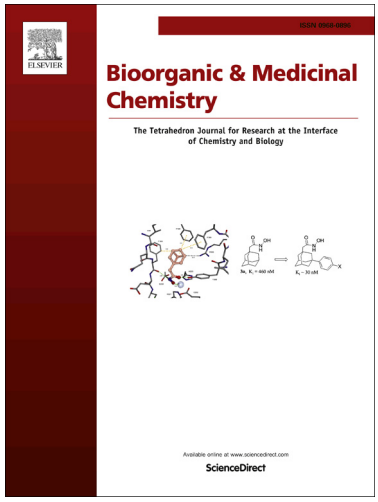

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Discovery of a novel class anti-proliferative agents and potential inhibitors of EGFR tyrosine kinases based on 4-anilinotetrahydropyrido[4,3-*d*]pyrimidine scaffold: design, synthesis and biological evaluations

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Keywords

tetrahydropyrido[4,3-*d*]pyrimidine (THPP); anti-proliferative agents; EGFR tyrosine kinase inhibitor.

Abstract

A novel series of 4-arylamino-6/7-substituted-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines were designed, synthesized and their biological activities as the potential anti-proliferative agents and EGFR kinase inhibitors were evaluated. Both of *N*-acrylamide fragment in THPPs and 4-aniline groups with substituents played key roles for their significant anti-proliferative activities against four cancer cell lines (HT29, A549, H460 and H1975). Especially inhibitory activity of Gefitinib-resistant H1975 were showed more favorable, which could be observed from compounds **13b**, **13c**, **13n**, **13o**, **13p**, **13r**, **13s**, **13u** and **24c** obviously. By evaluation of inhibiting EGFR and HER2 kinases, seven compounds (**13b**, **13g**, **13n**, **13o**, **13p**, **13r** and **13s**) showed stronger EGFR potency with $IC_{50} \leq 18$ nM, which could also be understood by preliminary docking study of **13b** with EGFR kinase. In view of the primary SAR, bisarylaniline derivatives (**13o**, **13p**, **13r** and **13s**) showed obvious improvements on HER2 inhibition, which indicated their being potential EGFR/HER2 dual kinase inhibitors.

1. Introduction

Non-small cell lung cancer (NSCLC) is one of important causes of cancer mortality globally.[1] Most of the NSCLC patients present with advanced, unresectable or metastatic disease, and a 5-year survival with less than ca. 15%.[2] Therefore, novel therapeutic strategies including the design of safe and potent anti-NSCLC drugs are highly desired. One of the most important strategies for the treatment of NSCLC is to target the epidermal growth factor receptor (EGFR).[3,4] which belongs to the erbB family of receptor tyrosine kinases (RTKs) and contains a subfamily of four closely related receptor tyrosine kinases, EGFR (ErbB-1), erbB2/HER2, erbB3/HER3 and erbB4/HER4.[5] As EGFR kinase inhibitors, 4-anilinoquinazoline derivatives have attracted more and more attention since the successful marketing of Gefitinib in 2003 (**Figure 1**).[6] To date, it is remarkable that the other 5 kinase inhibitors with 4-anilinoquinazoline moiety have also received the approvals from Food and Drug Administration (FDA) to be utilized as anticancer drugs, including EGFR inhibitors Erlotinib[7] and Icotinib,[8] EGFR/HER2 dual inhibitors Lapatinib[9] and Afatinib,[10] VEGFR/EGFR dual inhibitor Vandetanib.[11]

(**Figure 1** should be listed here.)

More abundant and systematic achievements on the research and development of 4-aminoquinzaline derivatives triggered people's interest on its usefulness of being bioisosteres and exploration toward broad non-quinazolines structures. We focused on the well-known tetrahydropyrido[4,3-*d*]pyrimidine (THPP) core which have attracted considerable attention due to their biological and pharmaceutical properties such as anti-proliferative activity (Bcl-2 inhibitor,[12] mTOR kinase inhibitor,[13,14] PI3K inhibitor,[15] P_2Y_{14} receptor antagonist,[16] VEGFR-2 inhibitor[17] and Erks inhibitor[18]) and other biological activities (PDE10 inhibitor,[19] DPP-4 inhibitor,[20] TASK-3 channel antagonist,[21] TGR5 Agonist,[22] α_1-Adrenoceptor antagonist,[23] HIV attachment inhibitor[24] etc.), as shown in **Figure 2**.

(**Figure 2** should be listed here.)

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However, to the best of our knowledge, 4-arylamino-6-substituted-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines have never been investigated as EGFR kinase inhibitors. Herein, we disclosed our design and synthesis of this completely new class of EGFR kinase inhibitors which were also biologically evaluated.

2. Results and Discussion

2.1. Design

The skeleton of 4-arylamino-6-substituted-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine retains the stability of the quinazoline but also its 6-substitued side chain showed the more flexibility than quinazoline's on account of fusing saturated tetrahydropyridine in THPP. We propose such motif can mimic the famous 4-anilinquinazoline and influence kinase inhibitory activities with better physicochemical properties due to more free orientation of THPP core and different linker pattern of flexible *N*-substituted side chains. Based on the structure-activity relationship (SAR) studies of quinazolines as EGFR inhibitors,[25, 26] we installed the 3-chloro-4-fluoro aniline fragment at 4-position as the first choice because it has been widely utilized in designing many EGFR inhibitors, such as Gefitinib and Afatinib. It has been established that 4-arylamino fragment can well extend in the hydrophobic pocket in the back of the ATP-binding cleft and provide the key kinase inhibition. At the same time, many different linker patterns of 6-substituents (series I) were designed and introduced which pointed at the Phosphate-binding region. Further optimization of 4-arylamino moiety in the designed compound with the optimal linker pattern led to compound series II as the potential EGFR inhibitors as evaluated by cancer cell lines and kinases. On the other hand, 4-arylamino-7-substituted-THPP series III were also designed and evaluated in order to investigate the anticancer activity of outspreading orientation of the side chain due to different *N*-position in THPP. (**Figure. 3**).

(**Figure 3** should be listed here.)

In our initial study, structure **a1** was designed and aligned with Gefitinib and Erlotinib based on molecule field theory.[27, 28] The result suggested that all three compounds had reasonable overlap as shown in **Figure 4**, which indicated the 4-arylamino-6-substituted-THPPs could effectively mimic the 4-anilinquinazoline derivatives.

(**Figure 4.** should be listed here.)

2.2. Chemistry

The designed 4-arylamino-6-substituted-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines (series I and II) were synthesized as shown in **Schemes 1** and **2**. It was critical to introduce a robust *N*-protecting group in THPP core to avoid formation of byproducts occurring at arylamine in subsequent deprotection (especially for arylamines with Cl, Br or NO_2 groups).[29] Ethyl 1-benzyl-4-oxopiperidine-3-carboxylate (**1**) was therefore subjected to catalytic hydrogenation to give the intermediate **2**, which was protected again with CbzCl to yield intermediate **3**. Cyclization with formamidine acetate generated **4** efficiently. After chlorination with phosphorus oxychloride to get **5**, the optimal base and solvent were explored and identified to facilitate nucleophilic substitution with different anilines and afford the compounds **6a-q** in satisfactory yields. Deprotection in HBr and acetic acid yielded the key precursors **7a-q** cleanly. The target products **8**-**13** were obtained in various conditions as depicted in **Scheme 2**. Compound **7a** reacted with alkylsulfury chloride to obtain sulfonamides **8a** and **8b**, or underwent Michael addition or alkylation to get amines **9a-b**, or reacted with diethyl squarate and subsequently amines to afford compounds **10a-c**, or reacted with CDI and amines to prepare ureas **11a-c**, or coupled with chlorocarbonic ester or acyl chloride/aliphatic acid to afford carbamates **12a-d** or amides **13a-u** and **a1**, respectively.

(**Scheme 1** should be listed here.)

(**Scheme 2** should be listed here.)

On the other hand, in order to investigate the influence of nitrogen atom at different positions in THPP core, the 4-arylamino-7-alkyl substituted-5,6,7,8-tetrahydropyrido[3,4-*d*]pyrimidine compounds **20-23** and **24 a-c** (series III) were synthesized by adopting the similar methods as for 6-substituted compounds taking ethyl 1-benzyl-3-oxopiperidine-4-carboxylate as the starting material (**Schemes 3** and **4**).

(**Scheme 3** should be listed here.)

(**Scheme 4** should be listed here.)

2.3. In *vitro* anti-proliferative activity

In order to find the best linker pattern at 6-position in THPP, the compounds of series I were initially synthesized and evaluated by applying the MTT colorimetric assay using Gefitinib as positive control.[30,31] The compounds with different linkers were screened by anti-proliferative activity against HT29 (human colorectal cancer line), A549 (human lung cancer cell line) and H460 (large cell lung cancer line), as well as Gefitinib-resistant H1975 cancer line (which is a human lung cancer cell line expressed high levels of EGFR T790M/L858R mutation) as shown in **Table 1**. The unsubstituted compound **7a**, sulfonamides **8a-b,** *N*-alkylated compounds **9a-b** and compounds **10a-b** using cyclobutene dione as linkage showed poor activities in four cancer cell lines, as compared with Gefitinib, although compound **10c** in squarate derivatives displayed comparable activity against three cancer cell lines (IC_{50} = 20.21 μM for HT29, IC_{50} = 57.28 μM for A549, IC_{50} = 24.12 μM for H460), poor activity for H1975 (IC_{50} = 125.33 μM). With carbonyl as linkage, the urea **11a-c** and carbamates **12a-d** also didn't exhibit more potency. However, urea **11c** (IC_{50} = 12.49 μM for A549) and carbamate **12a** (IC_{50} = 38.32 μM for H1975) showed comparable anti-proliferative activities against certain cancer cell lines. Remarkably, amide derivatives **13b** with propenamide demonstrated significant activity (IC_{50} = 42.35 μM for HT29, IC_{50} = 22.27 μM for A549, IC_{50} = 29.73 μM for H460, especially IC_{50} = 6.32 μM for H1975). Subsequently the result of comparison of the anti-proliferative activity of propenamide **13b**, propynamide **13c** and propanamide **13d** was that both Michael acceptors with unsaturated bond displayed better cellar activities, especially against H1975, IC_{50} of compound **13c** is 6.21 μM.

(**Table 1** should be listed here.)

Keeping the *N*-acrylamide as the optimal fragment in THPP, further optimization and biological evaluations were focused on the 4-substitued anilines fragment of series II compounds (**Table 2**). In most cases, the designed compounds showed comparable or even more significant activities against four cell lines comparing with control compound Gefitinib, especially inhibitory activity against Gefitinib-resistant H1975. It should be noted that compounds with electron-withdrawing groups at *meta*-position of aniline showed stronger activities than those with *para*- or *ortho*-electron-withdrawing groups (**13g** *vs.* **13k**, **13n** *vs.* **13l**). Compound **13m** exhibited lower activity than compound **13f** against H1975 (IC_{50} = 34.03 μM *vs* 12.37 μM) could be perceived that substituted group at the *ortho* position could reduce activity such as compound **13h** and **13m**. The change of substituted group at *para* position of the compounds would not sharply decrease the activity. If introducing another aryl moiety at the *para*-position of 3-halogenaniline group and with N, O or S as the linker, the anti-proliferative activities could be further improved. The inhibition of compound **13p** with Cl (IC_{50} = 2.79 μM) against H1975 was 15 times more potent than compound **13q** (IC_{50} = 43.42 μM) with F at *meta* position of arylamine. In the derivatives with N linker between bisarylamines, urea **13u** had more anti-proliferative effect than amide **13t**. There were eight compounds with $IC_{50} \leq 7$μM against H1975 which showed more favorable activity than the positive control Gefitinib (IC_{50} = 12.87 μM). From the **Table 2** we could see that all the tested compounds demonstrated good activity against H29, especially compound **13n**, **13r**, **13s** and **13u** (IC_{50} = 7.56 μM, 5.91 μM, 7.48 μM and 5.28 μM respectively). Compound **13p** and **13r** demonstrated the stronger activity against A549 (IC_{50} = 9.40 μM and 5.88 μM respectively), and five compounds (**13i**, **13n**, **13r**, **13t**, **13u**) showed potent activity against H460 with $IC_{50} < 7$ μM.

(**Table 2** should be listed here.)

On the other hand, we also evaluated the anti-proliferative activities of 7-substituted THPPs (series III) in order to investigate the influence of the side chain orientation resulted from different *N* positions in THPPs (**Table 3**). Totally 7 designed compounds showed comparable or poorer activities compared with the corresponding 6-substituted compounds (**20a** *vs.* **7a**; **21** *vs.* **8a**; **22** *vs.* **11a**; **23** *vs.* **12a**; **24a** *vs.* **13e**; **24b** *vs.* **13b** and **24c** *vs.* **13p**). The results indicated *N*-side chain at 6-position of THPP may have positive effect on the anti-proliferative activities.

(**Table 3** should be listed here.)

2.4. In *vitro* EGFR and HER2 inhibitory activity

On the basis of the anti-proliferative results against four cancer cell lines, totally 7 designed compounds were selected for further in *vitro* EGFR and HER2 inhibitory activities studies in comparison with Staurosporine, which had good inhibitory activities against ERFR and HER2 kinase as a multiple protein kinases inhibitor.[34] The results were summarized in **Table 4.** All tested compounds exhibited excellent EGFR potency ($IC_{50} \leq 18$ nM). The single arylamine substituted compounds **13b**, **13g** and **13n** showed low HER2 inhibitory activities but compounds **13o**, **13p, 13r** and **13s** with another aryl moiety in aniline fragment displayed considerable HER2 inhibitory activities. These biological data revealed that the single arylamine substituted THPPs might be selective inhibitor of single target of EGFR kinase, and bisaryl-substituted THPPs might be dual selective inhibitors on the ERFR/HER2 kinase targets.

(**Table 4** should be listed here.)

2.5. Molecular docking

Compound **13b** was selected for molecular docking study in EGFR crystal structure (PDB ID: 4G5P) for better understanding the potency of this series of 6-substitued THPPs and further SAR study.[35] As shown in **Figure 5**, the docking result suggested that the THPP scaffold well fit into the ATP pocket and the *N*-1 atom had hydrogen bonding interaction with carbonyl oxygen in Met790 as the quinazoline core.[36] At the same time, carbonyl oxygen at the 6-acrylamide tail has hydrogen bonding interaction with NH in residue Cys797. Moreover, the carbon atom of Michael acceptor was situated 3.73 Å away from the sulfhydroyl group of Cys797, which was suitable for the formation of the covalent bond.[37] The three key interactions might be able to explain the corresponding biological activities of the designed 4-arylamino-6-substituted-THPPs as irreversible inhibitors of EGFR.

(**Figure 5** should be listed here.)

3. Conclusions

A novel series of 4-arylamino-6/7-substituted-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine derivatives were designed, synthesized and biologically evaluated as potential EGFR tyrosine kinase inhibitor. *N*-acrylamide fragment in THPPs and 4-aniline groups with substituents played key roles for the significant anti-proliferative activities against four cancer cell lines (HT29, A549, H460 and H1975). Especially inhibitory activity of Gefitinib-resistant H1975 were showed more favorable, which could be observed from compounds **13b**, **13c**, **13n**, **13o**, **13p**, **13r**, **13s**, **13u** and **24c** ($IC_{50} \leq 8$ μM) obviously. By evaluation of inhibiting EGFR and HER2 kinases, 7 compounds (**13b**, **13g**, **13n**, **13o**, **13p**, **13r** and **13s**) showed stronger EGFR potency with $IC_{50} \leq 18$ nM, which could also be mechanistically understood by preliminary docking study of **13b** with EGFR kinase. On the other hand, bisarylaniline derivatives (**13o**, **13p**, **13r** and **13s**) showed obvious improvements on HER2 inhibition activities, which indicated their potential being as EGFR/HER2 dual kinase inhibitors. The further structure scope expanding of these new series and evaluations of their pharmacological profile, as well as improvement of HER2 inhibition ability are still on-going.

4. Experimental

4.1. Chemistry

Unless otherwise noted, all materials were obtained from commercial suppliers and were used without further purification. Reactions' time and purity of the products were monitored by TLC on FLUKA silica gel aluminum cards (0.2 mm thickness) with fluorescent indicator 254 nm. Column chromatography was run on silica gel (200-300 mesh) from Qingdao Ocean Chemicals (Qingdao, Shandong, China). All melting points were obtained on a Büchi Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland) and were uncorrected. Mass spectra (MS) were taken in ESI mode on Agilent 1100 LC–MS (Agilent, Palo Alto, CA, USA).^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker ARX-400, 400 MHz; or BrukerARX-600, 600 MHz spectrometers (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. The IR spectra were recorded by means of the KBr pellet technique on a Bruker FTS 135 spectrometer. Elemental analysis was determined on a Carlo-Erba1106Elemental analysis instrument (Carlo Erba, Milan,

Italy).(In the mode of measurement C, H, and N, the sample into the combustion tube in pure oxygen atmosphere static combustion and products by a specific reagent after formation of CO_2, H_2O, N_2 and nitrogen oxides, uniform mixing under the atmospheric pressure. The thermal conductivity detector is used for determining the content of C, H and N from mixed gases.).

4.1.1. Ethyl 4-oxopiperidine-3-carboxylate hydrochloride (2)

To compound **1** (160 g, 0.54 mol) in ethanol (1000 mL) was added palladium on carbon (16 g, 10% wt.) and concentrated aqueous hydrogen chloride (16 mL) and the mixture was hydrogenated under hydrogen (5 atm.) at 40 °C for 8h. The solution was filtered through a bed of Cellulose microcrystalline and the solvent was removed in *vacuo* to give 107 g of compound **2** as a light brown solid without any further purification to be used in the next step. Light brown solid; Yield: 95 %; MS (ESI) m/z (%): 172.3$[M+H]^+$.

4.1.2. 1-benzyl 3-ethyl 4-oxopiperidine-1,3-dicarboxylate (3)

To a solution of compound **2** (87.7 g, 0.42 mol) and DIPEA (193 g, 1.05 mol) in DCM (600 mL) was added dropwise slowly CbzCl (86.4 g, 0.50 mol) at 0 °C for an hour. After addition, the reaction solution was allowed to r.t. and stirred for an hour. The reaction mixture was added water (1000 mL), separated two phase, the organic layer was washed by 1 N HCl (1000 mL) and brine (1000 mL), then dried ($MgSO_4$), filtered and concentrated to afford 165 g crude product **3**. White gray solid; Yield: 91%; MS (ESI) m/z (%): 304.0$[M-H]^-$.

4.1.3. Benzyl 4-hydroxy-7,8-dihydropyrido[4,3-*d*]pyrimidine-6(5*H*)-carboxylate (4)

To a slurry of sodium methoxide (7.1 g, 131 mmol) in anhydrous ethanol (60 mL) at room temperature was added formamidine acetate (4.4 g, 24.9 mmol) and compound **3** (10 g, 33.0 mmol) in one portion. After stirring at reflux for 6 h, the mixture was cooled to r.t.. Water (30 mL) was added, followed by con. HCl to adjust pH = 4. And the mixture was extracted by DCM (100 mL × 2), the combined organic layer was concentrated and slurred by MTBE (30 mL) to afford 6.2 g compound **4**. White solid; Yield: 62%; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.07 (s, 1H), 7.32-7.38 (m, 5H), 5.11 (s, 2H), 4.22 (br. s., 2H), 3.64 (br. s., 2H), 2.50-2.62 (t, J = 5.7 Hz, 2H); MS (ESI) m/z (%): 285.9$[M+H]^+$.

4.1.4. Benzyl 4-chloro-7,8-dihydropyrido[4,3-*d*]pyrimidine-6(5*H*)-carboxylate (5)

To a solution of compound **4** (12. g, 49.6 mmol) and DIPEA (8.9 g, 58.5 mmol) in CH_3CN (80 mL) was added phosphorus oxychloride (9.12 g, 59.6 mmol) at r.t., and the reaction solution was heated to reflux for an hour, then cooled to r.t.. The resulting mixture was quenched with saturated aqueous sodium bicarbonate solution (200 mL), the mixture was then extracted with DCM (100 mL × 2). The combined organic layers were concentrated to give 11 g compound **5** for next step directly. Brown oil; MS (ESI) m/z (%): 303.7$[M+H]^+$.

4.1.5. General procedure for preparation of benzyl 4-substitutedphenylamino-7,8-dihydropyrido[4,3-*d*] pyrimidine-6(5*H*)-carboxylate (6a-6q)

To a mixture of an appropriate aniline (0.83 mmol) and compound **4** (200 mg, 66 mmol) in THF (5 mL) was added slowly NaHMDS (92 mg, 1 mmol) in an ice bath. Upon the completion of addition, the reaction mixture was allowed to room temperature and stirred for 2 h. The mixture was added water (10 mL), extracted with EtOAc (10 mL × 2), washed with brine (20 mL × 2). The organic phase was separated, dried and evaporated, and the crude product was purified by chromatography to afford the compounds **6a-6q**.

4.1.5.1. Benzyl 4-((3-chloro-4-fluorophenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidine-6(5*H*)-carboxylate (6a)

White solid; Yield: 79.3%; M.p.: 182-183 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.76(s, 1H), 8.44(s, 1H),7.96 (s,

1H), 7.66 (s, 1H), 7.34-7.39(m, 6H), 5.17 (s, 2H), 4.54 (br. s., 2H), 3.73 (br. s., 2H), 2.75 (t, *J* = 5.7 Hz, 2H); MS (ESI) m/z (%): 413.4$[M+H]^+$.

4.1.5.2. Benzyl 4-((3-(trifluoromethyl) phenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidine-6(5*H*)-carboxylate (6b)

White solid; Yield: 74.9%; M.p.: 133-135 ℃; MS (ESI) m/z (%): 429.1$[M+H]^+$.

4.1.5.3. Benzyl 4-((3-bromophenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidine-6(5*H*)-carboxylate (6c)

White solid; Yield: 80.7%; M.p.: 165-168 ℃; MS (ESI) m/z (%): 439.0$[M+H]^+$.

4.1.5.4. Benzyl 4-((2-methyl-5-nitrophenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidine-6(5*H*)-carboxylate (6d)

White yellow solid; Yield: 65.4%; M.p.: 149-150 ℃; MS (ESI) m/z (%): 419.9$[M+H]^+$.

4.1.5.5. Benzyl 4-((3,4-dimethylphenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidine-6(5*H*)-carboxylate (6e)

White solid; Yield: 71.6 %; M.p.: 176-179 ℃; MS (ESI) m/z (%): 389.2$[M+H]^+$.

4.1.5.6. Benzyl 4-((3-bromo-5-methoxyphenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidine-6(5*H*)-carboxylate (6f)

White solid; Yield: 59.2%; M.p.: 157-159 ℃; MS (ESI) m/z (%): 469.3$[M+H]^+$.

4.1.5.7. Benzyl 4-((4-bromophenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidine-6(5*H*)-carboxylate (6g)

White solid; Yield: 77.3%; M.p.: 126-127 ℃; MS (ESI) m/z (%): 439.2$[M+H]^+$.

4.1.5.8. Benzyl 4-((2,4-dichlorophenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidine-6(5*H*)-carboxylate (6h)

White solid; Yield: 69.7%; M.p.: 138-139 ℃; MS (ESI) m/z (%): 428.8$[M+H]^+$.

4.1.5.9. Benzyl 4-((2-chloro-5-(trifluoromethyl) phenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidine-6(5*H*)-carboxylate (6i)

White solid; Yield: 68.0%; M.p.: 145-147 ℃; MS (ESI) m/z (%): 462.3$[M+H]^+$.

4.1.5.10. Benzyl 4-((3,5-dichlorophenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidine-6(5*H*)-carboxylate (6j)

White solid; Yield: 73.2 %; M.p.: 153-155 ℃; MS (ESI) m/z (%): 429.0$[M+H]^+$.

4.1.5.11. Benzyl 4-((3-chloro-4-(pyridin-2-ylmethoxy) phenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidine-6(5*H*)-carboxylate (6k)

White solid; Yield: 74.8%; M.p.: 194-196 ℃; MS (ESI) m/z (%): 502.1$[M+H]^+$.

4.1.5.12. Benzyl 4-((3-chloro-4-((3-fluorobenzyl) oxy) phenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidine-6(5*H*)-carboxylate (6l)

White solid; Yield: 69.5%; M.p.: 189-191 ℃; MS (ESI) m/z (%): 519.0$[M+H]^+$.

4.1.5.13. Benzyl 4-((3-fluoro-4-(pyridin-2-ylmethoxy) phenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidine-6(5*H*)-carboxylate (6m)

White solid; Yield: 63.2%; M.p.: 171-173 ℃; MS (ESI) m/z (%): 485.8$[M+H]^+$.

4.1.5.14. Benzyl 4-((3-chloro-4-(3-(trifluoromethyl) phenoxy) phenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidine-

6(5*H*)-carboxylate (6n)

White solid; Yield: 79.6%; M.p.: 192-194 ℃; MS (ESI) m/z (%): 555.0[M+H]$^+$.

4.1.5.15. Benzyl 4-((3-chloro-4-((3-fluorobenzyl) thio) phenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidine-6(5*H*)-carboxylate (6o)

White solid; Yield: 87.1%; M.p.: 183-184 ℃; MS (ESI) m/z (%): 535.2[M+H]$^+$.

4.1.5.16. Benzyl 4-((3-chloro-4-(3-fluorobenzamido) phenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidine-6(5*H*)-carboxylate (6p)

White solid; Yield: 48.7 %; M.p.: 187-188 ℃; MS (ESI) m/z (%): 532.3[M+H]$^+$.

4.1.5.17. Benzyl 4-((3-chloro-4-(3-(3-fluorophenyl) ureido) phenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidine-6(5*H*)-carboxylate (6q)

White solid; Yield: 53.4%; M.p.: 196-198 ℃; MS (ESI) m/z (%): 547.1[M+H]$^+$.

4.1.6. General procedure for preparation of *N*-phenyl-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4- substituted amine (7a-q)

Compound **6a-q** (5 g) was dissolved in acetic acid (10 mL) and HBr (10 mL), the reaction solution was heated and stirred at 50 ℃ for 2 h. After finished reaction, the solution was cooled to 0 ℃, added water (100 mL), adjusted pH = 10 with 5 N NaOH, extracted with Me-THF (100 mL × 3), washed by brine (100 mL), dried and removed the solvent then slurried by MTBE (20 mL) to afford the product (**7a-q**).

4.1.6.1. *N*-(3-chloro-4-fluorophenyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4-amine (7a)

Beige solid,Yield: 89.4%; M.p.: 170-173℃; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.46 (s, 2H), 8.04 (dd, *J* = 6.8, 2.7 Hz, 1H), 7.72 (ddd, *J* = 9.0, 4.4, 2.7 Hz, 1H), 7.42 (t, *J*=9.2 Hz, 1H), 3.80 (s, 2H), 3.05 (t, *J* = 5.8Hz, 2H), 2.69 (t, *J* = 5.7 Hz, 2H); MS (ESI) m/z (%): 276.5[M+H]$^+$; Anal. calcd. for $C_{15}H_{14}ClFN_4O$ (%): C, 56.02; H, 4.34; N, 20.10; Found (%): C, 56.11; H, 4.31; N, 20.20.

4.1.6.2. *N*-(3-(trifluoromethyl) phenyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4-amine (7b)

Beige solid, Yield: 86.2%; M.p.: 129-131 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.55 (s, 1H), 8.44 (s, 1H), 8.12 (s, 1H), 8.07 (d, *J* = 8.2 Hz, 1H), 7.54 (t, *J* = 7.9 Hz, 1H), 7.36 (d, *J* = 7.7 Hz, 1H), 3.81 (s, 2H), 3.02 (t, *J* = 5.7 Hz, 2H), 2.66 (t, *J* = 5.7 Hz, 2H); MS (ESI) m/z (%): 294.6[M+H]$^+$.

4.1.6.3. *N*-(3-bromophenyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4-amine (7c)

Beige solid, Yield: 76.5%; M.p.: 118-120 ℃; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.43 (s, 1H), 7.89-7.99 (m, 1H), 7.57-7.64 (m, 1H), 7.23-7.29 (m, 2H), 3.99 (s, 2H), 3.30 (t, *J* = 6.0 Hz, 2H), 2.89 (t, *J* = 6.0 Hz, 2H); MS (ESI) m/z (%): 303.1[M-H]$^-$.

4.1.6.4. *N*-(2-methyl-5-nitrophenyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4-amine (7d)

Light brown; Yield: 69.4%; M.p.: 84-87 ℃; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.27 (s, 1H), 8.21 (d, *J* = 2.3 Hz, 1H), 8.07 (dd, *J* = 8.4, 2.3 Hz, 1H), 7.54 (d, *J* = 8.4 Hz, 1H), 4.03 (s, 2H), 3.31-3.35 (m, 2H), 2.89 (t, *J* = 5.9 Hz, 2H), 2.34 (s, 3H); MS (ESI) m/z(%): 286.2[M+H]$^+$.

4.1.6.5. *N*-(3,4-dimethylphenyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4-amine (7e)

Beige solid; Yield: 74.6%; M.p.: 184-185 ℃; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.31 (s, 1H), 7.29 (s, 1H), 7.26 (dd, *J* = 8.1, 2.1 Hz, 1H), 7.11 (d, *J* = 8.1 Hz, 1H), 3.98 (s, 2H), 3.31-3.34 (m, 2H), 2.87 (t, *J* = 6.1 Hz, 2H), 2.27 (s, 3H), 2.26 (s, 3H);MS (ESI) m/z(%): 253.0[M-H]$^-$.

4.1.6.6. *N*-(3-bromo-5-methoxyphenyl)-5,6,7,8-tetrahydro[4,3-*d*]pyrimidin-4-amine (7f)

Beige solid; Yield: 67.8%; M.p.: 134-136 ℃; MS (ESI) m/z (%): 335.1[M+H]$^+$.

4.1.6.7. *N*-(4-bromophenyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4-amine (7g)

Beige solid; Yield: 73.2%; M.p.: 110-112 ℃; MS (ESI) m/z (%): 305.0[M+H]$^+$.

4.1.6.8. *N*-(2, 4-dichlorophenyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4-amine (7h)

Beige solid; Yield: 84.6%; M.p.: 135-137 ℃; MS (ESI) m/z (%): 295.1[M+H]$^+$.

4.1.6.9. *N*-(2-chloro-5-(trifluoromethyl) phenyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4-amine (7i)

Beige solid; Yield: 71.0%; M.p.: 121-123 ℃; MS (ESI) m/z (%): 329.2[M+H]$^+$.

4.1.6.10. *N*-(3,5-dichlorophenyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4-amine (7j)

White solid; Yield: 83.4%; M.p.: 146-148 ℃; MS (ESI) m/z (%): 295.1[M+H]$^+$.

4.1.6.11. *N*-(3-chloro-4-(pyridin-2-ylmethoxy) phenyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4-amine (7k)

White solid; Yield: 73.6%; M.p.: 140-141 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.59 (d, *J* = 4.3 Hz, 1H), 8.35 (s, 1H), 8.27 (s, 1H), 7.84-7.90 (m, 1H), 7.83 (d, *J* = 2.2 Hz, 1H), 7.51-7.60 (m, 2H), 7.32-7.39 (m, 1H), 7.19 (d, *J* = 9.0 Hz, 1H), 5.26 (s, 2H), 3.74 (br. s., 2H), 3.00 (t, *J* = 5.5 Hz, 2H), 2.59-2.65 (m, 2H). MS (ESI) m/z (%): 366.1[M-H]$^-$.

4.1.6.12. *N*-(3-chloro-4-((3-fluorobenzyl) oxy) phenyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4-amine (7l)

Beige solid; Yield: 81.4%; M.p.: 181-183 ℃; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.33 (s, 1H), 7.72 (d, *J* = 2.6 Hz, 1H), 7.34-7.44 (m, 2H), 7.18-7.30 (m, 2H), 6.97-7.08 (m, 2H), 5.14 (s, 2H), 3.95 (s, 2H), 3.27 (t, *J* = 5.9 Hz, 2H), 2.85 (t, *J* = 5.8 Hz, 2H);MS (ESI) m/z (%): 385.1[M+H]$^+$.

4.1.6.13. *N*-(3-fluoro-4-(pyridin-2-ylmethoxy) phenyl)-5,6,7,8-tetrahydropyrido[4, 3-*d*]pyrimidin-4-amine (7m)

Beige solid; Yield: 74.8%; M.p.: 152-184 ℃; MS (ESI) m/z (%): 351.8[M+H]$^+$.

4.1.6.14. *N*-(3-chloro-4-(3-(trifluoromethyl) phenoxy) phenyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4-amine (7n)

Light yellow; Yield: 93.7%; M.p.: 169-170 ℃; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.42 (s, 1H), 7.96 (d, *J* = 2.6 Hz, 1H), 7.62 (dd, *J* = 8.8, 2.6 Hz, 1H), 7.48-7.55 (m, 1H), 7.37 (d, *J* = 7.7 Hz, 1H), 7.12-7.19 (m, 3H), 3.95 (s, 2H), 3.22-3.27 (m, 2H), 2.80-2.88 (m, 2H);MS (ESI) m/z (%): 421.0[M+H]$^+$.

4.1.6.15. *N*-(3-chloro-4-((3-fluorobenzyl) thio) phenyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4-amine (7o)

Light brown; Yield: 58.1%; M.p.: 186-188 °C; MS (ESI) m/z (%): 398.5[M-H]$^-$.

4.1.6.16. *N*-(2-chloro-4-((5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4-yl) amino) phenyl)-3-fluorobenzamide (7p)

Beige solid; Yield: 65.7%; M.p.: 172-173 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.14 (s, 1H), 8.54 (s, 1H), 8.46 (s, 1H), 8.01 (d, *J* = 2.4 Hz, 1H), 7.86 (d, *J* = 7.8 Hz, 1H), 7.78 (d, *J* = 9.5 Hz, 1H), 7.72 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.56-7.65 (m, 1H), 7.42-7.52 (m, 2H), 3.87 (s, 2H), 3.10 (t, *J* = 5.7 Hz, 2H), 2.71 (t, *J* = 5.6 Hz, 2H); MS (ESI) m/z (%): 396.7[M-H]$^-$.

4.1.6.17. 1-(2-chloro-4-((5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4-yl)amino)phenyl)-3-(3-fluorophenyl)urea(7q)

Beige solid; Yield: 87.2 %; M.p.: 126-128 °C; MS (ESI) m/z (%): 413.2[M+H]$^+$.

4.1.7. General procedure for preparation of *N*-(3-chloro-4-fluorophenyl)-6-(substituted sulfonyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4-amine (8a-b)

To a solution of compound **7a** (200 mg, 0.72 mmol) and DIPEA (138 mg,1.08 mmol) in DCM (6 mL) was added dropwise slowly alkyl sulfonyl chloride (0.94 mmol) at °C. After addition, the reaction solution was allowed to r.t., and stirred for an hour. The reaction solution was washed by Na_2CO_3 (10 mL) aqueous and brine (10 mL), then dried by Na_2SO_4, filtered and concentrated, purified by column to afford compound **8a-b**.

4.1.7.1. *N*-(3-chloro-4-fluorophenyl)-6-(methylsulfonyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4-amine (8a)

White solid; Yield: 85.9%; M.p.: 210-212 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.61 (s, 1H), 7.80 (br. s., 1H), 7.41 (d, *J*=5.4 Hz, 1H), 7.16 (t, *J*=8.7 Hz, 1H), 6.44 (br. s., 1H), 4.34 (s, 2H), 3.57-3.75 (m, 2H), 3.05 (br. s., 2H), 2.98 (s, 3H); MS (ESI) m/z(%): 357.2[M+H]$^+$; Anal. calcd. for $C_{14}H_{14}ClFN_4O_2S$ (%): C, 47.13; H, 3.95; N, 15.70; Found (%): C, 47.32; H, 4.06; N, 15.77.

4.1.7.2. *N*-(3-chloro-4-fluorophenyl)-6-(cyclopropylsulfonyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4-amine (8b)

White solid; Yield: 96.6 %; M.p.: 203-204 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.72 (s, 1H), 8.46 (s, 1H), 7.94 (dd, *J* = 6.8, 2.6 Hz, 1H), 7.58-7.74 (m, 1H), 7.40 (t, *J* = 9.1 Hz, 1H), 4.38 (s, 2H), 3.60 (t, *J* = 5.9 Hz, 2H), 2.87 (t, *J* = 5.7 Hz, 2H), 2.60-2.72 (m, 1H), 0.87-1.14 (m, 4H); MS (ESI) m/z (%): 382.5[M+H]$^+$; Anal. calcd. for $C_{16}H_{16}ClFN_4O_2S$(%): C, 50.20; H, 4.21; N, 14.63; Found (%): C, 50.28; H, 4.20; N, 14.66.

4.1.8.1. 2-(4-((3-chloro-4-fluorophenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl)-1- morpholino ethanone (9a)

To a solution of compound **7a** (200 mg, 0.72 mmol) and K_2CO_3 (198 mg, 1.44 mmol) in acetone (6 mL) was added 2-chloro-1-morpholinoethanone (176 mg, 1.04 mmol). And the reaction mixture was heated at reflux for 4 h. The reaction mixture was cooled to r.t., filtered. And the filtrate was removed the solvent, purified by column to afford compound **9a**. Light yellow solid; Yield: 74.3%; M.p.: 187–189 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.56 (s, 1H), 7.69-7.77 (m, 1H), 7.32-7.44 (m, 1H), 7.12 (t, *J* = 8.6 Hz, 1H), 6.48 (br. s., 1H), 3.58-3.73 (m, 10H), 3.49 (br. s., 2H), 2.93 (br. s., 2H); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 168.0, 160.7, 156.5, 155.8, 135.1, 124.0, 121.5, 120.9, 116.5, 110.9, 66.9, 61.0, 50.1, 49.2, 46.3, 42.3, 32.1; MS (ESI) m/z (%): 404.5[M-H]$^-$; Anal. calcd. for $C_{19}H_{21}ClFN_5O_2$ (%): C, 56.23; H, 5.22; N, 17.26;Found (%): C, 56.49; H, 5.21; N, 17.17.

4.1.8.2. 3-(4-((3-chloro-4-fluorophenyl) amino)-7,8-dihydropyrid[4,3-*d*]pyrimidin-6(5*H*)-yl)propanamide (9b)

The solution of compound **7a** (200 mg, 0.72 mmol) and acrylamide (64 mg, 0.90 mmol) in ethanol (6 mL) was heated at reflux for 8 h. The reaction mixture was cooled to r.t., and removed the solvent, purified by column to afford compound **9b**. White solid; Yield: 96.2%; M.p.: 177-179 °C; ^{1}H NMR (DMSO-*d*6, 400 MHz): δ 8.50 (br. s., 1H), 8.41 (s, 1H), 7.96 (dd, *J* = 6.8, 2.7 Hz, 1H), 7.66 (ddd, *J* = 9.0, 4.3, 2.7 Hz, 1H), 7.39 (s, 1H), 7.34-7.38 (m, 1H), 6.80 (br. s., 1H), 3.48 (s, 2H), 2.79 (t, *J* = 7.2 Hz, 2H), 2.73 (s, 4H), 2.38 (t, *J* = 7.3 Hz, 2H); MS (ESI) m/z (%):349.8[M+H] $^+$; Anal. calcd. for $C_{16}H_{17}ClFN_5O$ (%): C, 54.94; H, 4.90; N, 20.02; Found (%): C, 55.13; H, 4.92; N, 20.08.

4.1.9.1. 3-(4-((3-chloro-4-fluorophenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl)-4-ethoxycyclobut-3-ene-1,2-dione (10a)

To a solution of compound **7a** (200 mg, 0.72 mmol) and TEA (146 mg, 1.44 mmol) in ethanol (6 mL) was added diethyl squarate (246 mg, 1.44 mmol) and heated at reflux for 4 h. The reaction solution was cooled to r.t., and removed the solvent, purified by column to afford compound **10a**. Light yellow; Yield: 72.6%; M.p.: 211-213°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.71-8.94 (m, 1H), 8.47 (s, 1H), 7.91 (br. s., 1H), 7.61 (br. s., 1H), 7.40 (t, *J* = 9.1 Hz, 1H), 4.52-5.05 (m, 4H), 3.72-4.21 (m, 2H), 2.92 (br. s., 2H), 1.41 (t, *J* = 6.5 Hz, 3H); MS (ESI) m/z (%): 401.3[M-H] $^-$; Anal. calcd. for $C_{19}H_{16}ClFN_4O_3$(%): C, 56.65; H, 4.00; N, 13.91; Found (%): C, 56.69; H, 4.11; N, 13.86.

4.1.9.2. 3-(4-((3-chloro-4-fluorophenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl)-4-(dimethylamino) cyclobut-3-ene-1,2-dione (10b)

To the solution of compound **7a** (200 mg, 0.72 mmol) and TEA (146 mg, 1.44 mmol) in ethanol (6 mL) was added diethyl squarate (246 mg, 1.44 mmol) and heated at reflux for 4 h. After added diethylamine (1.8 ml, 3.6 mmol), the reaction was continued to stir at the same temperature for 4 h, removed the solvent, purified by column to afford the compound **10b**. White solid; Yield: 77.9 %; M.p.: 266-267 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.68 (s, 1H), 8.46 (s, 1H), 7.90 (dd, *J* = 6.7, 2.4 Hz, 1H), 7.55-7.70 (m, 1H), 7.40 (t, *J* = 9.1 Hz, 1H), 4.69 (s, 2H), 4.60-4.76 (m, 2H), 3.93 (t, *J* = 5.6 Hz, 2H), 3.23 (s, 6H), 2.89 (t, *J* = 5.4 Hz, 2H); MS (ESI) m/z (%): 400.2[M-H] $^-$; Anal. calcd. for $C_{19}H_{17}ClFN_5O_2$ (%): C, 56.79; H, 4.26; N, 17.43; Found (%): C, 56.83; H, 4.28; N, 17.33.

4.1.9.3. 3-(4-((3-chloro-4-fluorophenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl)-4-((2-(dimethylamino) ethyl) amino) cyclobut-3-ene-1,2-dione (10c)

Preparation of compound **10c** is followed the procedure for compound **10b**. White solid; Yield: 81.4%; M.p.: 224-226 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.83 (br. s., 1H), 8.45 (s, 1H), 8.20 (br. s., 1H), 8.02 (d, *J* = 6.0 Hz, 1H), 7.63-7.78 (m, 1H), 7.37 (t, *J* = 9.0 Hz, 1H), 4.77 (br. s., 2H), 3.99 (br. s., 2H), 3.78 (d, *J* = 5.4 Hz, 2H), 2.85 (t, *J* = 5.4 Hz, 2H), 2.70 (br. s., 2H), 2.36 (br. s., 6H) ; MS (ESI) m/z (%): 443.2 [M-H] $^-$; Anal. calcd. for $C_{21}H_{22}ClFN_6O_2$ (%): C, 56.69; H, 4.98; N, 18.89; Found (%): C, 56.75; H, 4.92; N, 18.92.

4.1.10.1. 4-((3-chloro-4-fluorophenyl) amino)-*N*, *N*-dimethyl-7,8-dihydropyrid[4,3-*d*]pyrimidine-6(5*H*)-carboxamide (11a)

The solution of compound **7a** (200 mg, 0.72 mmol) and CDI (154 mg, 0.94 mmol) in DMF (2 mL) was heated at 80 °C for 4 h. After added diethylamine (1.8 ml,3.6 mmol), the reaction was continued to stir at the same temperature for 4 h, cooled to r.t., added water (10 mL), extracted by Me-THF (10 mL × 3), washed by brine (10 mL), dried by Na_2SO_4,

removed the solvent, purified by column to afford the compound **11a**. White solid; Yield: 78.4%; M.p.: 201-203 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.54 (s, 1H), 7.67-7.78 (m, 1H), 7.34-7.45 (m, 1H), 7.11 (t, *J* = 8.7 Hz, 1H), 7.05 (br. s., 1H), 4.27 (s, 2H), 3.44-3.54 (m, 2H), 2.95 (m, 2H), 2.91 (s, 6H); MS (ESI) m/z (%): 348.4[M-H] $^-$; Anal. calcd. for $C_{16}H_{17}ClFN_5O$ (%): C, 54.94; H, 4.90; N, 20.02; Found (%): C, 54.91; H, 4.87; N, 20.25.

4.1.10.2. (4-((3-chloro-4-fluorophenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl)(4-methylpiperazin-1-yl) methanone (11b)

Preparation of compound **11b** is followed the procedure for compound **11a.** White solid; Yield: 72.5 %; M.p.: 213-214 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.71 (s, 1H), 8.42 (s, 1H), 7.94 (dd, *J* = 6.8, 2.6 Hz, 1H), 7.65 (ddd, *J* = 9.0, 4.3, 2.7 Hz, 1H), 7.38 (t, *J* = 9.1 Hz, 1H), 4.26 (s, 2H), 3.46 (t, *J* = 5.6 Hz, 2H), 3.28 (br. s., 4H), 2.79 (t, *J* = 5.4 Hz, 2H), 2.45 (br. s., 4H), 2.27 (s, 3H); MS (ESI) m/z (%): 403.5[M-H] $^-$; Anal. calcd. for $C_{19}H_{22}ClFN_6O$ (%): C, 56.36; H, 5.48; N, 20.76; Found (%): C, 56.46; H, 5.52; N, 20.80.

4.1.10.3. 4-((3-chloro-4-fluorophenyl) amino)-*N*-(3-(dimethylamino) propyl)-7,8-dihydropyrid[4,3-*d*]pyrimidine-6(5*H*)-carboxamide (11c)

Preparation of compound **11c** is followed the procedure for compound **10a**. White solid; Yield: 72.8%; M.p.: 182-185 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.77 (s, 1H), 8.45 (s, 1H), 8.08 (dd, *J* = 6.8, 2.6 Hz, 1H), 7.75-7.81 (m, 1H), 7.37 (t, *J* = 9.1 Hz, 1H), 6.98 (t, *J* = 5.3 Hz, 1H), 4.51 (s, 2H), 3.64 (t, *J* = 5.6 Hz, 2H), 3.10-3.17 (m, 2H), 2.66-2.75 (m, 4H), 2.46 (s, 6H), 1.65-1.78 (m, 2H); MS (ESI) m/z (%): 407.3[M+H]$^+$; Anal. calcd. for $C_{19}H_{24}ClFN_6O$ (%): C, 56.09; H, 5.95; N, 20.65; Found (%): C, 56.24; H, 5.92; N, 20.71.

4.1.11.1. Methyl 4-((3-chloro-4-fluorophenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidine-6(5*H*)-carboxylate (12a)

To a solution of compound **7a** (200 mg, 0.72 mmol) and DIPEA (138 mg, 1.08 mmol) in DCM (6 mL) was added dropwise slowly methyl carbonochloridate (82 mg, 0.86 mmol) at 0 ℃. After addition, the reaction solution was allowed to r.t., and stirred for an hour. The reaction solution was washed by Na_2CO_3 (10 mL) aqueous solution and brine (10 mL), then dried by Na_2SO_4, filtered and concentrated, purified by column to afford compound **12a**. Gray solid; Yield: 83.2%; M.p.: 183-185 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.58 (br. s., 1H), 7.80 (br. s., 1H), 7.41 (d, *J* = 8.3 Hz, 1H), 7.06-7.17 (m, 1H), 6.82 (br. s., 1H), 4.53 (br. s., 2H), 3.77(s, 3H), 3.72-3.89 (m, 2H), 2.91 (br. s., 2H); MS (ESI) m/z(%): 335.2[M-H]$^-$; Anal. calcd. for $C_{15}H_{14}ClFN_4O_2$ (%): C, 53.50; H, 4.19; N, 16.64; Found (%): C, 53.71; H, 4.44; N, 16.61.

4.1.11.2. Ethyl 4-((3-chloro-4-fluorophenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidine-6(5*H*)-carboxylate (12b)

Preparation of compound **12b** is followed the procedure for compound **12a**. White solid; Yield: 93.2%; M.p.: 182-184 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.58 (s, 1H), 7.79 (br. s., 1H), 7.42 (d, *J* = 8.2 Hz, 1H), 7.15 (t, *J* = 8.7 Hz, 1H), 4.55 (br. s., 2H), 4.15-4.26 (m, 2H), 3.82 (t, *J* = 5.7 Hz, 2H), 2.96 (t, *J* = 5.7 Hz, 2H), 1.27-1.36 (m, 3H); MS (ESI) m/z(%): 348.7[M-H] $^-$; Anal. calcd. for $C_{16}H_{16}ClFN_4O_2$(%): C, 54.78; H, 4.60; N, 15.97; Found (%): C, 54.87; H, 4.63; N, 16.04.

4.1.11.3. 2-(4-(2-hydroxyethyl) piperazin-1-yl) ethyl 4-((3-chloro-4-fluorophenyl) amino)-7,8-dihydropyrido[4,3-*d*] pyrimidine-6(5*H*)-carboxylate (12c)

Preparation of compound **12c** is followed the procedure for compound **12a**.White solid; Yield: 59.1 %; M.p.: 174-176 ℃; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.41 (s, 1H), 7.86 (d, *J* = 4.4 Hz, 1H), 7.56 (br. s., 1H), 7.22 (t, *J* = 8.9 Hz, 1H), 4.48-4.67 (m, 2H), 4.32 (t, *J* = 5.6 Hz, 2H), 3.83 (br. s., 2H), 3.68 (br. s., 2H), 2.85 (t, *J* = 5.8 Hz, 2H), 2.73 (t, *J* = 5.6

Hz, 2H), 2.29-2.70 (m, 9H); MS (ESI) m/z (%): 476.6[M-H] $^-$; Anal. calcd. for $C_{22}H_{28}ClFN_6O_3$(%): C, 55.17; H, 5.89; N, 17.55; Found (%): C, 55.22; H, 5.90; N, 17.53.

4.1.11.4. 2-(dimethylamino) ethyl 4-((3-chloro-4-fluorophenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidine-6(5*H*)-carboxylate (12d)

Preparation of compound **12d** is followed the procedure for compound **12a**. White solid; Yield: 65.3%; M.p.:179-180 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.40 (s, 1H), 7.86 (d, *J* = 2.9 Hz, 1H), 7.54 (br. s., 1H), 7.22 (t, *J* = 8.9 Hz, 1H), 4.51-4.64 (m, 2H), 4.31 (t, *J* = 5.5 Hz, 2H), 3.81 (d, *J* = 2.9 Hz, 2H), 2.85 (t, *J* = 5.8 Hz, 2H), 2.73 (br. s., 2H), 2.37 (br. s., 6H); MS (ESI) m/z (%):391.6[M-H]$^-$; Anal. calcd. for $C_{18}H_{21}ClFN_5O_2$ (%): C, 54.89; H, 5.37; N, 17.78; Found (%): C, 54.96; H, 5.35; N, 17.79.

4.1.12.1. 1-(4-((3-chloro-4-fluorophenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl) ethanone (13a)

To a solution of compound **7a** (200 mg, 0.72 mmol) and DIPEA (138 mg, 1.08 mmol) in DCM (6 mL) was added dropwise slowly methyl acetyl chloride(67.5 mg, 0.86 mmol) at 0 °C. After addition, the reaction solution was allowed to r.t., and stirred for an hour. The reaction solution was washed by Na_2CO_3 (10 mL) aqueous solution and brine (10 mL), then dried by Na_2SO_4, filtered and concentrated, purified by column to afford compound **13a**. White solid; Yield: 91.7%; M.p.: 159-160 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.54 (s, 1H), 7.94 (br. s., 1H), 7.74 (dd, *J* = 6.5, 2.5 Hz, 1H), 7.39-7.52 (m, 1H), 7.13 (t, *J* = 8.7 Hz, 1H), 4.74 (s, 2H), 3.79 (t, *J* = 5.9 Hz, 2H), 2.98 (t, *J* = 5.6 Hz, 2H), 2.17 (s, 3H); MS (ESI) m/z (%): 318.9[M-H]$^-$; Anal. calcd. for $C_{15}H_{14}ClFN_4O$ (%): C, 56.17; H, 4.40; N, 17.47; Found (%): C, 56.31; H, 4.38; N, 17.52.

4.1.12.2. 1-(4-((3-chloro-4-fluorophenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl) prop-2-en-1-one (13b)

Preparation of compound **13b** is followed the procedure for compound **13a**. White solid; Yield: 81.2%; M.p.: 166-168 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.58 (s, 1H), 7.79 (dd, *J* = 6.4, 2.3 Hz, 1H), 7.44 (dt, *J* = 8.8, 3.4 Hz, 1H), 7.26 (br. s., 1H), 7.12 (t, *J* = 8.7 Hz, 1H), 6.69 (dd, *J* = 16.9, 10.6 Hz, 1H), 6.28 (d, *J* = 16.9 Hz, 1H), 5.80 (dd, *J* = 10.6, 1.5 Hz, 1H), 4.77 (s, 2H), 3.91 (t, *J* = 5.7 Hz, 2H), 2.98 (t, *J* = 5.6 Hz, 2H); ^{13}C NMR (DMSO-d_6, 101 MHz): δ 165.2, 160.9, 156.6, 155.5, 151.9, 137.2, 128.7, 128.3, 123.8, 122.7, 119.2, 116.8, 111.6, 42.3, 38.3, 31.9; MS (ESI) m/z (%): 330.7[M-H] $^-$; IR (KBr) cm^{-1}: 3482.5, 3324.6, 3218.8, 3121.5, 3010.2, 1645.5, 1615.1, 1497.6, 1423.5, 1349.3, 1264.1, 1213.6, 1133.8, 1056.5, 962.3, 923.4, 879.4, 787.8, 692.9, 563.6, 543.7, 515.1, 501.8; Anal. calcd. for $C_{16}H_{14}ClFN_4O$ (%): C, 57.75; H, 4.24; N, 16.84; Found (%): C, 57.81; H, 4.27; N, 16.77.

4.1.12.3. 1-(4-((3-chloro-4-fluorophenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl) prop-2-yn-1-one (13c)

To a mixture of compound **7a** (200 mg, 0.72 mmol) and propargylic acid (75 mg, 1.08 mmol) in CH_3CN (6 mL) was added EDCI.HCl (208 mg, 1.08 mmol) and HOBT.H_2O (166 mg, 1.08 mmol), then added dropwise DIPEA (186 mg, 2.88 mmol) at r.t.. After addition, the reaction solution was heated at 60 °C for 6 hour. The reaction solution was added water (10 mL), extracted with EtOAc (10 mL × 3), washed by brine (10 mL), then dried by Na_2SO_4, filtered and concentrated, purified by column to afford compound **13c**.White solid; Yield: 58.4%; M.p.: 171-174 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.80 (br. s., 1H), 8.31-8.55 (m, 1H), 7.96 (dd, *J* = 6.8, 2.6 Hz, 1H), 7.56-7.73 (m, 1H),

7.13-7.43 (m, 1H), 4.53-4.86 (m, 2H), 4.60 (s, 1H), 3.71-4.14 (m, 2H), 2.65-2.91 (m, 2H); MS (ESI) m/z (%): 329.2[M-H] $^-$; Anal. calcd. for $C_{16}H_{12}ClFN_4O$ (%): C, 58.10; H, 3.66; N, 16.94; Found (%): C, 58.27; H, 3.65; N, 16.89.

4.1.12.4. 1-(4-((3-chloro-4-fluorophenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl) propan-1-one (13d)

Preparation of compound **13d** is followed the procedure for compound **13c**. White solid; Yield: 93.7%; M.p.: 163-166 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.73 (br. s., 1H), 8.43 (s, 1H), 7.80-8.05 (m, 1H), 7.57-7.75 (m, 1H), 7.27-7.49 (m, 1H), 4.43-4.67 (m, 2H), 3.63-3.85 (m, 2H), 2.61-2.87 (m, 2H), 2.35-2.57 (m, 3H), 1.05 (t, *J* = 7.3 Hz, 3H); MS (ESI) m/z (%): 333.3[M-H] $^-$; Anal. calcd. for $C_{16}H_{16}ClFN_4O$ (%): C, 57.40; H, 4.82; N, 16.74; Found (%): C, 57.55; H, 4.79; N, 16.81.

4.1.12.5. 1-(4-((3-chloro-4-fluorophenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl)-2-(dimethylamino) ethanone (13e)

Preparation of compound **13e** is followed the procedure for compound **13c**.White solid; Yield: 84.7%; M.p.: 165-168 ℃; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.34-8.40 (m, 1H), 7.78-7.90 (m, 1H), 7.44-7.60 (m, 1H), 7.08-7.22 (m, 1H), 4.56-4.66 (m, 2H), 3.87 (t, *J* = 5.5 Hz, 2H), 3.34-3.45 (m, 2H), 2.74-2.92 (m, 2H), 2.34 (s, 6H); MS (ESI) m/z (%): 362.4[M-H] $^-$; Anal. calcd. for $C_{17}H_{19}ClFN_5O$(%): C, 56.12; H, 5.26; N, 19.25; Found (%): C, 56.33; H, 5.30; N, 19.41.

4.1.12.6. 1-(4-((3-chloro-4-fluorophenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl)-4-(dimethylamino) butan-1-one (a1)

Preparation of compound **a1** is followed the procedure for compound **13e**. White solid; Yield: 89.2%; M.p.: 165-167 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.09-9.48 (m, 1H), 8.59 (s, 1H), 7.91 (dd, *J* = 6.8, 2.6 Hz, 1H), 7.65 (ddd, *J* = 9.0, 4.3, 2.7 Hz, 1H), 7.40 (t, *J* = 9.0 Hz, 1H), 4.62 (s, 2H), 3.77-3.81 (m, 2H), 3.07-3.16 (m, 2H), 2.81 (s, 6H), 2.61 (t, *J* = 7.0 Hz, 2H), 2.51 (dt, *J* = 3.8, 1.8 Hz, 2H), 1.81-2.01 (m, 2H); MS (ESI) m/z (%): 390.5 [M-H] $^-$; Anal. calcd. for $C_{19}H_{23}ClFN_5O$(%): C, 58.23; H, 5.92; N, 17.87; Found (%): C, 58.41; H, 5.99; N, 17,64.

4.1.12.7. 1-(4-((3-(trifluoromethyl) phenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl) prop-2-en-1-one (13f)

Preparation of compound **13f** is followed the procedure for compound **13a**. White solid; Yield: 82.0%; M.p.: 167-169 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.62 (s, 1H), 7.96 (br. s., 1H), 7.84 (d, *J* = 8.1 Hz, 1H), 7.48 (t, *J* = 7.9 Hz, 1H), 7.38 (d, *J* = 7.6 Hz, 1H), 7.21 (br. s., 1H), 6.70 (dd, *J* = 16.8, 10.6 Hz, 1H), 6.32 (d, *J* = 16.6 Hz, 1H), 5.81 (dd, *J* = 10.6, 1.3 Hz, 1H), 4.80 (s, 2H), 3.92 (t, *J* = 5.6 Hz, 2H), 3.00 (t, *J* = 5.6 Hz, 2H); MS (ESI) m/z (%): 347.1[M-H]$^-$; Anal. calcd. for $C_{17}H_{15}F_3N_4O$ (%): C, 58.62; H, 4.34; N, 16.08; Found (%): C, 58.67; H, 4.29; N, 16.13.

4.1.12.8. 1-(4-((3-bromophenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl) prop-2-en-1-one (13g)

Preparation of compound **13g** is followed the procedure for compound **13a**. White solid; Yield: 92.4 %; M.p.: 154-155 ℃; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.61-8.93 (m, 1H), 8.51 (s, 1H), 7.96-8.18 (m, 1H), 7.79 (d, *J* = 8.1 Hz, 1H), 7.22-7.47 (m, 2H), 7.00 (dt, *J* = 16.4, 10.9 Hz, 1H), 6.09-6.39 (m, 1H), 5.63-5.94 (m, 1H), 4.63-4.86 (m, 2H), 3.80-4.01 (m, 2H), 2.70-2.91 (m, 2H); ^{13}C NMR (DMSO-d_6, 101 MHz): δ 165.1, 160.7, 156.1,155.5 141.8, 130.7, 128.7, 128.3, 125.8, 124.4, 121.5, 121.0, 110.1, 42.3, 37.9, 32.5; MS (ESI) m/z (%): 357.4[M-H]$^-$; IR (KBr) cm^{-1}: 3424.6, 3286.8, 3185.2, 3106.1, 2999.6, 2923.2, 2807.1, 1645.5, 1617.9, 1575.1, 1511.3, 1475.6, 1428.0, 1346.0, 1242.8, 1228.9, 1139.3, 1072.1, 921.6, 864.8, 782.0, 683.4, 561.9, 475.9; Anal. calcd. for $C_{16}H_{15}BrN_4O$(%): C, 53.50; H, 4.21; N, 15.60; Found (%): C, 53.57; H, 4.27; N, 15.53.

4.1.12.9. 1-(4-((2-methyl-5-nitrophenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl) prop-2-en-1-one (13h)

Preparation of compound **13h** is followed the procedure for compound **13a**. Light brown solid; Yield: 81.7%; M.p.: 193-196 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.49 (s, 1H), 8.40 (br. s., 1H), 7.93-8.05 (m, 1H), 7.66 (br. s., 1H), 7.41 (d, *J* = 8.3 Hz, 1H), 7.33 (br. s., 1H), 6.50-6.64 (m, 1H), 6.02 (d, *J* = 16.8 Hz, 1H), 5.60 (d, *J* = 10.6 Hz, 1H), 4.86 (br. s., 2H), 3.90 (br. s., 2H), 2.96 (t, *J* = 5.0 Hz, 2H), 2.34 (s, 3H); MS (ESI) m/z (%):338.4[M-H]$^-$; Anal. calcd. for $C_{17}H_{17}N_5O_3$ (%): C, 60.17; H, 5.05; N, 20.64; Found (%): C, 60.15; H, 5.14; N, 20.59.

4.1.12.10. 1-(4-((3, 4-dimethylphenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl) prop-2-en-1-one (13i)

Preparation of compound **13i** is followed the procedure for compound **13a**. Light yellow solid; Yield: 87.0%; M.p.: 145-147 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.53 (s, 1H), 7.22-7.27 (m, 2H), 7.11 (d, *J* = 7.9 Hz, 1H), 6.83 (br. s., 1H), 6.67 (dd, *J* = 16.8, 10.6 Hz, 1H), 6.32 (d, *J* = 16.6 Hz, 1H), 5.78 (dd, *J* = 10.6, 1.1 Hz, 1H), 4.69 (s, 2H), 3.87 (t, *J* = 5.6 Hz, 2H), 2.92 (t, *J* = 5.6 Hz, 2H), 2.26 (s, 3H), 2.24 (s, 3H); MS (ESI) m/z(%): 307.4[M-H]$^-$; Anal. calcd. for $C_{18}H_{20}N_4O$ (%): C, 70.11; H, 6.54; N, 18.17; Found (%): C, 70.23; H, 6.49; N, 18.21.

4.1.12.11. 1-(4-((3-bromo-5-methoxyphenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl) prop-2-en-1-one (13j)

Preparation of compound **13j** is followed the procedure for compound **13a**. White solid; Yield: 77.3%; M.p.: 169-161 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.82 (br. s., 1H), 8.67 (s, 1H), 7.16 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.05 (br. s., 1H), 6.79 (d, *J* = 8.7 Hz, 1H), 6.70 (dd, *J* = 16.8, 10.6 Hz, 1H), 6.41 (dd, *J* = 16.8, 1.3 Hz, 1H), 5.83 (dd, *J* = 10.6, 1.3 Hz, 1H), 4.69 (br. s., 2H), 3.94 (s, 3H), 3.90 (br. s., 2H), 2.97 (t, *J* = 5.3 Hz, 2H); MS (ESI) m/z (%): 387.2[M-H]$^-$; Anal. calcd. for $C_{17}H_{17}BrN_4O_2$ (%): C, 52.46; H, 4.40; N, 14.39; Found (%): C, 52.41; H, 4.39; N, 14.48.

4.1.12.12. 1-(4-((4-bromophenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl) prop-2-en-1-one (13k)

Preparation of compound **13k** is followed the procedure for compound **13a**. White solid; Yield: 90.1 %; M.p.:155-158 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.54 (br. s., 1H), 8.07 (br. s., 1H), 7.36-7.61 (m, 4H), 6.65 (dd, *J* = 16.1, 10.8 Hz, 1H), 6.20 (d, *J* = 16.6 Hz, 1H), 5.77 (d, *J* = 10.5 Hz, 1H), 4.82 (br. s., 2H), 3.88 (br. s., 2H), 3.02 (br. s., 2H); MS (ESI) m/z(%):357.2[M-H]$^-$; Anal. calcd. for $C_{16}H_{15}BrN_4O$(%): C, 53.50; H, 4.21; N, 15.60; Found (%): C, 53.47; H, 4.23; N, 15.67.

4.1.12.13. 1-(4-((2, 4-dichlorophenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl) prop-2-en-1-one (13l)

Preparation of compound **13l** is followed the procedure for compound **13a**. White solid; Yield: 87.5 %; M.p.:181-183 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.63 (s, 1H), 8.42 (d, *J* = 8.4 Hz, 1H), 7.46 (d, *J* = 2.3 Hz, 1H), 7.32 (dd, *J* = 8.9, 2.1 Hz, 1H), 6.84 (br. s., 1H), 6.70 (dd, *J* = 16.8, 10.5 Hz, 1H), 6.40 (d, *J* = 16.8 Hz, 1H), 5.83 (d, *J* = 10.5 Hz, 1H), 4.75 (br. s., 2H), 3.93 (br. s., 2H), 3.00 (t, *J* = 5.1 Hz, 2H); MS (ESI) m/z (%): 347.1[M-H]$^-$; Anal. calcd. for $C_{16}H_{14}C_{l2}N_4O$ (%): C, 55.03; H, 4.04; N, 16.04; Found (%): C, 55.19; H, 4.07; N, 16.11.

4.1.12.14. 1-(4-((2-chloro-5-(trifluoromethyl) phenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl) prop-2-en-1-one (13m)

Preparation of compound **13m** is followed the procedure for compound **13a**. White solid; Yield: 91.3%; M.p.: 185-187

°C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.95 (br. s., 1H), 8.70 (s, 1H), 7.56 (d, *J* = 8.3 Hz, 1H), 7.32 (d, *J* = 8.2 Hz, 1H), 7.03 (br. s., 1H), 6.70 (dd, *J* = 16.8, 10.6 Hz, 1H), 6.40 (dd, *J* = 16.8, 1.2 Hz, 1H), 5.83 (d, *J* = 10.5 Hz, 1H), 4.78 (br. s., 2H), 3.93 (br. s., 2H), 3.01 (t, *J* = 5.4 Hz, 2H); MS (ESI) m/z (%): 381.4[M-H]$^-$; Anal. calcd. for $C_{17}H_{14}ClF_3N_4O$ (%): C, 53.34; H, 3.69; N, 14.64; Found (%): C, 53.41; H, 3.71; N, 14.58.

4.1.12.14. 1-(4-((3, 5-dichlorophenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl) prop-2-en-1-one (13n)

Preparation of compound **13n** is followed the procedure for compound **13a**. White solid; Yield: 79.3 %; M.p.: 177-179 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.50 (br. s., 1H), 7.78 (s, 2H), 7.14 (s, 1H), 6.92 (dd, *J* = 16.7, 10.7 Hz, 1H), 6.31 (d, *J* = 16.8 Hz, 1H), 5.85 (d, *J* = 10.8 Hz, 1H), 4.73 (br. s., 2H), 3.98 (t, *J* = 5.9 Hz, 2H), 2.83-2.99 (m, 2H); MS (ESI) m/z(%): 347.2[M-H]$^-$; Anal. calcd. for $C_{16}H_{14}C_{l2}N_4O$ (%): C, 55.03; H, 4.04; N, 16.04; Found (%): C, 55.23; H, 4.07; N, 16.11.

4.1.12.15. 1-(4-((3-chloro-4-(pyridin-2-ylmethoxy) phenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl) prop-2-en-1-one (13o)

Preparation of compound **13o** is followed the procedure for compound **13a**. Light gray solid; Yield: 90.5%; M.p.: 178-180 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.62 (d, *J* = 4.4 Hz, 1H), 8.47-8.77 (m, 1H), 8.43 (br. s., 1H), 7.76-7.99 (m, 2H), 7.61 (d, *J* = 7.6 Hz, 2H), 7.33-7.43 (m, 1H), 7.16-7.32 (m, 1H), 6.82-7.08 (m, 1H), 6.11-6.35 (m, 1H), 5.63-5.88 (m, 1H), 5.30 (s, 2H), 4.55-4.81 (m, 2H), 3.74-4.02 (m, 2H), 2.64-2.94 (m, 2H); ^{13}C NMR (DMSO-d_6, 101MHz): δ 165.4, 160.6, 160.5, 156.9, 155.6, 149.6, 137.5, 134.0, 128.9, 128.3, 124.5, 123.5, 121.8, 121.2, 114.5, 111.2, 110.6, 71.6, 42.3, 38.5, 32.5, 31.2; MS (ESI) m/z (%): 420.3[M-H]$^-$; IR (KBr) cm^{-1}: 3491.9, 3159.5, 3094.0, 2923.4, 2852.9, 1643.3, 1592.0, 1501.5, 1453.7, 1423.1, 1348.2, 1302.3, 1270.5, 1220.1, 1133.9, 1097.0, 1065.8, 1037.5, 977.9, 923.5, 864.0, 805.1, 747.7, 664.3, 623.7, 575.0, 500.0; Anal. calcd. for $C_{22}H_{20}ClN_5O_2$(%): C, 62.63; H, 4.78; N, 16.60; Found (%): C, 62.71; H, 4.83; N, 16.70.

4.1.12.16. 1-(4-((3-chloro-4-((3-fluorobenzyl) oxy) phenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl) prop-2-en-1-one (13p)

Preparation of compound **13p** is followed the procedure for compound **13a**. White solid; Yield: 77.1%; M.p.: 195-197 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.53 (s, 1H), 7.65 (d, *J* = 2.2 Hz, 1H), 7.42 (s, 1H), 7.32-7.40 (m, 2H), 7.18-7.25 (m, 2H), 6.98-7.06 (m, 1H), 6.91 (d, *J* = 8.9 Hz, 1H), 6.65 (dd, *J* = 16.8, 10.6 Hz, 1H), 6.23 (d, *J* = 16.8 Hz, 1H), 5.75 (dd, *J* = 10.7, 1.2 Hz, 1H), 5.13 (s, 2H), 4.77 (s, 2H), 3.88 (t, *J* = 5.6 Hz, 2H), 2.94 (t, *J* = 5.4 Hz, 2H); ^{13}C NMR (CD_3OD, 101 MHz): δ 166.0, 163.9, 161.4, 159.1, 156.7, 155.7, 150.5, 138.8, 132.2, 129.8, 128.7, 126.7, 124.8, 123.0, 122.0, 114.5, 113.9, 113.6, 109.5, 70.1, 42.4, 39.2, 30.6; MS (ESI) m/z (%): 437.3[M-H]$^-$; IR (KBr) cm^{-1}: 3422.7, 3321.7, 2923.5, 2954.3, 1643.6, 1605.0, 1498.4, 1447.9, 1423.2, 1384.3, 1269.8, 1251.3, 1218.1, 1136.0, 1060.0, 926.0, 859.5, 804.8, 780.3, 682.0, 573.4, 521.8; Anal. calcd. for $C_{23}H_{20}ClFN_4O_2$(%): C, 62.94; H, 4.59; N, 12.77; Found (%): C, 62.98; H, 4.64; N, 12.69.

4.1.12.17. 1-(4-((3-fluoro-4-(pyridin-2-ylmethoxy) phenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl) prop-2-en-1-one (13q)

Preparation of compound **13q** is followed the procedure for compound **13a**. White solid; Yield: 83.3%; M.p.: 187-189 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.60 (d, *J* = 4.3 Hz, 1H), 8.55 (s, 1H), 7.75 (t, *J* = 7.2 Hz, 1H), 7.50-7.63 (m, 2H), 7.25 (d, *J* = 6.8 Hz, 1H), 7.16 (d, *J* = 8.7 Hz, 1H), 6.99 (t, *J* = 8.9 Hz, 1H), 6.66 (dd, *J* = 16.5, 10.8 Hz, 1H), 6.27 (d, *J* = 16.8 Hz, 1H), 5.77 (d, *J* = 11.1 Hz, 1H), 5.27 (s, 2H), 4.73 (br. s., 2H), 3.89 (br. s., 2H), 2.99 (br. s., 2H); ^{13}C NMR (CD_3OD, 101 MHz): δ 165.9, 159.1, 156.5, 155.6, 153.2, 150.7, 148.8, 142.8, 136.6, 132.0, 128.7, 126.6, 122.5, 121.0, 117.7, 115.1, 111.3, 109.5, 71.8, 42.3, 39.1, 31.7; MS (ESI) m/z (%): 406.1[M+H]$^+$; IR (KBr) cm^{-1}: 3425.3, 3312.9,

3113.4, 2922.9, 1645.9, 1645.9, 1615.5, 1574.9, 1522.5, 1449.1, 1433.7, 1300.7, 1253.3, 1216.4, 1129.1, 1094.9, 1052.7, 982.9, 906.7, 867.5, 807.7, 761.0, 660.6, 559.8, 470.3; Anal. calcd. for $C_{22}H_{20}FN_5O_2$ (%): C, 65.17; H, 4.97; N, 17.27; Found (%): C, 65.21; H, 4.54; N, 17.23.

4.1.12.18. 1-(4-((3-chloro-4-(3-(trifluoromethyl) phenoxy) phenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl) prop-2-en-1-one (13r)

Preparation of compound **13r** is followed the procedure for compound **13a**. White solid; Yield: 76.8 %; M.p.: 247-248 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.57 (s, 1H), 8.25 (s, 1H), 7.87 (d, *J* = 2.3 Hz, 1H), 7.59 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.37-7.43 (m, 1H), 7.31 (d, *J* = 7.7 Hz, 1H), 7.16 (s, 1H), 7.09 (d, *J* = 8.1 Hz, 1H), 7.01 (d, *J* = 8.8 Hz, 1H), 6.67 (dd, *J* = 16.9, 10.8 Hz, 1H), 6.19 (d, *J* = 16.9 Hz, 1H), 5.75 (d, *J* = 11.5 Hz, 1H), 4.88 (s, 2H), 3.89 (t, *J* = 5.6 Hz, 2H), 2.92-3.00 (m, 2H); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 166.6, 159.8, 158.0, 157.1, 156.0, 147.1, 136.8, 132.3, 130.5, 129.3, 127.4, 126.6, 124.6, 122.3, 122.1, 120.3, 119.7, 114.0, 110.8, 43.0, 40.1, 32.3; MS (ESI) m/z (%): 473.0[M-H]$^-$; Anal. calcd. for $C_{23}H_{18}ClF_3N_4O_2$(%): C, 58.17; H, 3.82; N, 11.80; Found (%): C, 58.21; H, 3.78; N, 11.74.

4.1.12.19. 1-(4-((3-chloro-4-((3-fluorobenzyl) thio) phenyl) amino)-7,8-dihydropyrido[4,3-*d*]pyrimidin-6(5*H*)-yl) prop-2-en-1-one (13s)

Preparation of compound **13s** is followed the procedure for compound **13a**. Light yellow solid; Yield: 75.7%; M.p.: 223-226 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.44 (br. s., 1H), 7.84 (br. s., 1H), 7.47 (d, *J* = 8.6 Hz, 1H), 7.19-7.32 (m, 3H), 7.10 (d, *J* = 7.7 Hz, 1H), 7.04 (d, *J* = 9.9 Hz, 1H), 6.81-6.97 (m, 2H), 6.29 (d, *J* = 16.8 Hz, 1H), 5.83 (d, *J* = 10.6 Hz, 1H), 4.66 (br. s., 2H), 4.09-4.17 (m, 2H), 3.94 (t, *J* = 5.9 Hz, 2H), 2.79-2.96 (m, 2H); ^{13}C NMR (CD_3OD, 101 MHz): δ 167.8, 165.1, 162.5, 158.9, 155.2, 141.3, 139.6, 132.6, 131.1, 130.6, 129.9, 129.4, 128.8, 125.8, 124.3, 122.0, 116.5, 114.9, 112.3, 43.2, 39.9, 38.2, 31.9; MS (ESI) m/z (%): 455.2[M+H]$^+$; Anal. calcd. for $C_{20}H_{18}ClFN_4S$ (%): C, 59.92; H, 4.53; N, 13.98; Found (%): C, 60.03; H, 4.50; N, 13.92.

4.1.12.20. *N*-(4-((6-acryloyl-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4-yl) amino)-2-chlorophenyl)-3-fluoro benzamide (13t)

Preparation of compound **13t** is followed the procedure for compound **13a**. White solid; Yield: 93.1 %; M.p.: 242-243 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.18 (s, 1H), 8.61-9.02 (m, 1H), 8.51 (br. s., 1H), 7.97-8.09 (m, 1H), 7.89 (d, *J* = 7.8 Hz, 1H), 7.82 (d, *J* = 9.5 Hz, 1H), 7.76 (d, *J* = 8.6 Hz, 1H), 7.63 (td, *J* = 7.9, 6.1 Hz, 1H), 7.43-7.57 (m, 2H), 6.85-7.12 (m, 1H), 6.11-6.36 (m, 1H), 5.57-5.94 (m, 1H), 4.55-4.81 (m, 2H), 3.57-4.00 (m, 2H), 2.71-2.92 (m, 2H); ^{13}C NMR (DMSO-d_6, 101 MHz): δ 165.4, 164.6, 163.6, 161.2, 160.9, 156.6, 155.5, 139.4, 136.7, 131.1, 129.9, 129.0, 128.6, 124.3, 122.6, 121.1, 119.1, 115.0, 114.8, 111.8, 42.2, 38.1, 32.5; MS (ESI) m/z (%): 450.2[M-H]$^-$; Anal. calcd. for $C_{23}H_{19}ClFN_5O_2$(%): C, 61.13; H, 4.24; N, 15.50; Found (%): C, 61.17; H, 4.23; N, 15.60.

4.1.12.21. 1-(4-((6-acryloyl-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidin-4-yl) amino)-2-chlorophenyl)-3-(3-fluoro phenyl) urea (13u)

Preparation of compound **13u** is followed the procedure for compound **13a**. White solid; Yield: 84.5 %; M.p.: 274-276 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.45 (br. s., 1H), 8.09 (d, *J* = 8.9 Hz, 1H), 7.81-7.88 (m, 1H), 7.43-7.53 (m, 2H), 7.28 (td, *J* = 8.1, 6.7 Hz, 1H), 7.10 (dd, *J* = 8.2, 1.2 Hz, 1H), 6.87-7.01 (m, 1H), 6.75 (td, *J* = 8.3, 2.1 Hz, 1H), 6.32 (d, *J* = 16.5 Hz, 1H), 5.85 (d, *J* = 10.6 Hz, 1H), 4.71 (br. s., 2H), 3.98 (t, *J* = 5.8 Hz, 2H), 2.82-2.97 (m, 2H); MS (ESI) m/z

(%):465.2[M-H]$^-$; Anal. calcd. for $C_{23}H_{20}ClFN_6O_2$(%): C, 59.17; H, 4.32; N, 18.00; Found (%): C, 59.28; H, 4.29; N, 18.15.

4.1.13. Ethyl 3-oxopiperidine-4-carboxylate hydrochloride (15)

Preparation of compound **15** is followed the procedure for compound **2**. Light brown solid; Yield: 91.4%; MS (ESI) m/z (%): 172.2[M+H]$^+$.

4.1.14. 1-benzyl 4-ethyl 3-oxopiperidine-1, 4-dicarboxylate (16)

Preparation of compound **16** is followed the procedure for compound **3**. White solid; Yield: 87.3 %; ^{1}H NMR (METHANOL-d_4, 400 MHz): δ 7.20-7.48 (m, 6H), 5.13 (s, 2H), 4.21 (q, J = 7.1 Hz, 2H), 4.05 (br. s., 2H), 3.52 (br. s., 2H), 2.28 (br. s., 2H), 1.28 (t, J = 7.1 Hz, 3H); MS (ESI) m/z (%): 304.0[M-H]$^-$.

4.1.15. Benzyl 4-hydroxy-5,6-dihydropyrido[3,4-*d*]pyrimidine-7(8*H*)-carboxylate (17)

Preparation of compound **17** is followed the procedure for compound **4**. White solid; Yield: 91.6%; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.39 (br. s., 1H), 8.06 (s, 1H), 7.28-7.45 (m, 5H), 5.12 (s, 2H), 4.31 (br. s., 2H), 3.60 (br. s., 2H), 2.43 (t, J = 5.7 Hz, 2H); MS (ESI) m/z (%): 286.1[M+H]$^+$.

4.1.16. Benzyl 4-chloro-5,6-dihydropyrido[3,4-*d*]pyrimidine-7(8*H*)-carboxylate (18)

Preparation of compound **18** is followed the procedure for compound **5**. Brown oil; Yield: 96%; MS (ESI) m/z (%): 303.7[M+H]$^+$.

4.1.17.1. Benzyl 4-(3-chloro-4-fluorophenylamino)-5,6-dihydropyrido[3,4-*d*]pyrimidine-7(8*H*)-carboxylate (19a)

Preparation of compound **19a** is followed the procedure for compound **6a**. White solid; Yield: 73.6 %; M.p.: 184-185 °C; MS (ESI) m/z (%): 413.1[M+H]$^+$.

4.1.17.2. Benzyl 4-((3-chloro-4-((3-fluorobenzyl) oxy) phenyl) amino)-5,6-dihydropyrido[3,4-*d*]pyrimidine-7(8*H*)-carboxylate (19b)

Preparation of compound **19b** is followed the procedure for compound **6a**. White solid; Yield: 77.1 %; M.p.: 196-197 °C; MS (ESI) m/z (%): 519.2[M+H]$^+$.

4.1.18.1. *N*-(3-chloro-4-fluorophenyl)-5,6,7,8-tetrahydropyrido[3,4-*d*]pyrimidin-4-amine (20a)

Preparation of compound **20a** is followed the procedure for compound **7a**. Gray solid; Yield: 78.3 %; M.p.: 177-179 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.36 (s, 1H), 7.88 (dd, J = 6.7, 2.6 Hz, 1H), 7.55 (ddd, J = 8.9, 4.0, 2.7 Hz, 1H), 7.20 (t, J = 8.9 Hz, 1H), 3.84 (s, 2H), 3.17 (t, J = 5.9 Hz, 2H), 2.62 (t, J = 5.7 Hz, 2H); MS (ESI) m/z (%): 278.6[M+H]$^+$; Anal. calcd. for $C_{13}H_{12}ClFN_4$(%): C, 56.02; H, 4.34; N, 20.10; Found (%): C, 56.18; H, 4.33; N, 20.16.

4.1.18.2. *N*-(3-chloro-4-((3-fluorobenzyl) oxy) phenyl)-5,6,7,8-tetrahydropyrido[3,4-*d*]pyrimidin-4-amine (20b)

Preparation of compound **20b** is followed the procedure for compound **7a**. White solid; Yield: 69.6%; M.p.: 180-181 °C; MS (ESI) m/z (%): 385.1[M+H]$^+$.

4.1.19. *N*-(3-chloro-4-fluorophenyl)-7-(methylsulfonyl)-5,6,7,8-tetrahydropyrido[3,4-*d*]pyrimidin-4-amine (21)

Preparation of compound **21** is followed the procedure for compound **8a**. White solid; Yield: 79.6 %; M.p.: 223-225 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.42 (s, 1H), 7.89 (dd, *J* = 6.7, 2.6 Hz, 1H), 7.50-7.62 (m, 1H), 7.22 (t, *J* = 9.0 Hz, 1H), 4.33 (s, 2H), 3.64 (t, *J* = 5.9 Hz, 2H), 2.98 (s, 3H), 2.80 (t, *J* = 5.7 Hz, 2H); MS (ESI) m/z (%): 356.5$[M+H]^+$; Anal. calcd. for $C_{14}H_{14}ClFN_4O_2S$ (%): C, 47.13; H, 3.95; N, 15.70; Found (%): C, 47.38; H, 3.84; N, 15.90.

4.1.20. 4-((3-chloro-4-fluorophenyl) amino)-*N*, *N*-dimethyl-5,6-dihydropyrido[3,4-*d*]pyrimidine-7(8H)-carboxamide (22)

Preparation of compound **22** is followed the procedure for compound **11a**. White solid; Yield: 67.9 %; M.p.:188-190 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.52 (s, 1H), 7.76 (dd, *J* = 6.5, 2.6 Hz, 1H), 7.55-7.71 (m, 1H), 7.45-7.53 (m, 1H), 7.28 (s, 1H), 7.13 (t, *J* = 8.7 Hz, 1H), 4.45 (s, 2H), 3.61 (t, *J* = 5.6 Hz, 2H), 2.89 (s, 6H), 2.82 (t, *J* = 5.4 Hz, 2H); MS (ESI) m/z (%): 348.3$[M-H]^-$; Anal. calcd. for $C_{16}H_{17}ClFN_5O$ (%): C, 54.94; H, 4.90; N, 20.02; Found (%): C, 55.18; H, 4.85; N, 20.13.

4.1.21. Methyl 4-((3-chloro-4-fluorophenyl) amino)-5,6-dihydropyrido[3,4-*d*]pyrimidine-7(8*H*)-carboxylate (23)

Preparation of compound **23** is followed the procedure for compound **13a**. White solid; Yield: 86.5%; M.p.:185-187 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.53 (s, 1H), 7.80 (d, *J* = 4.8 Hz, 1H), 7.40-7.57 (m, 1H), 7.14 (t, *J* = 8.7 Hz, 1H), 4.66 (s, 2H), 3.84 (br. s., 2H), 3.76 (s, 3H), 2.77 (br. s., 2H); MS (ESI) m/z (%): 335.4$[M-H]^-$; Anal. calcd. for $C_{15}H_{14}ClFN_4O_2$(%): C, 53.50; H, 4.19; N, 16.64; Found (%): C, 53.64; H, 4.25; N, 16.65.

4.1.22.1. 1-(4-((3-chloro-4-fluorophenyl) amino)-5,6-dihydropyrido[3,4-*d*]pyrimidin-7(8*H*)-yl)-2-(dimethylamino) ethanone (24a)

Preparation of compound **24a** is followed the procedure for compound **13c**. White solid; Yield: 79.8%; M.p.: 176-178 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.76 (br. s., 1H), 8.46 (s, 1H), 7.99-8.08 (m, 1H), 7.63-7.75 (m, 1H), 7.38 (t, *J* = 9.1 Hz, 1H), 4.69 (s, 1H), 4.48 (s, 1H), 3.85 (t, *J* = 5.6 Hz, 1H), 3.74-3.79 (m, 1H), 3.11-3.21 (m, 2H), 2.58-2.80 (m, 2H), 2.12-2.24 (m, 6H); MS (ESI) m/z (%): 364.2$[M+H]^+$; Anal. calcd. for $C_{17}H_{19}ClFN_5O$ (%): C, 56.12; H, 5.26; N, 19.25; Found (%): C, 56.29; H, 5.37; N, 19.41.

4.1.23.1. 1-(4-((3-chloro-4-fluorophenyl) amino)-5,6-dihydropyrido[3,4-*d*]pyrimidin-7(8*H*)-yl) prop-2-en-1-one (24b)

Preparation of compound **24b** is followed the procedure for compound **13a**. White solid; Yield: 83.2%; M.p.: 177-179 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.69 (d, *J* = 8.2 Hz, 1H), 8.46 (s, 1H), 7.99 (d, *J* = 4.2 Hz, 1H), 7.60-7.72 (m, 1H), 7.38 (t, *J* = 9.1 Hz, 1H), 6.79-7.05 (m, 1H), 6.09-6.23 (m, 1H), 5.64-5.83 (m, 1H), 4.52-4.70 (m, 2H), 3.77-3.99 (m, 2H), 2.69 (d, *J* = 18.2 Hz, 2H); MS (ESI) m/z (%): 332.5$[M+H]^+$; Anal. calcd. for $C_{16}H_{14}ClFN_4O$ (%): C, 57.75; H, 4.24; N, 16.84; Found (%): C, 57.81; H, 4.23; N, 16.89.

4.1.23.2. 1-(4-((3-chloro-4-((3-fluorobenzyl) oxy) phenyl) amino)-5,6-dihydropyrido[3,4-*d*]pyrimidin-7(8*H*)-yl) prop-2-en-1-one (24c)

Preparation of compound **24c** is followed the procedure for compound **13a**. White solid; Yield: 76.0%; M.p.: 194-196 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.34 (s, 1H), 7.71 (br. s., 1H), 7.35-7.46 (m, 2H), 7.28 (d, *J* = 7.7 Hz, 1H), 7.23 (d, *J* = 9.9 Hz, 1H), 7.09 (d, *J* = 8.9 Hz, 1H), 7.04 (td, *J* = 8.6, 2.4 Hz, 1H), 6.74-6.94 (m, 1H), 6.27 (d, *J* = 16.5 Hz, 1H), 5.81 (d, *J* = 10.8 Hz, 1H), 5.18 (s, 2H), 4.68 (s, 2H), 3.97 (t, *J* = 5.9 Hz, 2H), 2.70 (m, 2H); MS (ESI) m/z(%): 439.1$[M+H]^+$; Anal. calcd. for $C_{23}H_{20}ClFN_4O_2$ (%): C, 62.94; H, 4.59; N, 12.77; Found (%): C, 62.87; H, 4.57; N, 12.90.

4.2 Cytotoxicity assay

The anti-proliferative activities of compounds **7a-24d** were evaluated against HT29, A549, H460 and H1975 cell lines by the standard MTT assay *in vitro*, with Gefitinib as the positive control.[38] The cancer cell lines were cultured in minimum essential medium (MEM) supplement with 10% fetal bovine serum (FBS). Approximate 4×10^3 cells, suspended in MEM medium, were plated into each well of a 96-well plate and incubated in 5% CO_2 at 37 ºC for 24 h. The tested compounds at the indicated final concentrations were added to the culture medium and incubated for 72 h. Fresh MTT was added to each well at the terminal concentration of 5 μg/mL, and incubated with cells at 37 ºC for 4 h. The formazan crystals in each well were dissolved in 100 μL DMSO, and the absorbency at 492 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength) was measured with an ELISA reader. All of the compounds were tested three times in each of the cell lines. The results, expressed as IC_{50} (inhibitory concentration 50%), were the averages of three determinations and calculated by the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

4.3 In vitro enzymatic activity assay

The EGFR and HER2 kinase assays of tested compounds were performed by homogeneous time-resolved fluorescence (HTRF) assay as previously reported protocol.[39] Briefly, 20 μg/mL poly (Glu, Tyr) 4:1 (Sigma) was preloaded as a substrate in 384-well plates. Then 50 μL of 10 mM ATP (Invitrogen) solution diluted in kinase reaction buffer (50 mM HEPES, Ph 7.0, 1 mM DTT, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 0.1% NaN_3) was added to each well. Various concentrations of compounds were diluted in 10 μL of 1% DMSO (v/v), with blank DMSO solution as the negative control. The kinase reaction was initiated by the addition of purified tyrosine kinase proteins diluted in 39 μL of kinase reaction buffer solution. Reactions were incubated for 30 min at 25 ºC and stopped by the addition of 5 μL Streptavidin-XL665 and 5 μL Tk Antibody Cryptate working solution to all of wells. The plate was read by Envision (Perkinelmer) at 320 nm and 615 nm. IC_{50} values were calculated from the inhibition curves.

4.4 Molecular docking

The crystal structures of the proteins complex were retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). The three-dimensional structures of Afatinib with EGFR kinase was selected as the docking model (PDB ID: 4G5P). The docking simulation was conducted with Glide XP (Schrödinger 2014), since Glide uses ahierarchical series of filters to search for possible locations of the ligand in the active-site region of the receptor. The shape and properties of the receptor were represented on a grid by several different sets of fields that provide progressively more accurate scoring of the ligand poses. The image files were generated by AccelrysDS visualizer 4.0 system. The binding model was exemplified by the interaction of compound **13b** with EGFR.

Acknowledgments

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References and notes

1. Bruce, E. J.; Daniel, C. I.; Mary, J. M.; Paul, A. B.; Alan, Z.; Robert, W. M.; Anita, J.; Martin, H. C.; Eli, G.; John, D. M. *J. Med. Chem.* **1986**, *80*, 1103-10.
2. Douglas, A. A.; Michael, P. K.; Bruno, D.;Steven, L. K.; Scott, R. B. S.; Marie, D. B.; Mark, D. I.; Robert, M. S. *Cancer Immunol.*

Immun. **2000**, *49*, 63-70.

3. Sharma, S. V.; Bell, D. W.; Settleman, J.; Haber, D. A. *Nat. Rev. Cancer.* **2007**, *7*, 169-181**.**
4. Janku, F.; Stewart, D. J.; Kurzrock, R. *Nat. Rev. Clin. Oncol*. **2011**, *8*, 384-385.
5. Burgess, A. W.; Cho, H. S.; Eigenbrot, C.; Ferguson, K. M.; Garrett, T. P.; Leahy, D. J.; Lemmon, M. A.; Sliwkowski, M. X.; Ward, C. W.; Yokoyama, S. *Mol. Cell.* **2003**, *12*, 541-552.
6. Barker, A. J.; Gibson, K. H.; Grundy, W.; Godfrey, A. A.; Barlow, J. J.; Healy, M. P.; Woodburn, J. R.; Ashton, S. E.; Curry, B. J.; Scarlett, L.; Henthorn, L.; Richards, L. *Med. Chem. Lett.* **2001**, *11*, 1911-1914.
7. Sorbera, L. A.; Castañer, J.; Silvestre, J. S.; Bayes, M. *Drugs Future* **2002**, *27*, 923.
8. Tan, F.; Shen, X.; Wang, D.; Xie, G.; Zhang, X.; Ding, L.; Hu, Y.; He, W.; Wang, Y.; Wang, Y. *Lung Cancer.* **2012**, *76*, 177.
9. Wood, E. R.; Truesdale, A. T.; McDonald, O. B.; Yuan, D.; Hassell, A.; Dickerson, S. H.; Ellis, B.; Pennisi, C.; Horne, E.; Lackey, K.; Alligood, K. J.; Rusnak, D. W.; Gilmer, T. M.; Shewchuk, L. *Cancer Res.* **2004**, *64*, 6652-6659.
10. Eskens, F. A. L. M.; Mom, C. H.; Planting, A. S. T.; Gietema, J. A.; Amelsberg, A.; Huisman, H.; Doorn, L.; Burger, H.; Stopfer, P.; Verweij, J.; Vries, E. G. *Br. J. Cancer.* **2008**, *98*, 80-85.
11. Hennequin, L. F.; Stokes, E.S.; Thomas, A. P.; Johnstone, C.; Ple, P. A.; Ogilvie, D. J.; Dukes, M.; Wedge, S. R.; Kendrew, J.; Curwen, J. O. *J. Med. Chem.* **2002**,*45,* 1300.
12. Barry, T.; Karen, M. M.; Naeem, Y.; Lawrence, P.; Michael, D.; Carol, J.; Walter, M.; Lucian, D.; Simon, P.; Michael, M.; Francois, L.; Madelene, H.; Rajesh, K.; Clayton, S.; John, S.; Kymberly, L.; Catherine, F.; Xie, X.; Raviraj, K.; Kara, H.; Dale, P.; Michael, V. *ACS Med Chem Lett.* **2013**, *4*, 186−190.
13. Michael, F. T.; Koehler, P. B.; Elizabeth, B.; Krista, K. B.; Chen, Y. H.; Gauri, D.; Ding, X.; Jennifer, E.; Kevin, L.; Leslie, L.; Liu, L. C.; Ly, C.; Shiva, M.; Jim, N.; Jason, O.; Daniel, F.; Ortwine, D. S.; Steve, S.; Lan, T.; Tom, T.; Wu, J. S.; Pei, Z. H.; Joseph, P. L.; Orally, B. *J. Med. Chem.* **2012**, *55*, 10958-10971.
14. Anthony, A. E.; Daniel, G. S.; Elizabeth, B.; Yung-Hsiang, C.; Gauri, D.; Xiao, D.; Antonio, G; Jennifer, A. E.; Lori, S. F.; Michael, F. T.; Lichuan, L.; Shiva, M.; Daniel, F. O. Zhonghua, P.; Steve, S.; Frederic, S.; Lan, T.; Tom, T.; Joseph, P. L. *J. Med. Chem.* **2013**, *56*, 3090−3101.
15. Cooke, N. G.; Fernandes, G. S.; Paulo, A.; Furet, P.; Hebach, C.; Hoegenauer, K.; Hollingworth, G.; Kalis, C.; Lewis, I.; Smith, A. B.; Soldermann, N. *From PCT Int. Appl.* **2013**, WO 2013088404.
16. Guay, D.; Beaulieu, C.; Belley, M.; Crane, S. N.; DeLuca, J.; Gareau, Y.; Hamel, M.; Henault, M.; Hyjazie, H.; Kargman, S. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2832-2835.
17. Artman, G. D.; Elliott, J. M.; Ji, N.; Liu, D. l.; Ma, F.; Mainolfi, N.; Meredith, E.; Miranda, K.; Powers, J. J.; Rao, C. *From PCT. Int. Appl.* **2010**, WO 2010066684.
18. James, F. B.; John, J. G.; Jason, D. M.; Peter, M.; Mark, C.; Tian, H.; Rustam, G.; Allen, T.; Christopher, S. S.; Michael, B. W.; Gabrielle, K.; Robert, K.; Michael, B.; Matthew, M.; Chen, H.; Brian, D.; Danette, A. D.; Stephen, E. G.; Patricia, P.; Sheerin, S. L.; Wang, W.; Kristina, W.; Yin, J.; John, M.; Jacob, B. S. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 2635–2639.
19. Izzat, T. R.; Michael, J. B.; Christine, F.; Joy, F.; Nicole, H.; Sarah, H.; Monika, K.; Somang, H. K.; Bennett, M.; Georgia, M.; John, J. R.; John, D. S.; Sujata, S.; Sean, S.; Jason, U.; Yan, Y. W.; Paul, J. C.; Christopher, D. C. *Bioorg. Med. Chem. Lett*. **2012**, *22*, 5903-5908.
20. Lim, K. S.; Cho, J.; Kim, B. H. *et al. Brit. J. Clin. Pharmaco.* **2009**, *68*, 883-890.
21. Craig, A. C.; Luo, Y. F.; Cui, M. X; Wang, J. B; Richard, S.; Dong, J. C.; Hu, B.; Michael A. L.; Vincent, P. S.; Richard, L. K.; Yun, G.; Wang, Y.; Steven, V. F.; Jacquelyn, B.; Scott, M. D.; Duane, R. R.; Pamela, L. T.; Anthony, L. G.; Peter, T. M.; John, J. R. *ChemMedChem.* **2012**, *7*, 123-133.
22. Piotrowski, D. W.; Futatsugi, K.; Warmus, J. S.; Orr, S. T. M.; Freeman-Cook, K. D.; Londregan, A. T.; Wei, L. Q.; Jennings, S. M.; Herr, M.; Coffey, S. B. *Med. Chem. Lett.*. **2013**, *4*, 63-68.
23. Terrence, J. C.; Michael, M.; Keshab, S. *Org. Process Res. & Dev.* **2005**, *9*, 80-87.
24. Regueiro-Ren, A.; Swidorski, J.; Liu, Z.; Wang, T.; Zhang, Z; Hamann, L. G.; Meanwel, N. A.; Carini, D. J.; Li, W. *From PCT. Int. Appl.* **2009**, WO 2009158396.
25. Tsou, H. R.; Mamuya, N.; Johnson, B. D.; Reich, M. F.; Gruber, B. C.; Ye, F.; Nilakantan, R.; Shen, R.; Discafani, C.; DeBlanc, R.; Davis, R.; Koehn, F. E.; Greenberger, L. M.; Wang, Y. F.; Wissner, A. *J. Med. Chem.* **2001**, *44*, 2719-2734.

26. Rewcastle, G. W.; Denny, W. A.; Bridges, A. J.; Zhou, H.; Cody, D. R.; McMichael, A.; Fry, D. W. *J. Med. Chem.* **1995**, *38*, 3482-3487.

27. Totrov, M. *Chem. Biol. Drug Des.* **2008**, *71*, 15-27.

28. Cheeseright, T.; Mackey, M.; Rose, S.; Vinter, A. *J. Chem. Inf. Model.* **2006**, *46,* 665-76.

29. Treder, A. P.; Andruszkiewicz, R.; Zgoda, W.; Ford, C.; Hudson, A. L. *Bioorg. Med. Chem. Lett*. **2009**, *19*, 1009-1011.

30. Han, C.; Wan, L. D.; Ji, H. B.; Ding, K.; Huang, Z. J.; Lai, Y. S.; Peng, S. X.; Zhang, Y. H.. *Eur. J. Med. Chem*. **2014**, *77*, 75-83.

31. Mao, Y. J.; Zhu, W. X.; Kong, X. G.; Wang, Z.; Xie, H.; Ding, J.; Nicholas, K. T.; Shen, J. K.; Shen, J. S. *Bioorg. Med. Chem*. **2013**, *21*, 3090-3104.

32. Hwei-Ru, T.; Elsebe, G. O. K.; William, A. H.; Marvin, F. R. M.; Brawner, F.; Bernard, D. J.; Ronald, S. M.; Ramaswamy, N.; Carolyn, D.; Jonathan, G.; Sridhar, K. R.; Ru, S.; Shi, X. Q.; Wang, Y. F.; Janis, U.; Allan, W. *J. Med. Chem.* **2005**, *48*, 1107-1131.

33. Siavosh, M.; Andreas, S.; Matthias, W.; Emerich, E.; Herwig, P.; Thomas, C.; Thomas, B.; Thomas, M.; Thomas, B. *J. Med. Chem.* **2010**, *53*, 8546–8555.

34. Karaman, M. W.; Herrgard, S.; Treiber, D. K.; Gallant, P.; Atteridge, C. E.; Campbell, B. T.; Chan, K. W.; Ciceri, P.; Davis, M. I.; Edeen, P. T.; Faraoni, R.; Floyd, M.; Hunt, J. P.; Lockhart, D. J.; Milanov, Z. V.; Morrison, M. J.; Pallares, G.; Patel, H. K.; Pritchard, S.; Wodicka, L. M.; Zarrinkar, P. P. *Nat. Biotechnol*. **2008**, *26*, 127-132.

35. Solca, F.; Dahl, G.; Zoephel, A.; Bader, G.; Sanderson, M.; Klein, C.; Kraemer, O.; Himmelsbach, F.; Haaksma, E.; Adolf, G. R. *J. Pharmacol. Exp. Ther.* **2012**,*343*, 342-350.

36. Zhou, W.; Liu, X. F.; Tu, Z. C.; Zhang, L. W.; Ku, X.; Bai, F.; Zhao, Z. J.; Xu, Y. F.; Ding, K.; Li, H. L. *J. Med. Chem.* **2013**, *56*, 7821-7837.

37. Allan, W.; Philip, R. H.; Ramaswamy, N.; Lee, M. G.; Ye, F.; Timothy, A. R.; Frank, L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1411-1416.

38. Tiedt, R.; Degenkolbe, E.; Furet, P.; Appleton, B. A.; Wagner, S.; Schoepfer, J.; Buck, E.; Ruddy, D. A.; Monahan, J. E.; Jones, M. D.; Blank, J.; Haasen, D.; Drueckes, P.; Wartmann, M.; McCarthy, C.; Sellers, W. R.; Hofmann, F. *Cancer Res.* **2011**, *71*, 5255.

39. Li, H. Q.; Li, D. D.; Lu, X.; Xu, Y. Y.; Zhu, H. L. *Bioorg. Med. Chem*. **2012**, *20*, 317-323.

Legends

Figure 1 Representative EGFR inhibitors with 4-arylamino-quinazoline.

Figure 2 Representative THPP derivatives with biological and pharmaceutical properties.

Figure 3 Design strategy, screening and optimization of 4-arylamino-6-substituted-THPPs.

Figure 4 The structure of compound **a1** and overlay of compound **a1** with **Gefitinib** and **Erlotinib**. Carbon atom in compound **a1** was shown in purple, cyan for **Gefitinib**, and grey for **Erlotinib**. Nitrogen was colored in blue, oxygen in red, chlorine in green.

Scheme 1 Reagents and conditions: (a) H_2, Pd/C, 5 atm., HCl, ethanol, 40 ℃, 8 h; (b) CbzCl, DIPEA, DCM, r.t., 2 h; (c) formamidine acetate, ethanol, $NaOCH_3$, 6 h, reflux; (d) $POCl_3$, DIPEA, CH_3CN, reflux, 6 h; (e) NH_2ArR_1, NaHMDS, THF, 0 ℃, 2 h; (f) HBr, acetic acid, 50 ℃, 2 h.

Scheme 2 Reagents and conditions: (a) ClO_2SR_2, DIPEA, DCM, 0 ℃, 2h; (b) $ClCH_2R_2$, DIPEA, DCM, 0 ℃, 1 h or CH_2CHR_2, ethanol, 60 ℃, 8 h; (c) i) diethyl squarate, ethanol, 60 ℃, 4 h; ii) NR_1R_2, ethanol, 60 ℃, 4 h; (d) i) CDI, ethanol, reflux, 4 h; ii) NR_1R_2, ethanol, reflux, 12h; (e) $ClOCOR_2$, DIPEA, CH_3CN, 0 ℃, 1 h;(f) $ClOCR_2$, DIPEA, DCM, 0 ℃, 1 h or HO_2CR_2 EDCI, HOBT, DIPEA, CH_3CN, 60 ℃, 6 h.

Scheme 3 Reagents and conditions: (a) H_2, Pd/C, 5 atm., ethanol, 40 ℃, 8 h; (b) CbzCl, DIPEA, DCM, r.t., 2 h; (c) formamidine acetate, ethanol, $NaOCH_3$, 6 h, reflux; (d) $POCl_3$, DIPEA, DCE, reflux, 6 h; (e) NH_2ArR_1, NaHMDS, THF, 0 ℃, 2 h; (f) HBr, acetic acid, 50 ℃, 2 h.

Scheme 4 Reagents and conditions: (a) ClO_2SCH_3, DIPEA, DCM, 0 ℃, 2 h; (b) CDI, ethanol, reflux, 4 h; ii) $N(CH_3)_2$, ethanol, reflux, 12 h; (c) $ClOCOCH_3$, DIPEA, CH_3CN, 0 ℃, 1 h; (d) $ClOCR_2$, DIPEA, DCM, 0 ℃, 1 h or HO_2CR_2, EDCI, HOBT, DIPEA, CH_3CN, 60 ℃, 6 h.

Table 1

Structures and cytotoxicities of compounds **7a-13e** against HT29, A549, H460 and H1975 cancer cell lines in *vitro*.

Table 2

Structures and cytotoxicities of compounds **13f-u** against HT29, A549, H460 and H1975 cancer cell lines in *vitro*.

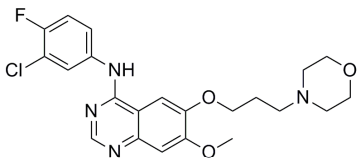
Table 3

Structures and cytotoxicities of compounds **20-24c** against HT29, A549, H460 and H1975 cancer cell lines in *vitro*.

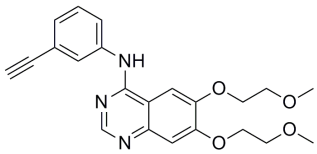
Table 4

EGFR and HER2 kinase activity of selected compounds **13b**, **13g**, **13n**, **13o**, **13p**, **13r** and **13s** in *vitro*.

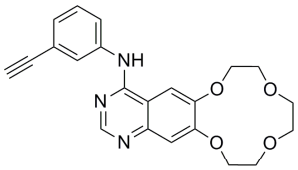
Figure 5 Proposed binding model for compound **13b** bound to the kinase domain of EGFR. The described hydrogen bonds and key distance to Cys-797 are shown.



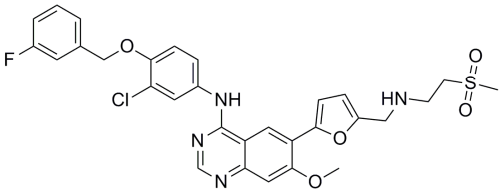
Gefitinib



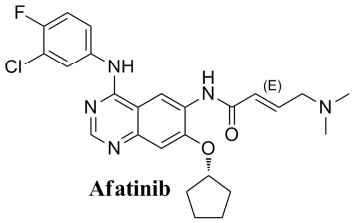
Erlotinib



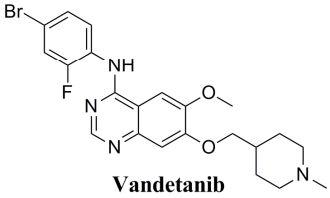
Icotinib



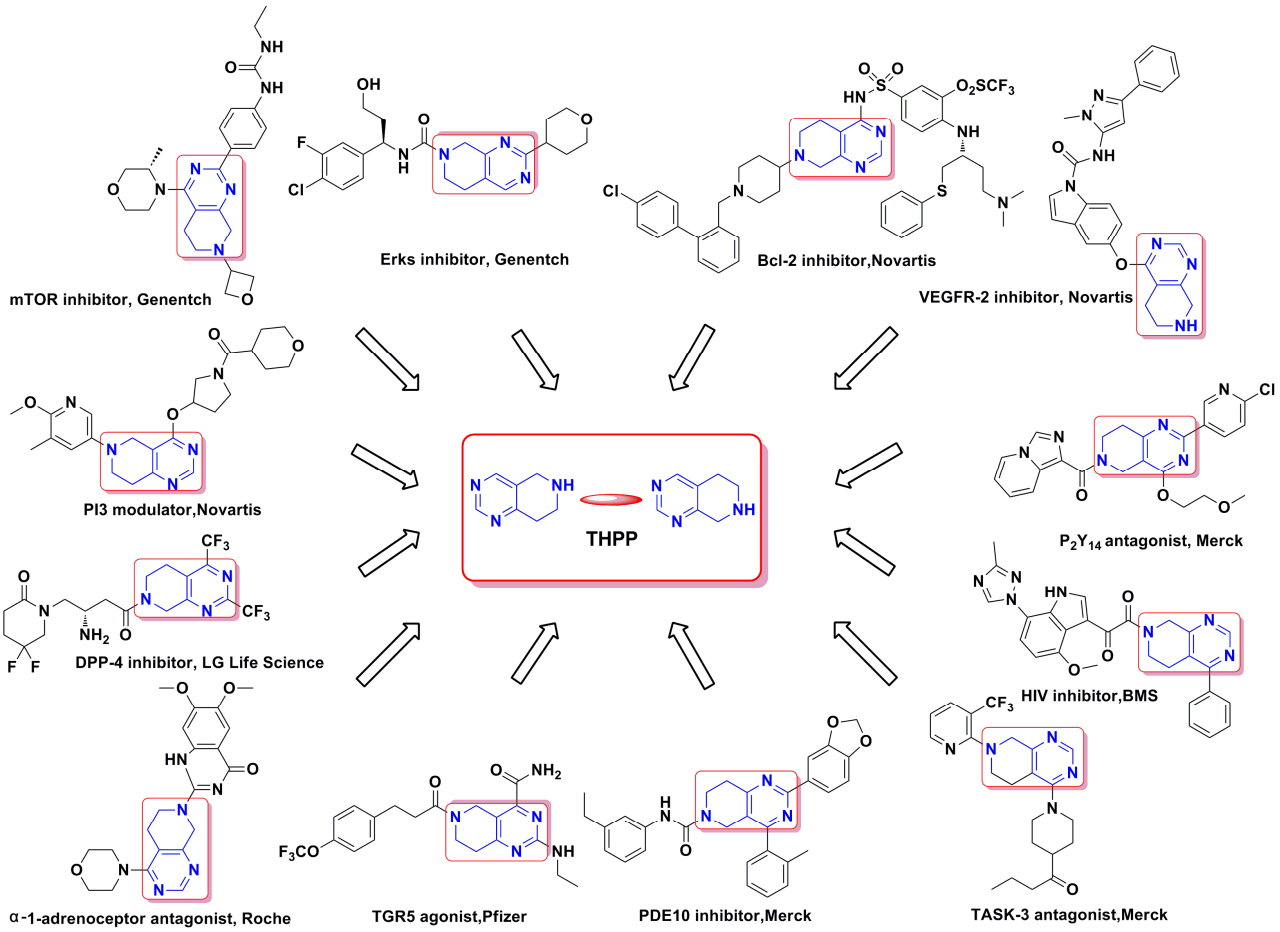
Lapatinib

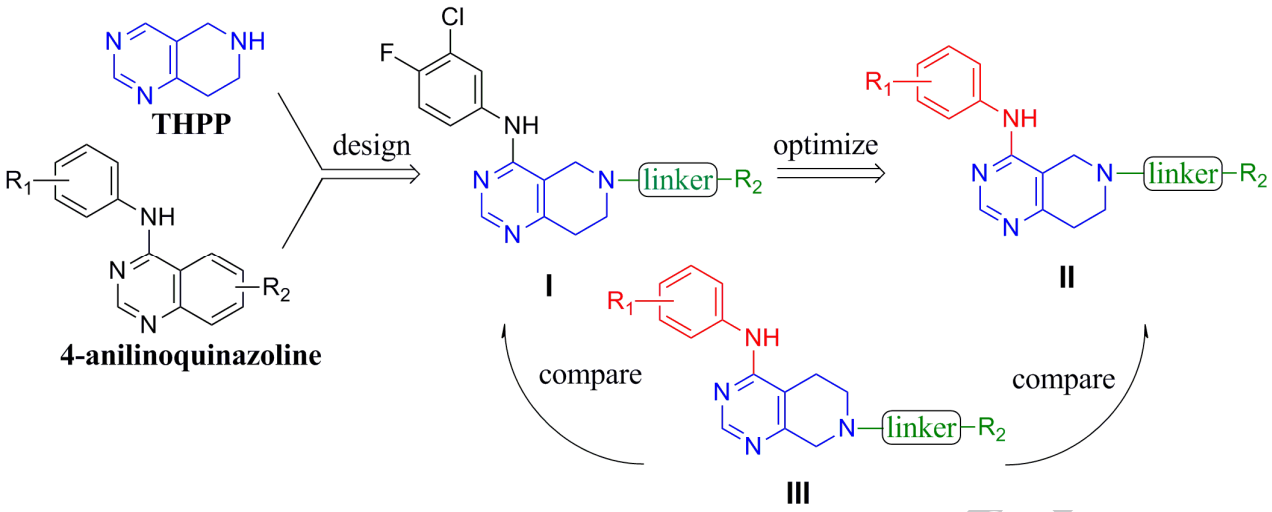


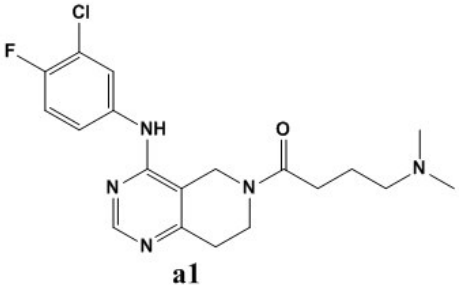
Afatinib



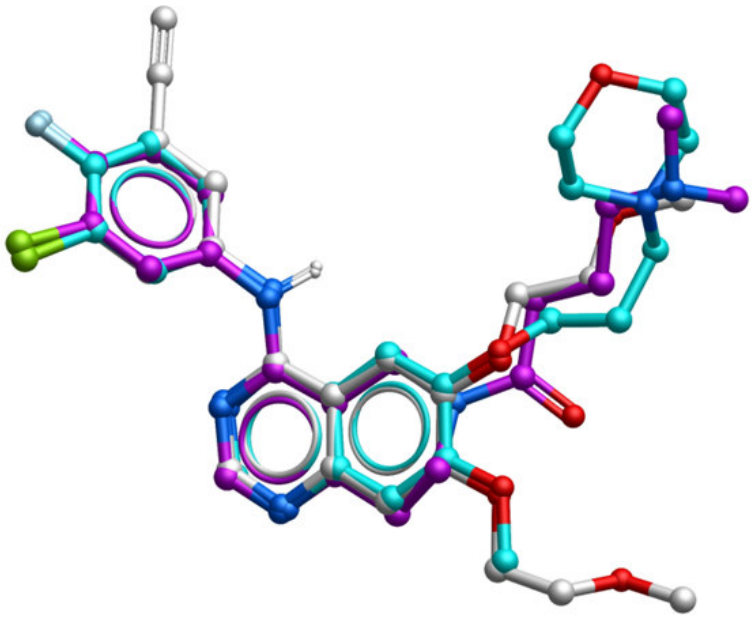
Vandetanib

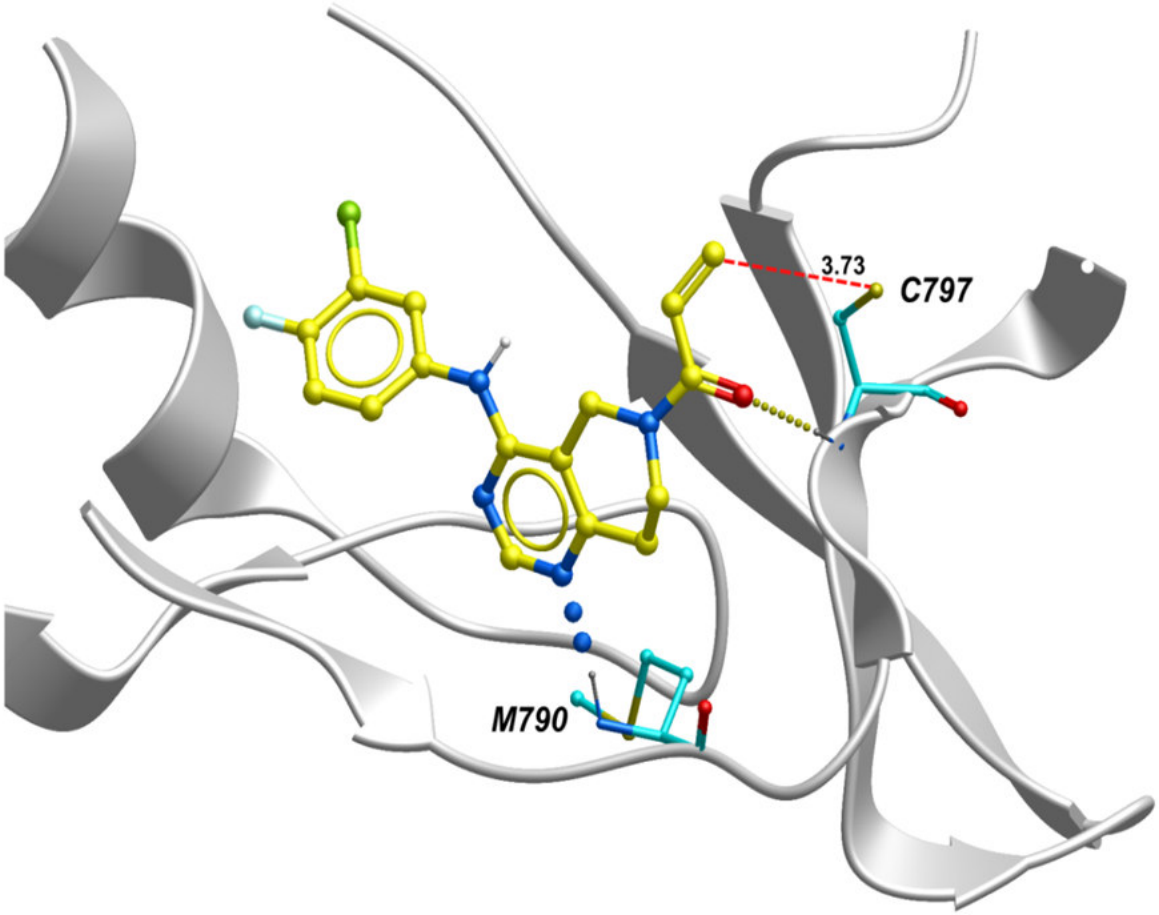


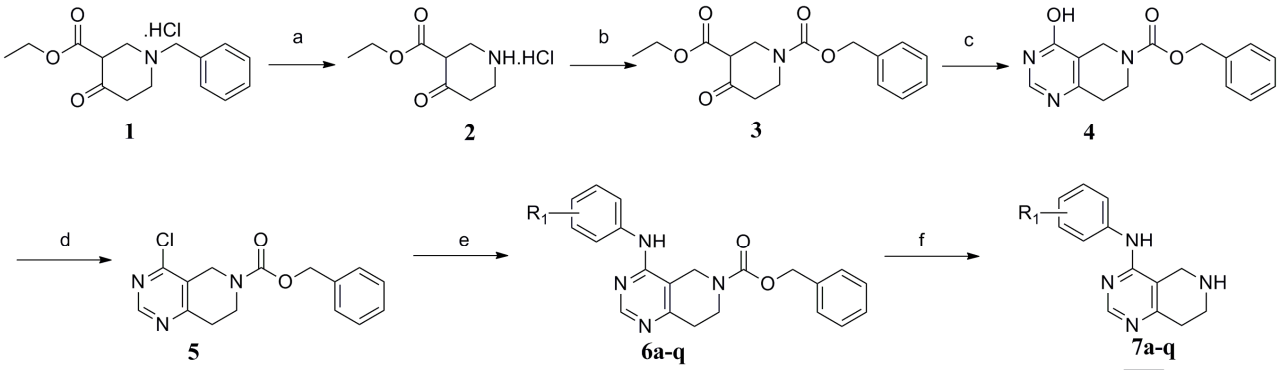


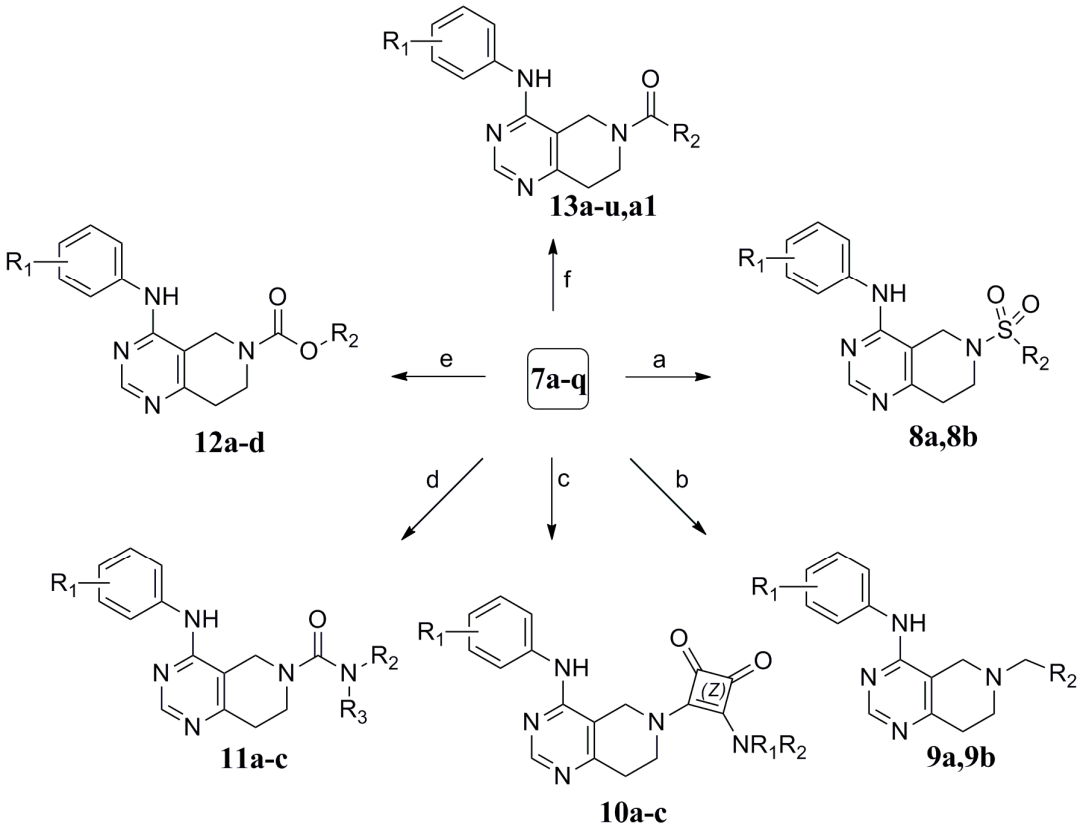


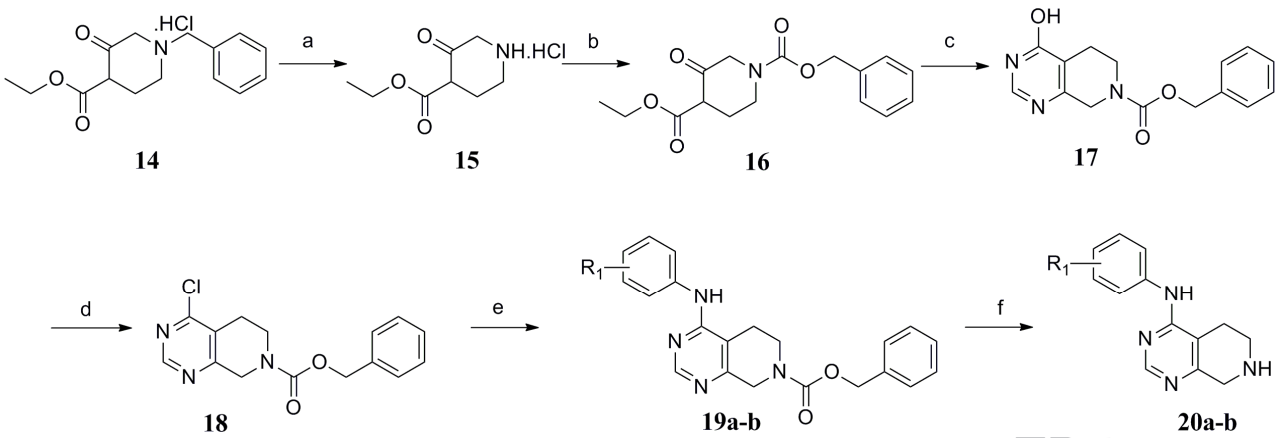
a1











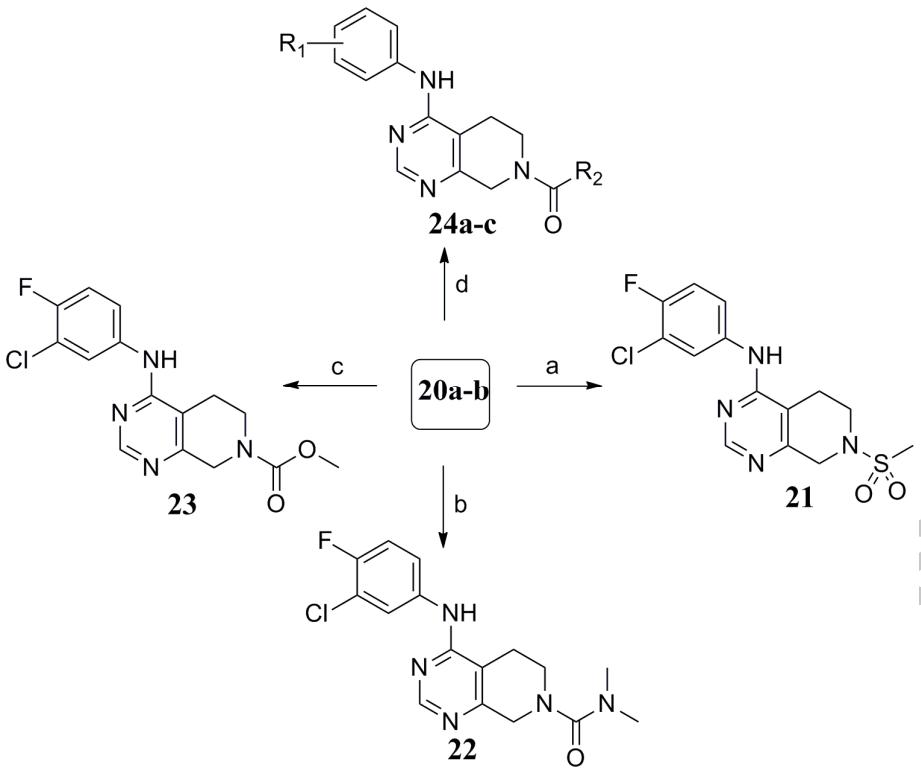
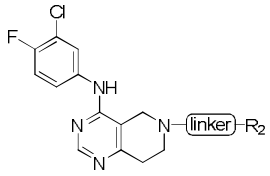
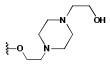


Table 1

Structures and cytotoxicities of compounds **7a-13e** against HT29, A549, H460 and H1975 cancer cell lines in *vitro*.



Compd.	linker	R_2	IC_{50}[a] (μmol/L)			
			HT29	A549	H460	H1975
7a			111.45	50.42	129.68	120.75
8a			74.27	46.21	68.62	48.82
8b			54.60	52.46	156.24	130.03
9a			>200	>200	>200	>200
9b			183.53	>200	>200	132.83
10a			115.48	130.54	>200	75.56
10b			98.41	100.08	>200	101.34
10c			20.21	57.28	24.14	125.33
11a			46.64	54.33	87.53	157.70
11b			49.56	50.47	52.57	130.49
11c			10.35	12.49	18.48	102.47
12a			46.44	49.37	116.06	38.32
12b			196.03	106.44	>200	87.06
12c			50.9	112.36	>200	>200
12d			65.86	77.67	103.44	116.28
13a			165.12	133.36	>200	130.67
13b			42.35	22.27	29.73	6.32
13c			2.69	17.46	160.82	6.21

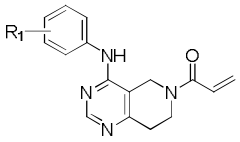
13d			108.65	139.88	170.33	82.56
13e			64.32	95.60	>200	74.27
a1			47.53	32.05	101.56	66.58
Gefitinib[b]			18.44	15.67	19.43	12.87

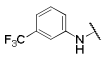
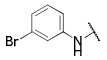
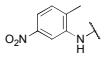
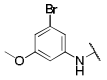
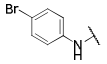
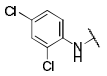
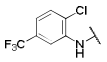
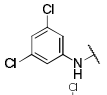
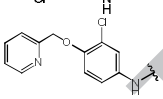
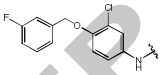
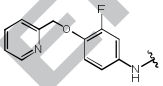
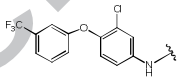
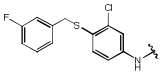
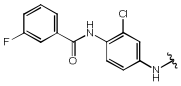
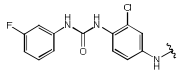
[a]Values are the means of at least three independent experiments SD < 10%.

[b]Used as positive control.

Table 2

Structures and cytotoxicities of compounds **13f-u** against HT29, A549, H460 and H1975 cancer cell lines in *vitro*.



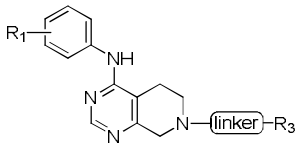
Compd.	R_1ArNH	IC_{50}[a] (μmol/L)			
		HT29	A549	H460	H1975
13f		21.53	21.08	21.08	12.37
13g		13.21	18.02	25.69	15.32
13h		24.62	43.25	48.17	55.16
13i		21.48	63.33	3.83	18.54
13j		17.03	35.75	24.07	16.76
13k		24.38	70.71	30.70	22.64
13l		24.37	35.23	40.64	33.32
13m		26.55	46.07	45.53	34.05
13n		7.56	15.40	6.16	7.00
13o		13.87	20.02	18.13	2.79
13p		11.69	9.40	11.03	3.34
13q		52.33	36.72	23.03	43.42
13r		7.91	5.88	3.99	6.93
13s		7.48	>200	34.54	3.96
13t		25.52	37.40	5.28	21.56
13u		5.28	18.92	4.21	6.34
Gefitinib[b]		18.44	15.67	19.43	12.87

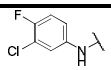
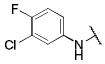
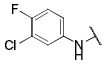
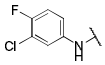
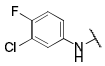
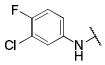
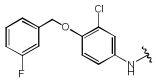
[a]Values are the means of at least three independent experiments, SD < 10%.

[b]Used as positive control.

Table 3

Structures and cytotoxicities of compounds **20-24c** against HT29, A549, H460 and H1975 cancer cell lines in *vitro*.



Compd.	R_1ArNH	linker	R_3	IC_{50}[a] (μmol/L)			
				HT29	A549	H460	H1975
20a				119.05	89.25	64.89	77.17
21				88.94	136.08	60.84	69.06
22				44.76	71.13	135.59	88.40
23				45.59	67.90	154.41	71.65
24a				60.54	136.76	>200	93.02
24b				40.18	28.99	49.41	13.51
24c				11.00	6.63	9.46	3.30
Gefitinib[b]				18.44	15.67	19.43	12.87

[a]Values are the means of at least three independent experiments, SD < 10%.

[b]Used as positive control.

Table 4

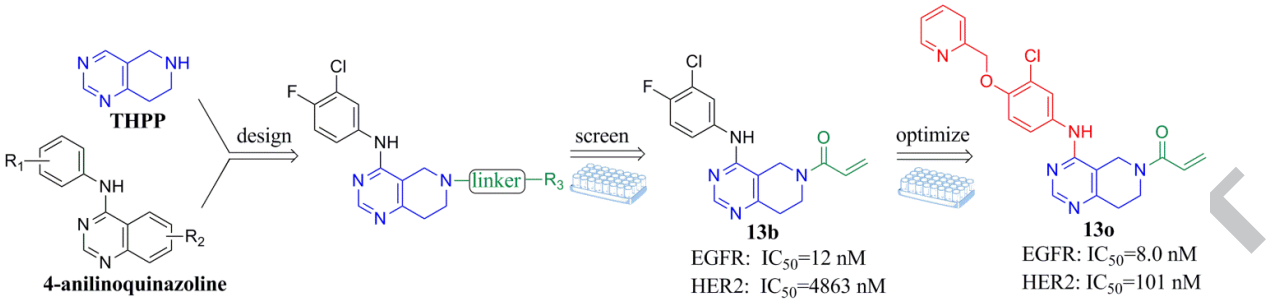
EGFR and HER2 kinase activity of selected compounds **13b, 13g, 13n, 13o, 13p, 13r** and **13s** *in vitro*.

Compd.	IC_{50}[a] on EGFR(nM)	IC_{50}[a] on HER2 (nM)
13b	12	4863
13g	9	2670
13n	6	4142
13o	8	101
13p	9	64
13r	18	35
13s	8	67
Staurosporine[b]	68	321

[a]Values are the means of at least three independent experiments, SD < 10%.

[b]Used as positive control.

Graphic abstract



The discovery of 4-anilinotetrahydropyrido[4,3-*d*]pyrimidines as the novel anti-proliferative agents and EGFR kinase inhibitors were reported.