. The reaction progress was monitored by thin-layer chromatography. After completion, the reaction mixture was allowed to cool to room temperature and left undisturbed for 12 h. The resulting solid was filtered, washed with cold ethanol and recrystallized from a mixture of *n*-hexane and benzene (1:1 v/v) to afford 2-substituted-4-(methylthio)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**5**) as a crystalline solid. The product was dried under vacuum to ensure purity.

Synthesis of 4-(substituted phenylamino)-2-substituted-6-oxo-1,6-dihydropyrimidine-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-**

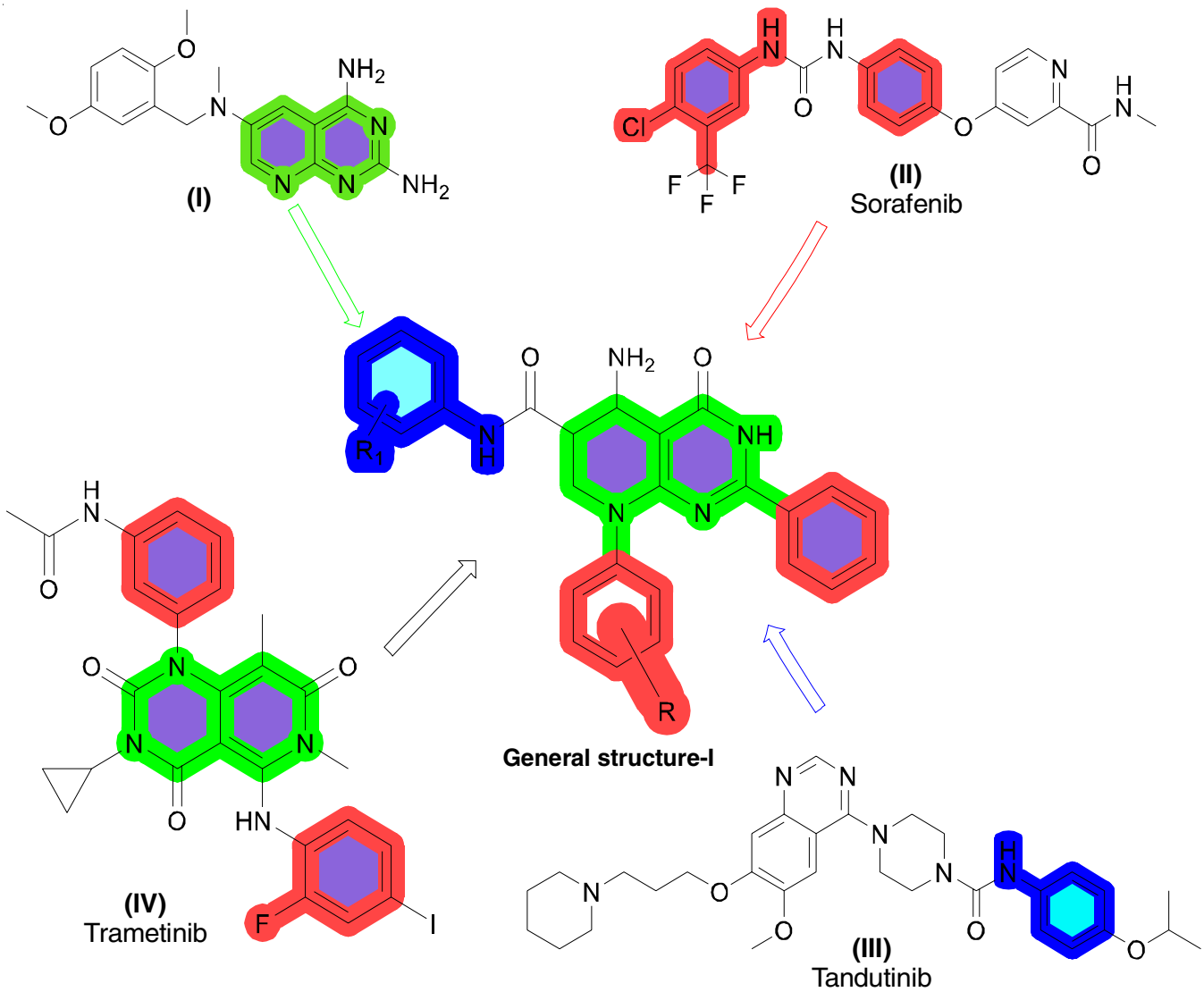


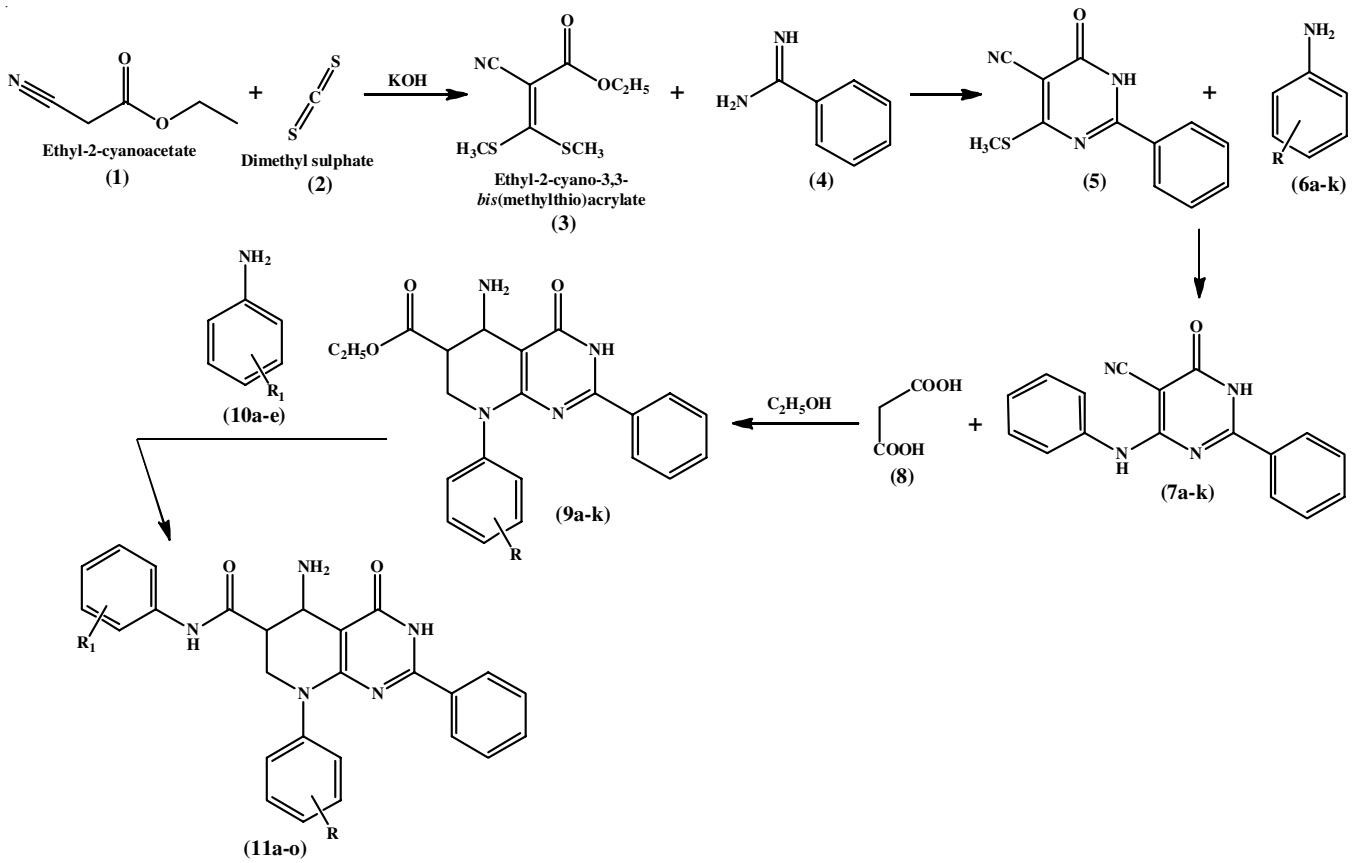
Fig. 1. Design of 5-amino-4-oxo-N-2,8-substituted triphenyl-3,4,5,6,7,8-hexahydropyrido[2,3-*d*]-pyrimidine-6-carboxamide (**11a-o**)

k, 0.01 mol) in 30 mL ethanol was refluxed for 1 h. The reaction progress was monitored by TLC. After completion, the reaction mixture was allowed to cool to room temperature and left undisturbed for 24 h. The precipitated solid was filtered, washed with cold ethanol and recrystallized from *n*-hexane to yield 4-(substituted phenylamino)-2-substituted-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**7a-k**) as pure compounds. The products were dried under vacuum to remove any residual solvents.

Synthesis of ethyl-5-amino-8-(substituted phenyl)-2-substituted-4,7-dioxo-3,4,5,6,7,8-hexahydropyrido[2,3-*d*]-pyrimidine-6-carboxylate (9a-k): A mixture of 4-(4-halophenylamino)-2-substituted-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**7a-k**, 0.01 mol), malonic acid (**8**, 0.02 mol) and 30 mL ethanol was refluxed for 1 h. The reaction progress was monitored by TLC. After completion, the reaction mixture was allowed to cool to room temperature and left undisturbed for 24 h. The resulting solid was filtered, washed with cold ethanol and recrystallized from a mixture of benzene and *n*-hexane (1:1 v/v) to yield ethyl-5-amino-8-(substituted phenyl)-2-substituted-4,7-dioxo-3,4,5,6,7,8-hexahydropyrido[2,3-*d*]-pyrimidine-6-carboxylate (**9a-k**) as pure compounds. The products were dried under vacuum to ensure purity.

Synthesis 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-hexahydropyrido-[2,3-*d*]pyrimidine-6-carboxylate (**9a-k**, 0.02 mol) and substituted anilines (**10a-e**, 0.01 mol) in 30 mL ethanol was refluxed for 1 h. The reaction progress was monitored by TLC. After completion, the reaction mixture was allowed to cool to room temperature and left undisturbed for 24 h. The resulting solid was filtered, washed with cold ethanol and recrystallized from *n*-hexane to yield 5-amino-4-oxo-*N*-substituted-2,8-triphenyl-3,4,5,6,7,8-hexahydropyrido[2,3-*d*]pyrimidine-6-carboxamide derivatives (**11a-o**) as pure products (**Scheme-I**). The final compounds were dried under vacuum to remove any residual solvents and characterized using spectroscopic techniques.

5-Amino-*N*-(4-fluorophenyl)-4-oxo-2,8-diphenyl-3,4,5,6,7,8-hexahydropyrido[2,3-*d*]pyrimidine-6-carboxamide (11a): Yield: 56%, m.p.: 106-108 ºC, m.f.: $C_{26}H_{22}N_5O_2F$ (m.w.:



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

471), R_f: 0.6, IR (KBr, ν_{max}, cm^{-1}): 3408.28 (NH *str.*), 3045.12 (C-H arom. *str.*), 2980.10 (C-H aliphatic *str.*), 1590.18 (C=C arom. *str.*), 1228.45 (C=S *str.*); ^{1}H NMR (400 MHz, $CDCl_3$, 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); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 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 $[M+4]^+$; Elemental analysis of $C_{26}H_{22}FN_5O_2$; calcd. (found) %: C, 68.56 (68.50); H, 4.87 (4.81); N, 15.38 (15.30).

5-Amino-*N*-(4-chlorophenyl)-4-oxo-2,8-diphenyl-3,4,5,6,7,8-hexahydropyrido[2,3-*d*]pyrimidine-6-carboxamide (11b): Yield: 70%, m.p.: 102-104 ºC, m.f.: $C_{26}H_{22}N_5O_2Cl$ (m.w.: 207), R_f: 0.5, IR (KBr, ν_{max}, cm^{-1}): 3340.99 (NH *str.*), 3058.56 (C-H arom. *str.*), 2976.58 (C-H aliph. *str.*), 1591.58 (C=C arom. *str.*), 1222.35 (C=S *str.*); ^{1}H NMR (400 MHz $CDCl_3$, 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); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 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: *m/z*: 348 $[M+H]^+$, 350 $[M+2]^+$, 352 $[M+4]^+$; Elemental analysis of $C_{26}H_{22}ClN_5O_2$; calcd. (found) %: C, 66.17 (66.01); H, 4.70 (4.74); N, 14.84 (14.80).

5-Amino-*N*-(4-bromophenyl)-4-oxo-2,8-diphenyl-3,4,5,6,7,8-hexahydropyrido[2,3-*d*]pyrimidine-6-carboxamide (11c): Yield: 65%, m.p.: 106-108 ºC, m.f.: $C_{26}H_{22}N_5O_2Br$ (m.w.: 394), R_f: 0.6, IR (KBr, ν_{max}, cm^{-1}): 3408.28 (NH *str.*), 3045.12 (C-H arom. *str.*), 2980.10 (C-H aliph. *str.*), 1590.18 (C=C arom. *str.*), 1228.45 (C=S *str.*); ^{1}H NMR (400 MHz, $CDCl_3$, 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). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 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 *m/z*: 395 $[M+2]^+$, 397 $[M+4]^+$; Elemental analysis of $C_{26}H_{22}BrN_5O_2$; calcd. (found) %: C, 48.86 (48.86); H, 3.59 (3.50); N, 14.25 (14.24); S, 8.15 (8.10).

5-Amino-*N*-(4-nitrophenyl)-4-oxo-2,8-diphenyl-3,4,5,6,7,8-hexahydropyrido[2,3-*d*]-pyrimidine-6-carboxamide (11d): Yield: 75%, m.p.: 130-132 ºC, m.f.: $C_{26}H_{22}N_6O_4$ (m.w.: 482), R_f: 0.4, IR (KBr, ν_{max}, cm^{-1}): 3481.20 (NH *str.*), 3062.10 (C-H arom. *str.*), 2960.10 (C-H aliph. *str.*), 1570.04 (C=C arom. *str.*), 1254.18 (C=S, *str.*); ^{1}H NMR (400 MHz $CDCl_3$, 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); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 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 *m/z*: 332 $[M+H]^+$, 334 $[M+2]^+$, 336 $[M+2]^+$; Elemental analysis of $C_{26}H_{22}N_6O_4$; calcd. (found) %: C, 64.72 (64.70); H, 4.60 (4.65); N, 17.42 (17.40).

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): Yield: 74%, m.p.: 168-170 ºC, m.f.: $C_{26}H_{21}N_5O_2ClF$ (m.w.: 489), R_f: 0.5, IR (KBr, ν_{max}, cm^{-1}): 3551.56 (NH *str.*), 3080.32 (C-H arom. *str.*), 2931.73 (C-H aliph. *str.*), 1585.20 (C=C arom. *str.*), 1266.18 (C=S *str.*); ^{1}H NMR (400 MHz, $CDCl_3$, 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 (100 MHz, $CDCl_3$, δ ppm): 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 *m/z*: 385 $[M+2]^+$, 387 $[M+4]^+$, 389 $[M+6]^+$; Elemental analysis of $C_{26}H_{21}ClFN_5O_2$; calcd. (found) %: C, 63.74 (63.70); H, 4.32 (4.30); N, 14.29 (14.21).

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): Yield: 54%, m.p.: 180-182 ºC, m.f.: $C_{19}H_{14}N_5OBr_3$ (m.w.: 506), R_f: 0.8, IR (KBr, ν_{max}, cm^{-1}): 3551.56 (NH *str.*), 3080.32 (C-H arom. *str.*), 2931.73 (C-H aliph. *str.*), 1585.20 (C=C arom. *str.*), 1266.18 (C=S *str.*); ^{1}H NMR (400 MHz, $CDCl_3$, 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, arom. 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 (100 MHz, $CDCl_3$, δ ppm): 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 *m/z*: 385 $[M+2]^+$, 387 $[M+4]^+$, 389 $[M+6]^+$; Elemental analysis of $C_{26}H_{21}Cl_2N_5O_2$; calcd. (found) %: C, 61.67 (61.60); H, 4.18 (4.11); N, 13.83 (13.80).

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): Yield: 70%, m.p.: 158-160 ºC, m.f.: $C_{19}H_{15}N_6O_3Br$ (m.w.: 455), R_f: 0.5, IR (KBr, ν_{max}, cm^{-1}): 3420.10 (NH *str.*), 3061.10 (C-H arom. *str.*), 2968.18 (C-H aliph. *str.*), 1578.04 (C=C arom. *str.*), 1258.10 (C=S *str.*); ^{1}H NMR (400 MHz, $CDCl_3$, 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 (100 MHz, $CDCl_3$, δ ppm): 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 *m/z*: 365 $[M+H]^+$, 367 $[M+2]^+$, 369 $[M+4]^+$; Elemental analysis of $C_{26}H_{21}BrClN_5O_2$; calcd. (found) %: C, 56.69 (56.64); H, 3.84 (3.80); Br, 14.51 (14.56); N, 12.71 (12.70).

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): Yield: 38%, m.p.: 118-120 ºC, m.f.: $C_{19}H_{15}N_5OBrCl$ (m.w.: 444), R_f: 0.7, IR (KBr, ν_{max}, cm^{-1}): 3420.10 (NH *str.*), 3052.01 (C-H arom. *str.*), 2980.10 (C-H aliph. *str.*), 1580.10 (C=C arom. *str.*), 1265.40 (C=S *str.*); ^{1}H NMR (400 MHz, $CDCl_3$, 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 (100 MHz, $CDCl_3$, δ ppm): 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 *m/z*: 361 $[M+2]^+$; Elemental analysis of $C_{26}H_{21}ClN_6O_4$; calcd. (found) %: C, 60.41 (60.40); H, 4.09 (4.15); N, 16.26 (16.20).

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): Yield: 64%, m.p.: 122-124 ºC, m.f.: $C_{19}H_{14}N_6O_3F_2$ (m.w.: 533), R_f: 0.5, IR (KBr, ν_{max}, cm^{-1}): 3551.56 (NH *str.*), 3080.32 (C-H arom. *str.*), 2931.73 (C-H aliph. *str.*), 1585.20 (C=C arom. *str.*), 1266.18 (C=S *str.*); ^{1}H NMR (400 MHz, $CDCl_3$, 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 (100 MHz, $CDCl_3$, δ ppm): 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 *m/z*: 385 $[M+2]^+$, 387 $[M+4]^+$, 389 $[M+6]^+$; Elemental analysis of $C_{26}H_{21}BrFN_5O_2$; calcd. (found) %: C, 58.44 (58.40); H, 3.96 (3.90); N, 13.11 (13.16).

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): Yield: 80%, m.p.: 150-152 ºC, m.f.: $C_{19}H_{14}N_6O_3Br_2$ (m.w.: 531), R_f: 0.7, IR (KBr, ν_{max}, cm^{-1}): 3420.10 (NH *str.*), 3061.10 (C-H arom. *str.*), 2968.18 (C-H aliph. *str.*), 1578.04 (C=C arom. *str.*), 1258.10 (C=S *str.*); ^{1}H NMR (400 MHz, $CDCl_3$, 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 (100 MHz, $CDCl_3$, δ ppm): 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 *m/z*: 365 $[M+H]^+$, 367 $[M+2]^+$, 369 $[M+4]^+$; Elemental analysis of $C_{26}H_{21}BrClN_5O_2$; calcd. (found) %: C, 56.69 (56.60); H, 3.84 (3.80); N, 12.71 (12.70).

5-Amino-8-(4-bromophenyl)--N-(4-bromophenyl)-4-oxo-2-phenyl-3,4,5,6,7,8-hexahydropyrido[2,3-*d*]pyrimidine-

6-carboxamide (11k): Yield: 70%, m.p.: 162-164 ºC, m.f.: $C_{19}H_{15}N_7O_5$ (m.w.: 421), R_f: 0.5, IR (KBr, ν_{max}, cm^{-1}): 3431.10 (NH *str.*), 3082.18 (C-H arom. *str.*), 2950.61 (C-H aliph. *str.*), 1585.10 (C=C arom. *str.*), 1255.75 (C=S *str.*); ^{1}H NMR (400 MHz $CDCl_3$, 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); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 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 *m/z*: 409 $[M+H]^+$, 411 $[M+2]^+$, 413 $[M+4]^+$; Elemental analysis of $C_{16}H_{14}BrClN_4S$; calcd. (found) %: C, 46.90 (46.91); H, 3.44 (3.43); N, 13.67 (13.63); S, 7.83 (7.82).

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): Yield: 48%, m.p.: 150-152 ºC, m.f.: $C_{19}H_{15}N_6O_3Cl$ (m.w.: 410), R_f: 0.6, IR (KBr, ν_{max}, cm^{-1}): 3414.01 (NH *str.*), 3061.10 (C-H arom. *str.*), 2960.18 (C-H aliph. *str.*), 1570.03 (C=C arom. *str.*), 1226.10 (C=S *str.*); ^{1}H NMR (400 MHz, $CDCl_3$, 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); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 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 *m/z*: 348 $[M+H]^+$, 350 $[M+2]^+$, 352 $[M+4]^+$; Elemental analysis of $C_{26}H_{21}BrN_6O_4$; calcd. (found) %: C, 55.63 (55.60); H, 3.77 (3.70); N, 14.97 (14.90).

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): Yield: 62%, m.p.: 190-192 ºC, m.f.: $C_{19}H_{14}N_5OClF_2$ (m.w.: 401), R_f: 0.6, IR (KBr, ν_{max}, cm^{-1}): 3551.56 (NH *str.*), 3080.32 (C-H arom. *str.*), 2931.73 (C-H aliph. *str.*), 1585.20 (C=C arom. *str.*), 1266.18 (C=S *str.*); ^{1}H NMR (400 MHz, $CDCl_3$, 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 (100 MHz, $CDCl_3$, δ ppm): 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 *m/z*: 475 $[M+2]^+$, 477 $[M+4]^+$; Elemental analysis of $C_{26}H_{21}F_2N_5O_2$; calcd. (found) %: C, 65.95 (65.94); H, 4.47 (4.42); N, 14.79 (14.75).

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): Yield: 60%, m.p.: 196-198 ºC, m.f.: $C_{19}H_{14}N_5OBr_2Cl$ (m.w.: 520), R_f: 0.5, IR (KBr, ν_{max}, cm^{-1}): 3414.01 (NH *str.*), 3061.10 (C-H arom. *str.*), 2960.18 (C-H aliph. *str.*), 1570.03 (C=C arom. *str.*), 1226.10 (C=S *str.*); ^{1}H NMR (400 MHz, $CDCl_3$, 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); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 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 *m/z*: 491 $[M+2]^+$, 493 $[M+4]^+$; Elemental analysis of $C_{26}H_{21}F_2N_5O_2$; calcd. (found) %: C, 63.74 (63.70); H, 4.32 (4.30); N, 14.29 (14.25).

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): Yield: 54%, m.p.: 160-162 ºC, m.f.: $C_{19}H_{15}N_6O_3Cl$ (m.w.: 410), R_f: 0.6, IR (KBr, ν_{max}, cm^{-1}): 3551.56 (NH *str.*), 3080.32 (C-H arom. *str.*), 2931.73 (C-H aliph. *str.*), 1585.20 (C=C arom. *str.*), 1266.18 (C=S *str.*); ^{1}H NMR (400 MHz, $CDCl_3$, 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 (100 MHz, $CDCl_3$, δ ppm): 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 *m/z*: 385 $[M+2]^+$, 387 $[M+4]^+$, 389 $[M+6]^+$; Elemental analysis of $C_{26}H_{21}BrFN_5O_2$; calcd. (found) %: C, 61.67 (61.60); H, 4.18 (4.11); N, 13.83 (13.80).

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-carboxamides (**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). 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 [15]. 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 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).

MTT assay: The *in vitro* MTT assay was used to evaluate the anticancer activity of novel pyridopyrimidine derivatives, which have been identified for their broad spectrum of biological characteristics, including their anticancer properties. To analyze 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 1×10^5 cells/mL was distributed on 96 well microtiter plates and incubated overnight in minimal needed medium supplemented with fetal bovine serum. The compounds were dissolved in DMSO solvent to get a final concentration of 0.1 M. 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 µM. The MCF-7 breast cancer cells were cultured in a 96-well plate and treated with varying concentrations of the test compounds for 96 h at 37 ºC. The pH of the environment was regulated at a concentration of 5% CO_2. Afterwards, the cells were exposed to MTT reagent and kept in an incubator for an extra 4 h. The solution above each well, including both medium and MTT, was carefully removed. The cells produced a formazan material with a deep blue colour, which was then dissolved in 100 mL of DMSO. To evaluate the liveliness of cells, the measurement of absorbance at a specific wavelength of 570 nm was performed using a 96-well plate reader.

RESULTS AND DISCUSSION

All the target compounds were successfully synthesized according to the outlined synthetic scheme, yielding moderate to good quantities of the desired products. The structures of all compounds were confirmed using FTIR, ^{1}H NMR and ^{13}C NMR spectroscopy, with the observed peaks consistent with the expected chemical environments of the protons and carbons in each molecule. Additionally, the molecular masses of the compounds were verified using mass spectrometry, with the observed *m/z* values matching the theoretical molecular weights. These results collectively confirm the successful synthesis and structural integrity of the target compounds.

Molecular docking: The docking results, summarized in Table-1, revealed that the synthesized compounds effectively bound to the active sites of the target proteins. Compounds **11m** and **11e** emerged as the most potent inhibitors, exhibiting the highest binding affinities with docking scores of -12.401, -11.711 and -10.711 for **11m** and -11.057, -10.415 and -10.015 for **11e** against MCF-7 (6ENV), HeLa (7ACF) and MDA-MB-231 (6VJ3), respectively. These docking scores were significantly higher than those of the co-crystallized ligands, which had scores of -6.787, -8.068 and -10.068 for the respective targets, underscoring the superior binding affinity of compounds **11m** and **11e**.

Fig. 2 illustrates the 3D super-imposition of the docked ligand (erlotinib; pink) and the co-crystallized ligand (green) in the active site of MCF-7 (6ENV), with an RMSD value of 0.88 Å. The pyridopyrimidine ring of the docked ligand aligns closely with the scaffold of the co-crystallized ligand, indicating a similar binding mode and validating the docking approach.

TABLE-1
BINDING ENERGIES (Kcal/mol), NUMBER OF HYDROGEN BONDS AND BINDING SITES

Compound	Docking score		
	MCF-7 (6ENV)	HeLa (7ACF)	MDA-MB-231 (6VJ3)
11a	-6.971	-5.782	-6.102
11b	-5.707	-4.723	-5.124
11c	-7.861	-8.395	-7.125
11d	-5.146	-4.714	-5.254
11e	-11.057	-10.415	-10.015
11f	-6.181	-6.284	-7.114
11g	-6.381	-6.484	-6.214
11h	-5.712	-4.489	-4.105
11i	-4.942	-4.526	-4.142
11j	-5.386	-4.475	-4.434
11k	-6.386	-4.241	-4.547
11l	-5.934	-6.224	-6.021
11m	12.401	-11.711	-10.711
11n	-5.352	-6.149	-6.341
11o	-9.352	-9.721	-9.141
Co-crystal ligand	6.787	-8.068	-10.068

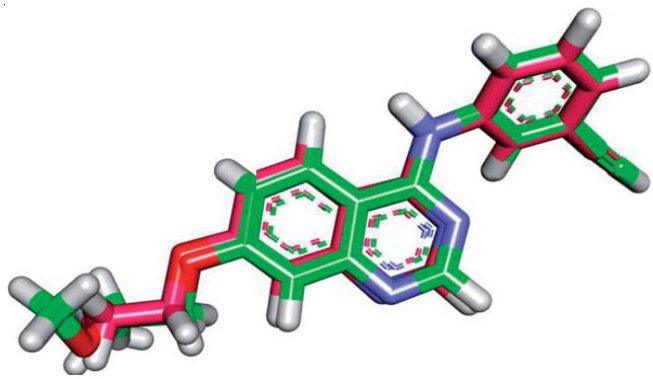


Fig. 2. 3D superimposition of the docked ligand (erlotinib; pink) and the original ligand (green) with RMSD value of 0.88 Å

Fig. 3 depicts the binding pose of compound **11e** in the active site of HeLa (7ACF). The compound forms critical hydrogen bonds with Met793 and a water-mediated hydrogen bond with Asp855, stabilizing its interaction with the target protein. These interactions likely contribute to the compound's high binding affinity and anticancer activity. Fig. 4 shows the docking position of compound **11m** in the active site of MDA-MB-231 (6VJ3). The binding mode reveals two key hydrogen bonds with Met793 and a water-mediated hydrogen bond with Asp855, further emphasizing the importance of these residues in stabilizing the ligand-protein complex.

The docking studies provide a comprehensive understanding of the binding interactions of the synthesized compounds with the target proteins. The high binding affinities and specific interactions observed for compounds **11m** and **11e** correlate well with their potent anticancer activity, suggesting their potential as lead candidates for further development. These findings are supported by the detailed binding modes and interactions illustrated in Figs. 2-4, which highlight the structural basis for the observed biological activity.

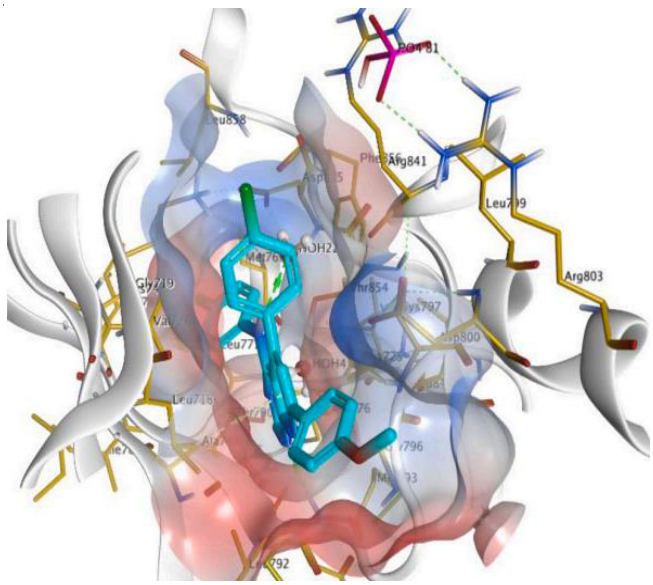


Fig. 3. Compound **11e** is positioned in the active site of HeLa (7ACF) in a docking posture. The binding mode of the compound reveals the major hydrogen bonds with Met793 and a water-mediated hydrogen bond with Asp855

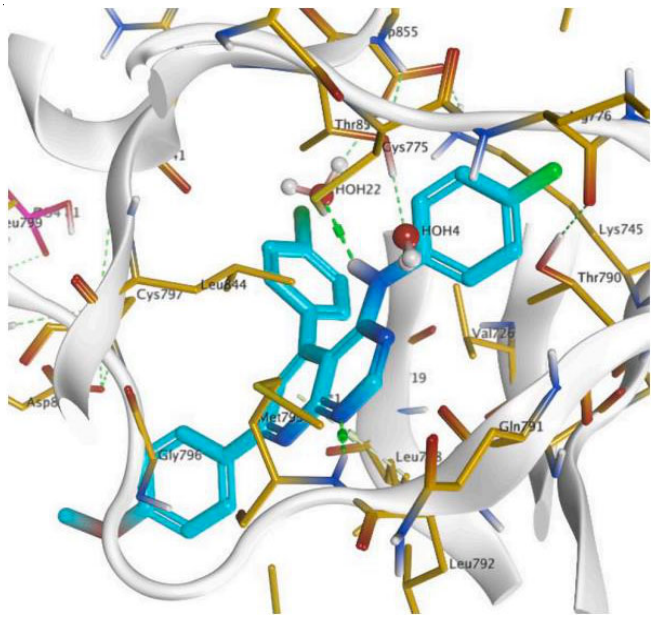


Fig. 4. Docking position of compound **11m** in the active site of MDA-MB-231 (6VJ3) is shown the binding mode shows two main hydrogen bonds with Met-793 and a water-mediated hydrogen bond with Asp855

Table-1 further highlights the binding energies and interactions of all synthesized compounds. Notably, compounds **11c**, **11o** and **11m** also exhibited strong binding affinities, with docking scores ranging from -7.861 to -12.401 kcal/mol across the three targets. These results suggest that the structural features of these compounds, particularly thpyridopyrimidine core and substituted phenyl groups, play a critical role in enhancing their binding interactions with the target proteins. The molecular docking studies not only validate the anticancer potential of the synthesized compounds but also provide valuable insights into their binding mechanisms. Compounds **11m** and **11e**, with their exceptional docking scores and interaction profiles, stand out as promising candidates for further optimization and development as anticancer agents.

Anticancer activity: The anticancer activity of the synthesized 5-amino-4-oxo-*N*-2,8-substituted triphenyl-3,4,5,6,7,8-hexahydropyrido[2,3-*d*]pyrimidine-6-carboxamide derivatives (**11a-o**) was evaluated against three human cancer cell lines *viz*. MDA-MB-231 (breast cancer), HeLa (cervical cancer) and MCF-7 (breast cancer). The results, expressed as half-maximal inhibitory concentration (IC_{50}) values, are summarized in Table-2. The IC_{50} values provide a quantitative measure of the compounds' potency, with lower values indicating higher anticancer activity.

Among the synthesized compounds, **11e** (R = 3-Cl, R_1 = 4-F) and **11m** (R = 4-F, R_1 = 4-F) emerged as the most potent inhibitors. Compound **11e** exhibited remarkable anticancer

TABLE-2
ANTICANCER 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**)

Compound	R	R_1	IC_{50} (μM)		
			MDA-MB-231	HeLa	MCF-7
11a	H	4-F	4.12 ± 0.03	4.20 ± 0.12	5.15 ± 0.12
11b	H	4-Cl	3.25 ± 0.24	3.84 ± 0.18	3.95 ± 0.25
11c	H	4-Br	3.28 ± 0.13	3.42 ± 0.03	3.26 ± 0.03
11d	H	4-NO_2	5.14 ± 0.42	5.62 ± 0.12	6.45 ± 0.03
11e	3-Cl	4-F	1.42 ± 0.12	1.54 ± 0.13	1.85 ± 0.23
11f	3-Cl	4-Cl	2.36 ± 0.03	2.96 ± 0.03	2.62 ± 0.02
11g	3-Cl	4-Br	3.65 ± 0.02	3.85 ± 0.03	5.95 ± 0.03
11h	3-Cl	4-NO_2	4.18 ± 0.13	4.38 ± 0.21	4.16 ± 0.13
11i	4-Br	4-F	3.11 ± 0.12	3.60 ± 0.32	3.45 ± 0.13
11j	4-Br	4-Cl	2.29 ± 0.18	2.34 ± 0.02	2.57 ± 0.12
11k	4-Br	4-Br	3.84 ± 0.14	3.86 ± 0.22	3.35 ± 0.28
11l	4-Br	4-NO_2	4.86 ± 0.13	4.02 ± 0.10	4.08 ± 0.24
11m	4-F	4-F	1.29 ± 0.18	1.34 ± 0.02	1.57 ± 0.12
11n	4-F	4-Cl	1.82 ± 0.05	1.91 ± 0.12	1.98 ± 0.20
11o	4-F	4-Br	1.92 ± 0.25	2.05 ± 0.03	2.10 ± 0.23
Cisplatin	–	–	1.14 ± 0.05	0.95 ± 0.02	1.02 ± 0.22

activity across all three cell lines, with IC_{50} values of 1.42 ± 0.12 μM, 1.54 ± 0.13 μM and 1.85 ± 0.23 μM against MDA-MB-231, HeLa and MCF-7, respectively. Compound **11m** demonstrated even greater potency, with IC_{50} values of 1.29 ± 0.18 μM, 1.34 ± 0.02 μM and 1.57 ± 0.12 μM against the same cell lines. These values are comparable to the reference drug cisplatin (IC_{50} values: 1.14 ± 0.05 μM, 0.95 ± 0.02 μM and 1.02 ± 0.22 μM), highlighting the potential of compound **11m** as a promising anticancer agent.

The presence of electron-withdrawing groups such as fluoro (F), chloro (Cl) and bromo (Br) at the R and R_1 positions significantly enhanced the anticancer activity. For instance, compounds **11e**, **11m** and **11n** (all containing 4-F or 4-Cl substituents) consistently showed the lower IC_{50} values compared to compounds with nitro (NO_2) or unsubstituted phenyl groups. The 3-Cl and 4-F substituents in compounds **11e** and **11m** appear to be particularly favourable, likely due to their ability to enhance binding interactions with target proteins, as supported by molecular docking studies.

Compounds with 4-F at R_1 position (*e.g.* **11a**, **11e**, **11i**, **11m**) generally exhibited higher activity than those with 4-Cl, 4-Br or 4-NO_2 substituents. For example, compound **11m** (4-F, 4-F) showed significantly lower IC_{50} values than **11l** (4-Br, 4-NO_2), which had IC_{50} values of 4.86 ± 0.13 μM, 4.02 ± 0.10 μM and 4.08 ± 0.24 μM against the respective cell lines. The introduction of a nitro (-NO_2) group at R_1 position (*e.g.* **11d**, **11h**, **11l**) resulted in reduced activity, suggesting that the strong electron-withdrawing nature of the nitro group may hinder optimal binding interactions.

The compounds demonstrated consistent activity across all three cancer cell lines, with HeLa cells generally being the most sensitive (lower IC_{50} values) and MCF-7 cells being slightly less sensitive. For example, compound **11m** showed IC_{50} values of 1.29 ± 0.18 μM (MDA-MB-231), 1.34 ± 0.02 μM (HeLa) and 1.57 ± 0.12 μM (MCF-7), indicating broad-spectrum anticancer potential. While cisplatin remains the most potent reference compound, the IC_{50} values of compounds **11m** and **11e** are remarkably close, suggesting that these compounds could serve as viable alternatives or complementary agents to cisplatin, particularly in cases of cisplatin resistance or toxicity.

The structure-activity relationship (SAR) analysis revealed that the pyridopyrimidine core is essential for anticancer activity, as it likely facilitates interactions with key residues in the target proteins. Electron-withdrawing groups at the R and R_1 positions enhance activity, with fluoro (F) and chloro (Cl) substituents being the most effective. The position of substituents also plays a critical role, with *para*-substitution (4-F, 4-Cl) generally yielding better activity than *meta*-substitution (3-Cl).

The anticancer activity data (Table-2) demonstrated that the synthesized pyridopyrimidine derivatives, particularly compounds **11m** and **11e**, exhibit potent and selective antiproliferative effects against multiple cancer cell lines. The SAR insights provide a foundation for further optimization of these compounds to enhance their efficacy and selectivity. These findings, combined with the molecular docking results, underscore the potential of these compounds as promising candidates for the development of novel anticancer therapies.

Conclusion

In conclusion, 15 novel pyrido[2,3-*d*]pyrimidine derivatives (**11a-o**), were synthesized, spectroscopically confirmed and evaluated for their anticancer 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 anticancer activity, with the most potent being pyrimidine derivatives. Compound **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 ± 0.18 μM, 1.34 ± 0.02 μM, 1.57 ± 0.12 μM 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 anticancer activity 1.42 ± 0.12 μM, 1.54 ± 0.13 μM, 1.85 ± 0.23 μM 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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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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