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Synthesis and biological activity of new 2,4,6-trisubstituted triazines as potential phosphoinositide 3-kinase inhibitors

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Abstract

Twenty-five novel 2,4,6-trisubstituted triazines were synthesized and biologically evaluated. Most of the compounds synthesized showed good antiproliferative activity against HCT-116 and MCF-7. Compounds **B18** and **B19** showed the best antiproliferative activity. Further study showed **B18** and **B19** inhibited four phosphoinositide 3-kinase isoforms and mammalian target of rapamycin with good potency. These results demonstrate that 2,4,6-trisubstituted triazines are potentially useful phosphoinositide 3-kinase inhibitors for the development of new anticancer drugs.

Keywords

anticancer, antiproliferative effects, PI3K inhibitor, synthesis, triazine derivatives

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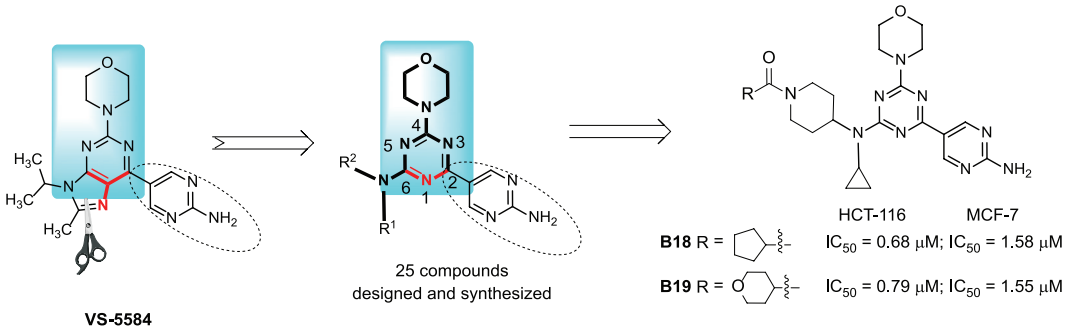

The phosphoinositide 3-kinase (PI3K) is a very important node within the PI3K/AKT/mTOR pathway that regulates a variety of cellular processes including cell proliferation, differentiation, survival, and migration.[1] Aberrant activation of PI3K signaling has been implicated in multiple cancer types including colorectal, breast, leukemia, and so on.[2] Therefore, PI3K is considered as a promising target for solid and hematologic tumor therapy.[3]

During the past 20 years, a great number of small molecule PI3K inhibitors have been investigated. Some early agents such as wortmannin and LY294002 did not enter clinical development due to their narrow therapeutic window and severe toxicity.[4] Fortunately, the selective second generation PI3K or PI3K/mTOR inhibitors, such as idelalisib (CAL-101, **1**),[5] copanlisib (BAY80-6946, **2**),[6] apitolisib (GDC-0980, **3**),[7] omipalisib (GSK2126458, **4**),[8] dactolisib (NVP-BEZ235, **5**),[9] buparlisib (NVP-BKM120, **6**),[10] and VS-5584 (**7**)[11] were advanced into clinical development, and some agents showed promising clinical efficacy (Figure 1). Particularly, idelalisib (selective PI3Kδ inhibitor) and copanlisib (pan-PI3K inhibitor) were recently approved by the Food and Drug Administration (FDA) for treatment of hematologic malignancy.[5,6] These encouraging

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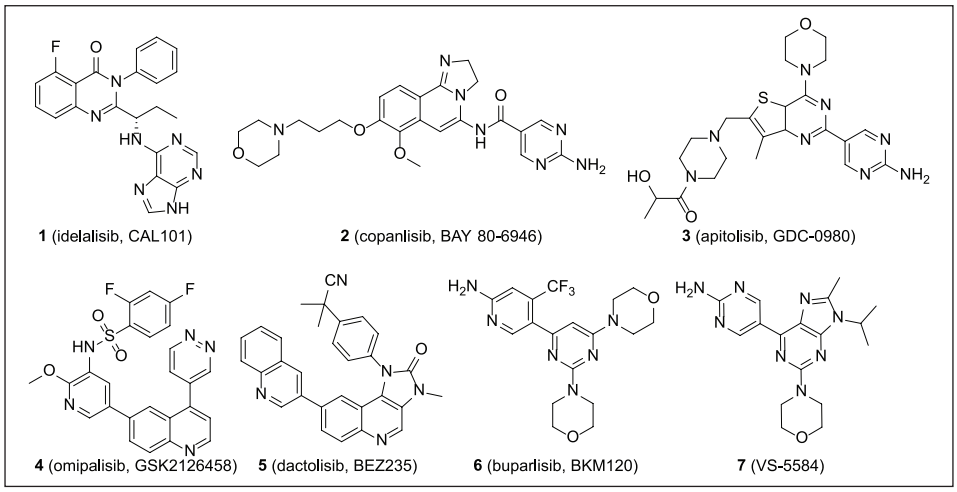


Figure 1. Representative structures of reported PI3K inhibitors.

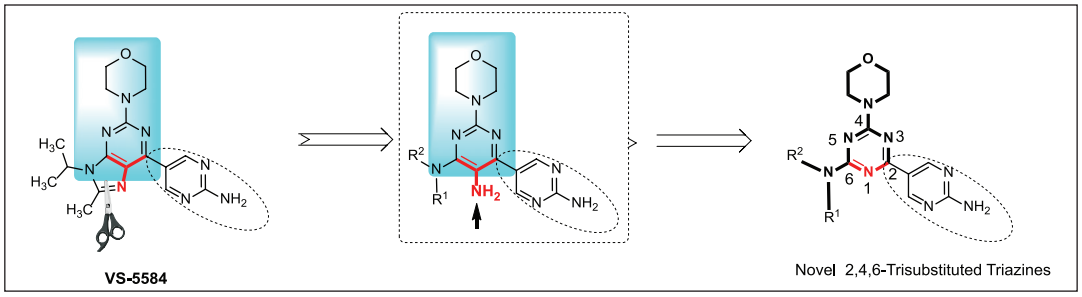


Figure 2. The design of novel 2,4,6-trisubstituted triazines.

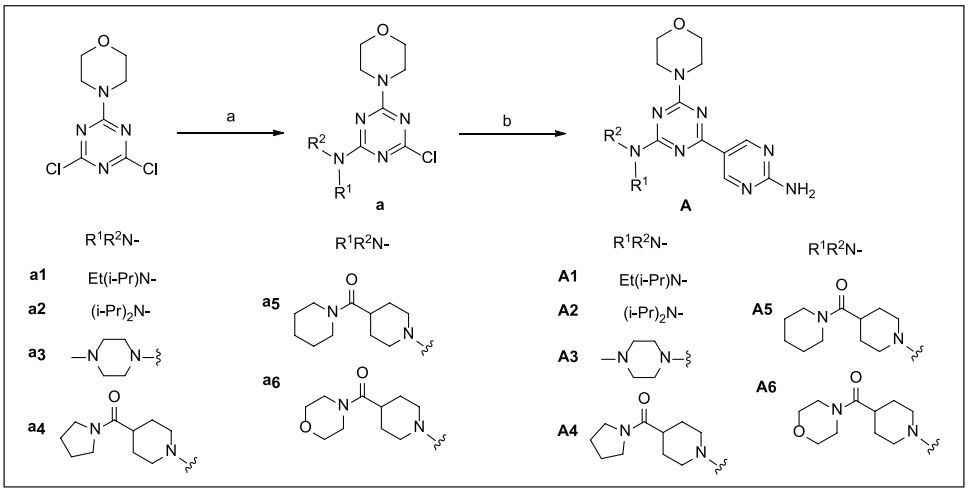
facts refueled the discovery and development of new PI3K inhibitors (Figure 1).

Recently, we reported our drug design and synthesis effort on the discovery of new potent PI3K inhibitors. The first strategy was to develop novel selective PI3Kδ inhibitors with distinct chemotypes which may elevate the therapeutic index and provide clinical benefit.[12,13] For example, several 6-aryl substituted quinazoline derivatives were synthesized and some showed promising potency against PI3Kδ in vitro.[14,15] The other strategy was to develop pan-PI3K or PI3K/mTOR inhibitors, exemplified by the preparation of several series of derivatives such as quinazolin-4(*3H*)-ones,[16,17] [1,2,4]triazolo[1,5-a]pyridines,[18] benzo[d]thiazoles,[19,20] 4-morpholinoquinazolines,[21] and 4-(morpholin-4-yl)-1,3,5-triazines.[22] In particular, in the series of 4-(morpholin-4-yl)-1,3,5-triazines, representative derivatives displayed potent antitumor effects in vitro and in vivo. Thus, in an attempt to develop new anticancer agents and based on our previous reports and the chemical structure of VS-5584, we intend to open the purine ring of VS-5584 and replace it with a triazine core so as to design a series of 1,3,5-triazines as new PI3K inhibitors (indicted in Figure 2). Certainly, the 4-(morpholin-4-yl)-1,3,5-triazine is facile to be constructed and its derivatives were reported to show good antitumor effects. Furthermore, the 4-(morpholin-4-yl)-1,3,5-triazine scaffold was a good mimic of the main pharmacophore of VS-5584 which generated several key H-bond interactions with PI3Kα. Therefore, in this paper we design and synthesize a series of 4-(morpholin-4-yl)-1,3,5-triazines by introducing a variety of aliphatic amino groups at the 6-position as potential PI3K inhibitors, and investigate the primary structure–activity relationship (Figure 2).

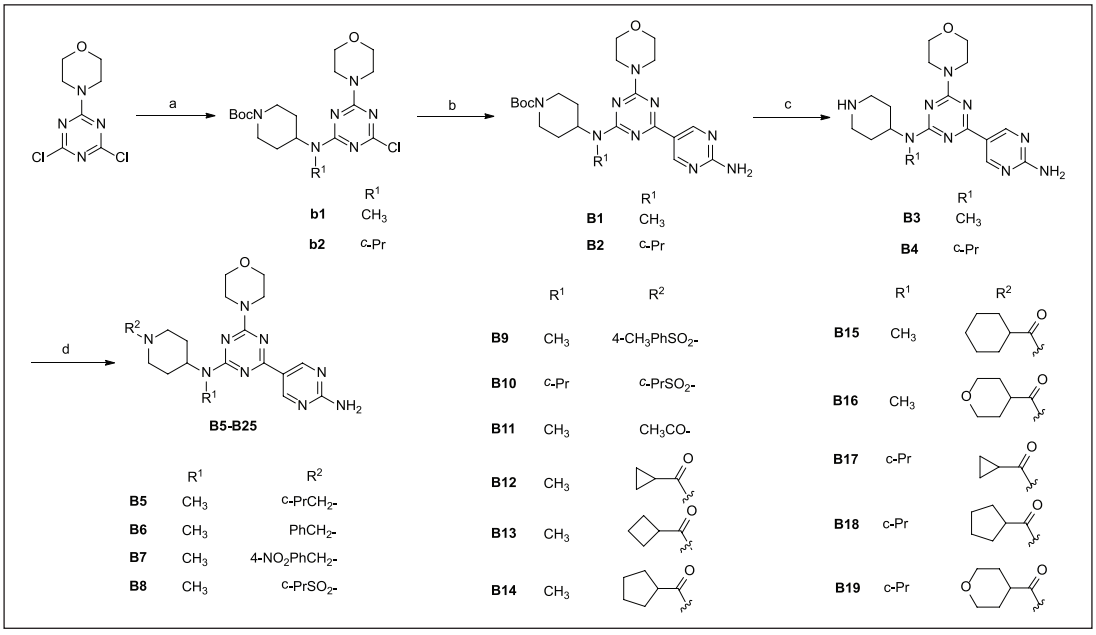
Results and discussion

The title compounds **A** were prepared by the route shown in Scheme 1. The key intermediates **a1**–**a6** were constructed by amination of the starting material 2,4-dichloro-6-morpholinyl-1,3,5-triazine with substituted aliphatic amines. The target compounds **A1**–**A6** were then synthesized in one-pot by $PdCl_2$(dppf)-catalyzed Suzuki coupling of **a1**–**a6** and the boronic ester intermediate 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidin-2-amine which was newly prepared from 2-amino-5-bromopyrimidine and bis(pinacolato)diboron using Miyaura borylation in the presence of $PdCl_2$(dppf) and potassium acetate (Scheme 1).

As outlined in Scheme 2, the compounds **B1**–**B2** were obtained similarly to Scheme 1. The compounds **B3**–**B4** were prepared by deprotection of the Boc group in **B1** or **B2** using trifluoroacetic acid (TFA) in dichloromethane. The reductive amination of **B3** with cyclopropanecarbaldehyde gave compound **B5**. The piperidinyl base is stronger than the amino group linked to the pyrimidinyl group, and so the nucleophilic substitution of **B3** with benzyl chloride or 4-nitrobenzyl bromide reacted at the piperidinyl site to



Scheme 1. Preparation of compounds **A1–A6**. Reagents and conditions: (a) R^1R^2NH, DIPEA, MeCN, rt, 4–6 h, 45%–83%; (b) (i) 2-amino-5-bromopyrimidine, bis(pinacolato)diboron, KOAc, $PdCl_2(dppf)$, dioxane, reflux; (ii) **a1–a6**, Na_2CO_3, $PdCl_2(dppf)$, dioxane/H_2O, reflux, 4–6 h; 66%–87% (two steps).

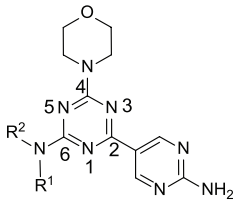


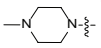
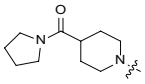
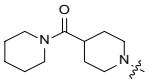
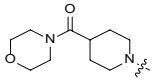
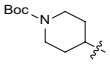
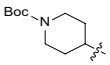
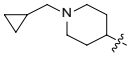
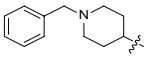
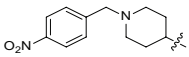
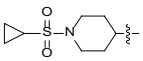
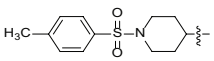
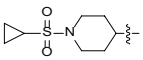
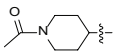
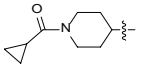
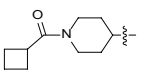
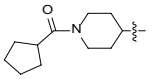
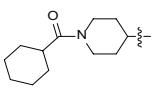
Scheme 2. Preparation of compounds **B1–B19**. Reagents and conditions: (a) amine, TEA, DCM, rt, 62% for **b1**; amine, DIPEA, THF, reflux, 71% for **b2**; (b) (i) 2-amino-5-bromopyrimidine, bis(pinacolato)diboron, KOAc, $PdCl_2(dppf)$, dioxane, reflux; (ii) Na_2CO_3, $PdCl_2(dppf)$, dioxane/H_2O, reflux, 4–6 h; 45%–74%; (c) TFA, DCM, rt, 2h, 85%–94%; (d) cyclo-PrCHO, $NaHB(OAc)_3$, for **B5**, 17%; BnCl, K_2CO_3 for **B6**, 89%; 4-NO_2BnBr, K_2CO_3 for **B7**, 87%; RSO_2Cl, DIPEA, MeCN for **B8–B10**, 62%–74%; RCO_2H, HATU, DIPEA, DCM for **B11–B19**, 46%–96%.

yield compound **B6** or **B7**. Similarly, the reaction of compound **B3** or **B4** with cyclopropanesulfonyl chloride, tosyl chloride, or acetic anhydride, respectively, produced the title compounds **B8**–**B11** and in the presence of a condensing agent, the reaction of **B3** or **B4** with a carboxylic acid provided the title compounds **B12**–**B19** (Scheme 2).

Compounds **A1**–**A6** and **B1**–**B19** were first evaluated for their antiproliferative activity by MTT assays.[16] Two cancer cell lines including HCT-116 (PI3CA mutant: H1047R) and MCF-7 (PI3CA mutant: E545K) were used in this evaluation, and the results are summarized in Table 1. Albeit sharing the same pharmacophore, 2-(2-aminopyrimidin-5-yl)-4-(morpholin-4-yl)triazine, the compounds **A1**–**A6** and **B1**–**B19** exhibited significantly different antiproliferation, which was mainly related to the 6-substituted groups linked to the triazine scaffold. Compounds **A1**–**A3** with a simple substituted amino group at the 6-position of the triazine showed weak antiproliferative effect and compounds **A4**–**A6** with a piperidinyl substituent displayed loss of cell-based activity. Subsequently, the piperid-1-yl substituent was moved to the piperidine-4-yl amino group leading to synthesis of compounds **B1**–**B7**. Compounds **B1** and **B2** with the Boc group attached to the 1-position of piperidine-4-yl ring, showed more potent antiproliferative activity. However, removal of the Boc group resulted in compounds **B3** and **B4** that showed

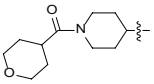
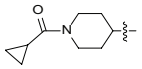
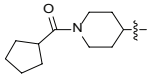
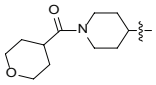
Table 1. Antiproliferative effects of compounds **A** and **B** ($\bar{x} \pm s$, n = 3).



Compound	R^1	R^2	IC_{50} (μM)	
			HCT-116	MCF-7
A1	−Et	−Pr-i	2.95 ± 0.19	4.45 ± 0.40
A2	−Pr-i	−Pr-i	9.54 ± 1.13	>10
A3			9.89 ± 0.60	>10
A4			>10	>10
A5			>10	>10
A6			>10	>10
B1	$-CH_3$		2.29 ± 0.44	3.60 ± 0.40
B2			1.89 ± 0.14	1.95 ± 0.08
B3	$-CH_3$		8.09 ± 0.36	7.23 ± 1.10
B4			5.34 ± 0.11	3.49 ± 0.50
B5	$-CH_3$		>10	>10
B6	$-CH_3$		>10	7.62 ± 1.50
B7	$-CH_3$		>10	6.98 ± 0.50
B8	$-CH_3$		2.84 ± 0.32	3.37 ± 0.52
B9	$-CH_3$		>10	>10
B10			1.39 ± 0.30	1.92 ± 0.05
B11	$-CH_3$		1.56 ± 0.23	2.93 ± 0.10
B12	$-CH_3$		2.33 ± 0.08	3.15 ± 0.15
B13	$-CH_3$		2.07 ± 0.06	3.06 ± 0.14
B14	$-CH_3$		1.42 ± 0.15	4.66 ± 0.10
B15	$-CH_3$		1.18 ± 0.11	2.34 ± 0.22

(Continued)

Table 1. (Continued)

Compound	R^1	R^2	IC_{50} (μM)	
			HCT-116	MCF-7
B16	$-CH_3$		1.31 ± 0.16	2.76 ± 0.44
B17			1.08 ± 0.12	2.18 ± 0.10
B18			0.68 ± 0.01	1.58 ± 0.12
B19			0.79 ± 0.03	1.55 ± 0.15
Dactolisib	–	–	0.37 ± 0.21	0.35 ± 0.31
VS-5584	–	–	0.60 ± 0.12	1.06 ± 0.10

weaker activity. These results suggested that the presence of the NH group of the piperidine-4-yl substituent was unfavorable. Thereafter, alkyl, sulfonyl, or acyl groups were evaluated (**B5–B19**). Compounds (**B5**–**B7**) with an alkyl group at the 1-position of the piperidinyl ring displayed weak or loss antiproliferative activity. A cyclopropyl sulfonyl group in **B8** and **B10** was tolerated; however, the larger 4-methylbenzenesulfonyl group (**B9**) was not. A survey of acyl substituents at the 1-position of the piperidinyl ring was investigated. As the data showed in Table 1, the antiproliferative activity of the acyl substituted compounds **B11–B19** was remarkably improved. In particular, the *N*-cyclopropyl-*N*-(1-acylpiperidin-4-yl)amino substituted compounds **B17–B19** showed more potent antiproliferative effect against both HCT-116 and MCF-7 cell lines, and more importantly, both compound **B18** and **B19** were comparable to the positive drug **VS-5584**, despite being slightly weaker than dactolisib (BEZ235). Building on their structure–activity relationship, it was found that the *N*-cyclopropyl-*N*-(1-acylpiperidin-4-yl)amino moiety is a suitable substituent at the 6-position of the triazine scaffold. Compounds **B18** and **B19** were the best antiproliferative agents, and were further examined for their enzymic activity (Table 1).

B18 and **B19** were evaluated for their inhibitory activity against PI3Ks and mTOR by performing an ATP depletion assay. **VS-5584** and Dactolisib were used as the positive drugs. As the data showed in Table 2, **B18** displayed good inhibitory activity against four PI3K isoforms and mTOR with IC_{50} values of 62, 286, 870, 627, and 772 nM, respectively, and **B19** showed respective IC_{50} values of 78, 423, 521, 422 and 263 nM. Although the IC_{50} values of both **B18** and **B19** were higher than that of **VS-5584**, it was noted that both **B18** and **B19** afforded comparable PI3Kα inhibition to Dactolisib. However, **B18** and **B19** showed potent antiproliferative activity in cell-based effects but weaker PI3K inhibition which may be attributed to their diverse efficacy (Table 2).

To explain further the good PI3Kα inhibitory activities of compound **B18**, docking studies with human PI3Kα (PDB code, 5UK8) were performed. As depicted in Figure 3, the oxygen atom of the morpholine moiety formed a hydrogen bond with Val851, and the two hydrogen atoms of the amino group on the pyrimidine ring formed hydrogen bonds with Asp810 and Asp802. In addition, the nitrogen atom of the pyrimidine ring formed hydrogen bonds with Asp933. The presence of these hydrogen bonds suggested that **B18** can undoubtedly interact with the catalytic domain of PI3Kα (Figure 3).

Table 2. Enzymatic activity of **B18** and **B19** (IC_{50}, nM, n = 2).

Compound	PI3Kα	PI3Kβ	PI3Kγ	PI3Kδ	mTOR
B18	62	286	870	627	772
B19	78	423	521	422	263
Dactolisib	49	478	72	138	44
VS-5584	7	23	9	9	52

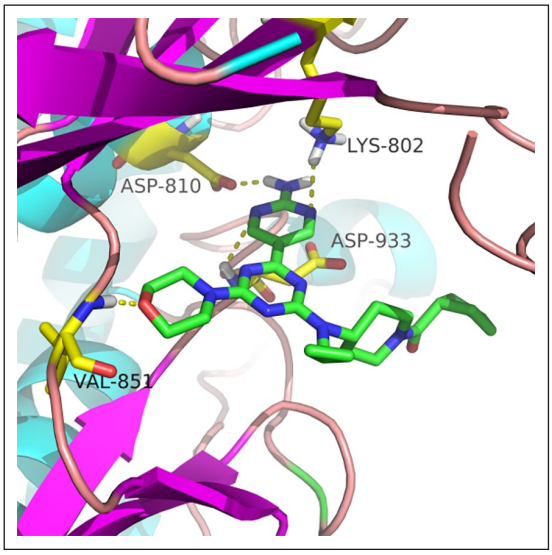


Figure 3. Compound **B18** docked into the ATP-binding site of PI3Kα (PDB code, 5UK8). **B18** is shown as sticks. Hydrogen bonds within 3.0 Å are shown as yellow dashed lines.

In summary, a series of novel 2,4,6-trisubstituted triazines were synthesized and characterized. Most compounds exhibited significant antiproliferative effects against HCT-116 and MCF-7 cell lines. The discussion of the SAR reveals that the antiproliferative effect is closely related to the substituent at the 6-position of the triazine, and the N-cyclopropyl-N-(1 acylpiperidin-4-yl)amino moiety is a suitable substituent at the 6-position contributing to the potent antiproliferation. The cell-based potencies of compounds **B18** and **B19** are comparable to that of **VS-5584**. The compounds **B18** and **B19** displayed good inhibitory activity against PI3Ks and mTOR. These results suggest that our compounds can serve as antiproliferative agents and potential PI3K inhibitors.

Experimental

All starting materials, reagents, and solvents were commercially available, unless specified otherwise. Reactions were monitored by thin-layer chromatography on silica gel plates (GF-254) and visualized with UV light. The melting points were determined on a Shanghai micro melting-point apparatus (model: SGW® X-4B) and are uncorrected. NMR spectra were recorded on a 400 Bruker NMR spectrometer with tetramethylsilane (TMS) as an internal reference. Chemical shifts are reported in parts per million (ppm). Mass measurements were performed using electrospray ionization (positive mode) on a quadrupole time-of-flight (QTOF) mass spectrometer (Skyray Instrument).

General procedure for the synthesis of intermediates **a1–a6**

To a solution of 4-(4,6-dichloro-1,3,5-triazin-2-yl)morpholine (0.10 g, 0.43 mmol) in dry dichloromethane (10 mL) was added dropwise a mixture of the substituted aliphatic amine (0.43 mmol) and triethylamine (178 μL) at 0 °C. The reaction mixture was stirred at room temperature until all the starting materials have been consumed. The solvent was removed under reduced pressure. Water (30 mL) was added to the residue and the mixture was stirred at room temperature for 10 min. The mixture was extracted with ethyl acetate (15 mL × 3). The organic extracts were combined and washed with water. After drying with anhydrous Na_2SO_4, the organic extracts were evaporated to provide compounds **a1–a6**.

4-Chloro-N-ethyl-N-isopropyl-6-morpholino-1,3,5-triazin-2-amine (**a1**):[23] colorless oil, yield 88%. ESI-MS m/z: 286.2 $[M+H]^+$.

4-Chloro-N,N-diisopropyl-6-morpholino-1,3,5-triazin-2-amine (**a2**):[23] Colorless oil, yield 83%. ESI-MS m/z: 300.2 $[M+H]^+$.

4-(4-Chloro-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-yl)morpholine (**a3**):[23] colorless oil, yield 80%. ESI-MS m/z: 299.2 $[M+H]^+$.

(1-(4-Chloro-6-morpholino-1,3,5-triazin-2-yl)piperidin-4-yl)(pyrrolidin-1-yl)methanone (**a4**): colorless oil, yield 71%. ^{1}H NMR ($CDCl_3$) δ 4.75–4.70 (m, 2H), 3.73–3.70 (m, 8H), 3.58–3.40 (m, 4H), 2.95–2.90 (m, 2H), 2.61 (d, J = 5.4 Hz, 1H), 1.98 (d, J = 6.1 Hz, 2H), 1.88–1.78 (m, 6H). ESI-MS m/z: 381.2 $[M+H]^+$.

(1-(4-Chloro-6-morpholino-1,3,5-triazin-2-yl)piperidin-4-yl)(piperidin-1-yl)methanone (**a5**): colorless oil, yield 60%. ^{1}H NMR ($CDCl_3$) δ 4.72–4.68 (m, 2H), 3.62–3.56 (m, 8H), 3.50–3.41 (m, 4H), 2.99–2.81 (m, 2H), 2.69 (d, J = 5.9 Hz, 1H), 1.68–1.55 (m, 6H), 1.50–1.45 (m, 4H). ESI-MS m/z: 395.2 $[M+H]^+$.

(1-(4-Chloro-6-morpholino-1,3,5-triazin-2-yl)piperidin-4-yl)(morpholino) methanone (**a6**): colorless oil, yield 65%. ^{1}H NMR ($CDCl_3$) δ 4.62–4.52 (m, 2H), 3.62–3.56 (m, 8H), 3.57–3.55 (m, 3H), 3.52–3.38 (m, 4H), 2.93–2.76 (m, 2H), 2.70–2.63 (m, 2H), 1.66–1.60 (m, 4H). ESI-MS m/z: 397.2 $[M+H]^+$.

General procedure for the synthesis of compound **A1–A6**

The mixture of 5-bromopyrimidin-2-amine (65 mg, 0.37 mmol), bis(pinacolato)diboron (113 mg, 0.44 mmol), potassium acetate (109 mg, 1.11 mmol), $PdCl_2$(dppf) (21 mg, 0.02 mmol), and 1,4-dioxane (10 mL) was heated under reflux under Ar atmosphere for 2.5 h, then concentrated under reduced pressure. To the resulting residue was added **b1** (98 mg, 0.25 mmol), K_2CO_3 (154 mg, 1.11 mmol), $PdCl_2$(dppf) (21 mg, 0.02 mmol), 1,4-dioxane (10 mL), and water (4 mL). The mixture obtained was heated under reflux in Ar atmosphere for 4 h. The volatile material was removed under reduced pressure and the residue was purified by a column chromatography on silica gel with CH_2Cl_2 / MeOH (v: v = 50:1) as eluent to produce **A1** as an off-white solid.

4-(2-Aminopyrimidin-5-yl)-N-ethyl-N-isopropyl-6-morpholino-1,3,5-triazin-2-amine (**A1**): off-white solid, yield 86%. mp 216–218 °C. ^{1}H NMR ($CDCl_3$) δ 9.19 (s, 2H), 5.41 (s, 2H), 5.22–4.86 (m, 1H), 3.90–3.85 (m, 4H), 3.80–3.68 (m, 4H), 3.60–3.39 (m, 2H), 1.22 (d, J = 5.2 Hz, 9H). ^{13}C NMR (DMSO-d_6): δ 165.2, 164.7 (2C), 164.1, 158.9 (2C), 119.4, 66.5 (2C), 46.0, 45.9, 43.7 (2C), 20.7, 20.5, 15.0. ESI-MS m/z: 345.2 $[M+H]^+$.

4-(2-Aminopyrimidin-5-yl)-N,N-diisopropyl-6-morpholino-1,3,5-triazin-2-amine (**A2**): off-white solid, yield 76%. mp 224–226 °C. ^{1}H NMR ($CDCl_3$) δ 9.19 (s, 2H), 5.40 (s, 2H), 4.45–4.40 (m, 2H), 3.90–3.85 (m, 4H), 3.79–3.72 (m, 4H), 1.37–1.30 (m, 12H). ^{13}C NMR (DMSO-d_6): δ 166.9, 165.2, 164.4, 164.1, 158.8 (2C), 119.5, 66.5 (2C), 45.4 (2C), 43.80 (2C), 20.9 (2C), 20.6 (2C). ESI-MS m/z: 359.8 $[M+H]^+$.

5-(4-(4-Methylpiperazin-1-yl)-6-morpholino-1,3,5-triazin-2-yl)pyrimidin-2-amine (**A3**): off-white solid, yield 86%. mp >250 °C. ^{1}H NMR ($CDCl_3$) δ 9.18 (s, 2H), 5.36 (s, 2H), 3.87 (s, 8H), 3.77–3.73 (m, 4H), 2.54–2.42 (m, 4H), 2.36 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 167.7, 165.3, 164.7, 164.3, 159.0 (2C), 119.1, 66.5 (2C), 54.9 (2C), 46.2 (2C), 43.7 (2C), 43.1. ESI-MS m/z: 357.2 $[M+H]^+$.

(1-(4-(2-Aminopyrimidin-5-yl)-6-morpholino-1,3,5-triazin-2-yl)piperidin-4-yl)(pyrrolidin-1-yl)methanone (**A4**): off-white solid, yield 87%. mp >250 °C. ^{1}H NMR ($CDCl_3$) δ 9.22 (s, 2H), 5.53 (s, 2H), 4.90–4.75 (m, 2H), 3.87–3.75 (m, 8H), 3.58–3.45 (m, 4H), 2.95–2.90 (m, 2H), 2.67–2.57 (m, 1H), 2.03–1.92 (m, 2H), 1.88–1.72

(m, 6H). ^{13}C NMR (DMSO-d_6) δ 172.6, 167.7, 165.3, 164.7, 164.4, 159.0 (2C), 119.1, 66.5 (2C), 63.3, 54.0 (2C), 46.2 (2C), 45.9 (2C), 26.1, 24.3, 18.5, 17.2. ESI-MS m/z: 440.2 $[M + H]^+$.

(1-(4-(2-Aminopyrimidin-5-yl)-6-morpholino-1,3,5-triazin-2-yl)piperidin-4-yl)(piperidin-1-yl)methanone (**A5**): white solid, yield 66%. mp >250 °C. ^{1}H NMR (DMSO-d_6) δ 9.03 (s, 2H), 7.25 (s, 2H), 4.90–4.74 (m, 2H), 3.72–3.68 (m, 8H,), 3.48–3.38 (m, 5H), 2.94–2.82 (m, 3H), 1.67–1.58 (m, 4H), 1.48–1.42 (m, 6H). ^{13}C NMR (DMSO-d_6) δ 172.4, 167.7, 165.2, 164.8, 164.4, 159.0 (2C), 119.4, 66.5 (2C), 55.4 (2C), 46.2 (2C), 42.5 (2C), 37.64, 28.7, 27.0, 25.8, 24.6 (2C). ESI-MS m/z: 454.2 $[M + H]^+$.

(1-(4-(2-Aminopyrimidin-5-yl)-6-morpholino-1,3,5-triazin-2-yl)piperidin-4-yl)(morpholino)methanone (**A6**): white solid, yield 76%. mp >250 °C. ^{1}H NMR (DMSO-d_6) δ 9.04 (s, 2H), 7.25 (s, 2H), 4.90–4.74 (m, 2H), 3.90–3.62 (m, 8H), 3.57–3.50 (m, 6H), 3.49–3.39 (m, 2H), 3.01–2.91 (m, 3H), 1.75–1.62 (m, 2H), 1.50–1.42 (m, 2H). ^{13}C NMR (DMSO-d_6) δ 172.9, 167.7, 165.2, 164.5, 164.4, 159.0 (2C), 119.1, 66.8, 66.7, 66.5 (2C), 56.5 (2C), 45.9 (2C), 42.0 (2C), 37.5, 28.5, 19.1. ESI-MS m/z: 456.2 $[M + H]^+$.

Tert-butyl 4-((4-chloro-6-morpholino-1,3,5-triazin-2-yl)(methyl)amino) piperidine-1-carboxylate (**b1**): To a solution of 4-(4,6-dichloro-1,3,5-triazin-2-yl)morpholine (4.00 g,17.0 mmol) in dry dichloromethane (20 mL) was added dropwise the mixture of *tert*-butyl 4-(methylamino)piperidine-1-carboxylate (3.65 g, 17.0 mmol) and triethylamine (4.71 mL) at 0 °C. The reaction mixture was stirred at room temperature until all starting materials consumed. The solvent was removed under reduced pressure. To the residue was added ethanol (4 mL) and water (30 mL), and the mixture was stirred at room temperature for 10 min. The solid was filtered, washed with water (5 mL× 3), and dried to afford compound **b1** (4.32 g) with a yield of 62%. mp 117–119 °C. ^{1}H NMR ($CDCl_3$) δ 4.11–3.80 (m, 8H), 3.76 (s, CH_3), 3.10–2.82 (m, 5H), 1.75–1.64 (m, 4H), 1.48 (s, 9H). ESI-MS m/z: 413.2 $[M + H]^+$.

Tert-Butyl 4-((4-chloro-6-morpholino-1,3,5-triazin-2-yl)(cyclopropyl) amino) piperidine-1-carboxylate (**b2**): To a solution of 4-(4,6-dichloro-1,3,5-triazin-2-yl)morpholine (1.76 g, 7.52 mmol) in dry tetrahydrofuran (20 mL) were added the mixture of *tert*-butyl 4-(cyclopropylamino) piperidine-1-carboxylate (2.16 g, 9.02 mmol), 4-methy lbenzenesulfonic acid (0.20 g, 1.16 mmol), and *N,N*-diisopropylethylamine (3.95 mL). The reaction mixture was heated at reflux for 4 h. The solvent was removed under reduced pressure. The residue was suspended in water (50 mL) and extracted with dichloromethane (20 mL × 3). The organic layer was washed by saturated sodium chloride, dried, filtered, and concentrated to afford compound **b2** (1.30 g). Yield: 71%. ^{1}H NMR ($CDCl_3$) δ 4.34–4.00 (m, 2H), 3.85–3.59 (m, 8H), 2.72–2.37 (m, 4H), 2.04–1.72 (s, 2H), 1.43 (s, 9H), 0.86–0.80 (m, 2H), 0.75–0.63 (m, 2H). ESI-MS m/z: 439.2 $[M + H]^+$.

General procedure for the synthesis of compound **B1** and **B2**

The mixture of 5-bromopyrimidin-2-amine (242 mg, 1.38 mmol), bis(pinacolato)diboron (386 mg, 1.52 mmol), KOAc (541 mg, 4.14 mmol), $PdCl_2$(dppf) (76 mg, 0.10 mmol), and 1,4-dioxane (20 mL) was heated at reflux under Ar atmosphere for 2.5 h, then concentrated in vacuum. To the resulted residue was added **b1** (400 mg, 0.97 mmol), K_2CO_3 (116 mg, 0.84 mmol), $PdCl_2$(dppf) (76 mg, 0.10 mmol), 1,4-dioxane (20 mL), and water (4 mL). The obtained mixture was heated at reflux under Ar atmosphere for 4 h. The volatile was removed under vacuum and the residue was purified through a column chromatography on silica gel with CH_2Cl_2 / MeOH (v:v = 50:1) as eluent to produce **B1** or **B2**.

Tert-Butyl-4-((4-(2-aminopyrimidin-5-yl)-6-morpholino-1,3,5-triazin-2-yl) (methyl)amino) piperidine-1-carboxylate (**B1**): white solid, yield 74%. mp 227–228 °C. ^{1}H NMR ($CDCl_3$) δ 9.18 (s, 2H), 5.45 (s, 2H), 4.11–3.80 (m, 8H), 3.77 (s, CH_3), 3.10–2.82 (m, 5H), 1.75–1.64 (m, 4H), 1.48 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 164.8, 164.1, 159.1 (3C), 154.7, 121.3, 79.7, 66.9 (2C), 52.3 (2C), 43.6 (2C), 28.3 (7C). ESI-MS m/z: 472.3 $[M + H]^+$.

Tert-Butyl-4-((4-(2-aminopyrimidin-5-yl)-6-morpholino-1,3,5-triazin-2-yl) (cyclopropyl)amino)piperidine-1-carboxylate (**B2**): off-white solid, yield 45%. mp 222–225 °C. ^{1}H NMR ($CDCl_3$) δ 9.19 (s, 2H), 5.48 (s, 2H), 4.34–4.00 (m, 2H), 3.85–3.59 (m, 8H), 2.72–2.37 (m, 4H), 2.04–1.72 (s, 2H), 1.43 (s, 9H), 0.86–0.80 (m, 2H), 0.75–0.63 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 167.1, 167.0, 164.6, 164.2, 159.1 (2C), 154.8, 121.4, 79.6, 66.8 (2C), 57.2, 43.6, 30.4, 28.5 (7C), 26.8, 9.2 (2C). ESI-MS m/z: 498.3 $[M + H]^+$.

General procedure for the synthesis of compound **B3** and **B4**

To a solution of **B1** or **B2** (300 mg, 0.63 mmol) in dichloromethane (10 mL), TFA (2 mL) was added. The resulting solution was stirred at room temperature for 2 h, concentrated in vacuum. The residue was suspended in saturated sodium carbonate solution (5.0 mL) and extracted with dichloromethane (20 mL × 3). The organic layer was washed by saturated sodium chloride, dried, filtered, and concentrated to afford compound **B3** or **B4** as a white solid.

4-(2-Aminopyrimidin-5-yl)-N-methyl-6-morpholino-N-(piperidin-4-yl)-1,3,5-triazin-2-amine (**B3**): white solid, yield 65%. mp >250 °C. ^{1}H NMR ($CDCl_3$) δ 9.19 (s, 2H), 5.38 (s, 2H), 4.00–3.75 (m, 8H), 4.87–4.65 (m, 1H), 3.71 (s, CH_3), 3.25–3.03 (m, 5H), 2.80–2.60 (m, 2H), 2.00–1.71 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 164.8 (2C), 164.0, 159.2 (3C), 121.5, 66.9 (2C), 52.4, 46.4 (3C), 43.6, 30.4, 30.2, 28.3. ESI-MS m/z: 372.2 $[M + H]^+$.

4-(2-Aminopyrimidin-5-yl)-N-cyclopropyl-6-morpholino-N-(piperidin-4-yl)-1,3,5-triazin-2-amine (**B4**): white solid. yield: 94%. mp: >250 °C. ^{1}H NMR (DMSO-d_6) δ 9.09 (s, 2H), 7.26 (s, 2H), 4.40–4.35 (m,

1H), 3.81–3.66 (m, 8H), 3.40–3.25 (m, 2H), 3.10–2.95 (m, 2H), 2.63–2.50 (m, 1H), 2.40–2.25 (m, 2H), 2.00–1.90 (m, 2H), 0.92–0.86 (m, 2H), 0.75–0.63 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 167.3, 166.9, 165.2, 164.4, 159.2 (2C), 66.52 (2C), 54.7, 43.7 (4C), 27.9, 27.4 (2C), 9.4 (2C). ESI-MS m/z: 398.3 $[M + H]^+$.

4-(2-Aminopyrimidin-5-yl)-N-(1-(cyclopropylmethyl) piperidin-4-yl)-N-methyl-6-morpholino-1,3,5-triazin-2-amine (**B5**): To a solution of **B3** (50 mg, 0.14 mmol), cyclopropylformaldehyde (10 mg, 11 μL), $NaBH(OAc)_3$ (45 mg, 0.22 mmol), and acetic acid (10 μL) were added. The resulted suspension was stirred overnight, quenched with aqueous sodium hydrogen carbonate and extracted with dichloromethane (10 mL×2). The combined organic phase was washed with brine, and dried over Na_2SO_4, filtered, concentrated in vacuum. The residue was purified by column chromatography on silica gel to produce **B5** (10 mg). Yield 17%. mp >250 °C. ^{1}H NMR ($CDCl_3$) δ 9.17 (s, 2H), 5.50 (s, 2H), 5.09–5.03 (m, 1H), 3.80–3.60 (m, 8H), 3.20–3.04 (m, 3H), 2.90–2.75 (m, 6H), 1.90–1.84 (m, 6H), 1.33–1.20 (m, 1H), 0.82–0.75 (m, 2H), 0.50–0.40 (m, 2H). ESI-MS m/z: 426.3 $[M + H]^+$.

General procedure for the synthesis of compounds **B6** and **B7**

The mixture containing compound **B3** (50 mg, 0.14 mmol), benzyl bromide (24 mg, 0.14 mmol), potassium carbonate (58 mg), and dimethylformamide (DMF) (10 mL) was stirred at room temperature overnight, concentrated in vacuum. The residue was purified by column chromatography on silica gel to produce **B6** or **B7**.

4-(2-Aminopyrimidin-5-yl)-N-(1-benzylpiperidin-4-yl)-N-methyl-6-morpholino-1,3,5-triazin-2-amine (**B6**): white solid, yield 89%. mp 232–234 °C. ^{1}H NMR ($CDCl_3$) δ 9.19 (s, 2H), 7.35–7.26 (m, 5H), 5.33 (s, 2H), 4.68–4.49 (m, 1H), 3.98–3.71 (m, 8H), 3.56 (s, 2H), 3.06–3.01 (m, 5H), 2.13–2.00 (m, 2H), 1.95–1.75 (m, 2H), 1.70–1.65 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 164.8, 164.0, 159.2 (2C), 129.2 (2C), 128.3 (4C), 127.1 (2C), 121.5, 66.9 (2C), 63.2 (2C), 53.32 (2C), 52.4, 43.6, 29.1, 28.9, 28.3. ESI-MS m/z: 462.3 $[M + H]^+$.

4-(2-Aminopyrimidin-5-yl)-N-methyl-6-morpholino-N-(1-(4-nitrobenzyl) piperidin-4-yl)-1,3,5-triazin-2-amine (**B7**): white solid, yield 87%. mp >250 °C. ^{1}H NMR ($CDCl_3$) δ 9.18 (s, 2H), 8.20 (d, J = 8.3 Hz, 2H), 7.53 (d, J = 7.6 Hz, 2H), 5.38 (s, 2H), 4.80–4.69 (m, 1H), 3.97–3.73 (m, 8H), 3.62 (s, 2H), 3.09–2.95 (m, 5H), 2.19–2.04 (m, 2H), 1.87–1.72 (m, 2H), 1.69–1.60 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 164.9, 164.0, 159.2 (2C), 147.1, 146.7, 129.5, 123.6 (4C), 121.9, 121.5, 66.9 (2C), 62.2 (2C), 53.4 (2C), 43.6 (2C), 29.2, 28.8, 28.3. ESI-MS m/z: 507.3 $[M + H]^+$.

General procedure for the synthesis of compound **B8** and **B10**

The mixture containing **B3** or **B4** (50 mg, 0.14 mmol), cyclopropylsulfonyl chloride (16 mg, 0.14 mmol), 4-dimethylaminopyridine (DMAP) (16 mg, 0.07 mmol), triethylamine (60 μL), and dichloromethane (15 mL) was stirred at room temperature for 2 h, concentrated in vacuum. The residue was purified by column chromatography on silica gel to produce **B8** or **B10**.

4-(2-Aminopyrimidin-5-yl)-N-(1-(cyclopropylsulfonyl) piperidin-4-yl)-N-methyl-6-morpholino-1,3,5-triazin-2-amine (**B8**): white solid, yield 65%. mp >250 °C. ^{1}H NMR ($CDCl_3$) δ 9.20 (s, 2H), 5.47 (s, 2H), 4.89–4.79 (m, 1H), 3.92–3.69 (m, 8H), 3.09–3.05 (m, 3H), 2.97–2.95 (m, 2H), 2.37–2.26 (m, 2H), 1.99–1.86 (m, 3H), 1.81–1.69 (m, 2H), 1.25–1.16 (m, 2H), 1.10–1.00 (m, 2H). ^{13}C NMR (DMSO-d_6) δ 167.5, 165.2, 164.8, 164.6, 159.0 (2C), 119.1, 66.51 (2C), 46.0 (4C), 43.6, 28.7, 28.5, 26.1, 26.0, 4.53 (2C). ESI-MS m/z: 498.2 $[M + Na]^+$.

4-(2-Aminopyrimidin-5-yl)-N-cyclopropyl-N-(1-(cyclopropylsulfonyl) piperidin-4-yl)-6-morpholino-1,3,5-triazin-2-amine (**B10**): white solid, yield 62%. mp 240–243 °C. ^{1}H NMR ($CDCl_3$) δ 9.19 (s, 2H), 5.52 (s, 2H), 4.41–4.38 (m, 1H), 3.95–3.75 (m, 9H), 2.90–2.85 (m, 2H), 2.50 (s, 1H), 2.28–2.19 (m, 4H), 1.95–1.90 (m, 2H), 1.22–1.16 (m, 2H), 1.07–0.99 (m, 2H), 0.95–0.90 (m, 2H), 0.81–0.70 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 167.1 (2C), 164.6, 164.0, 159.1 (2C), 121.2, 66.8 (2C), 58.4, 56.4, 46.6 (2C), 43.6, 30.2 (2C), 26.0, 18.4, 9.2 (2C), 4.4 (2C). ESI-MS m/z: 502.3 $[M + H]^+$.

4-(2-Aminopyrimidin-5-yl)-N-methyl-6-morpholino-N-(1-tosylpiperidin-4-yl)-1,3,5-triazin-2-amine (**B9**): To a solution of 4-methylbenzenesulfonyl chloride (26 mg, 0.14 mmol) in dry dichloromethane (10 mL) was added dropwise the mixture of **B3** (50 mg, 0.14 mmol), and triethylamine (4.71 mL) at 0 °C. The reaction mixture was stirred at room temperature until all starting materials consumed. The solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel with CH_2Cl_2 / MeOH (v: v = 50: 1) as eluent to produce **B9** as a white solid. Yield 74%. mp >250 °C. ^{1}H NMR ($CDCl_3$) δ 9.18 (s, 2H), 7.34 (d, J = 7.6 Hz, 2H), 7.22 (d, J = 7.9 Hz, 2H), 5.43 (s, 2H), 4.93–4.80 (m, 1H), 3.99–3.74 (m, 10H), 3.07–3.00 (m, 5H), 2.88–2.80 (m, 2H), 2.38 (s, 3H), 1.83–1.75 (m, 4H). ^{13}C NMR (DMS-d_6): δ 164.8, 164.6, 159.0, 143.9, 133.8, 130.4 (3C), 127.9 (4C), 119.1, 66.48 (2C), 56.50 (3C), 46.03 (2C), 43.6, 21.5, 19.0 (2C). ESI-MS m/z: 548.3 $[M + Na]^+$.

General procedure for the synthesis of compound **B11**–**B19**

A mixture of **B3** or **B4** (50 mg, 0.14 mmol), substituted carboxylic acid (0.14 mmol), HATU (54 mg, 0.14 mmol), N, N-diisopropylethylamine (0.42 mmol) and dichloromethane (15 mL) was stirred at room temperature for 2 h, then concentrated in vacuum. The residue was purified by column chromatography on silica with CH_2Cl_2 / MeOH (v: v = 50:1) as eluent to produce **B11–B19** as a white solid.

1-(4-((4-(2-Aminopyrimidin-5-yl)-6-morpholino-1,3,5-triazin-2-yl)(methyl)amino)piperidin-1-yl)ethan-1-one (**B11**): white solid, yield 56%. mp >250 °C. ^{1}H NMR ($CDCl_3$) δ 9.18 (s, 2H), 5.51 (s, 2H), 5.10–4.70 (m, 2H), 4.01–3.67 (m, 9H), 3.20–3.04 (m, 3H), 2.63–2.58 (m, 2H), 2.14 (s, 3H), 1.84–1.62 (m, 4H). ^{13}C NMR (DMSO-d_6) δ

168.5, 165.3 (2C), 164.7, 163.8, 159.0 (2C), 119.2, 66.5 (2C), 52.4, 49.1 (4C), 45.8, 43.7, 21.71 (2C). ESI-MS m/z: 414.3 $[M + H]^+$.

(4-((4-(2-Aminopyrimidin-5-yl)-6-morpholino-1,3,5-triazin-2-yl)(methyl)amino)piperidin-1-yl)(cyclopropyl) methanone (**B12**): white solid, yield 79%. mp:>250 °C. ^{1}H NMR ($CDCl_3$) δ 9.19 (s, 2H), 5.52 (s, 2H), 5.20–5.05 (m, 1H), 4.90–4.40 (m, 2H), 4.00–3.75 (m, 8H), 3.22–3.03 (m, 4H), 2.67–2.58 (m, 1H), 1.84–1.63 (m, 4H), 1.24 (t, J = 7.0 Hz, 1H), 1.00–0.90 (m, 2H), 0.80–0.75 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 171.3 (2C), 165.2 (2C), 164.6, 159.1, 159.0, 119.1, 66.5 (2C), 56.5, 55.4, 52.6 (2C), 44.9, 43.7, 28.7, 19.0, 10.8, 7.3 (2C). ESI-MS m/z: 462.3 $[M + Na]^+$.

(4-((4-(2-Aminopyrimidin-5-yl)-6-morpholino-1,3,5-triazin-2-yl)(methyl)amino)piperidin-1-yl)(cyclobutyl) methanone (**B13**): white solid, yield 51%. mp >250 °C. ^{1}H NMR ($CDCl_3$) δ 9.18 (s, 2H), 5.52 (s, 2H), 5.15–4.66 (m, 2H), 3.99–3.68 (m, 9H), 3.29–3.20 (m, 1H), 3.03–2.95 (m, 4H), 2.70–2.50 (m, 1H), 2.40–2.25 (m, 2H), 2.25–2.17 (m, 2H), 2.09–1.81 (m, 2H), 1.80–1.52 (m, 4H). ^{13}C NMR (DMSO-d_6): δ 172 (2C), 165.2 (2C), 164.6, 159.1, 159.0, 119.1, 66.5 (2C), 52.5, 52.4, 44.4, 44.3, 43.7, 41.2, 36.8, 28.6, 25.1 (2C), 17.8 (2C). ESI-MS m/z: 476.3 $[M + Na]^+$.

(4-((4-(2-Aminopyrimidin-5-yl)-6-morpholino-1,3,5-triazin-2-yl)(methyl)amino)piperidin-1-yl)(cyclopentyl) methanone (**B14**): white solid, yield 51%. mp 233–235 °C. ^{1}H NMR ($CDCl_3$) δ 9.19 (s, 2H), 5.45 (s, 2H), 5.20–4.75 (m, 2H), 4.12–4.05 (m, 1H), 4.02–3.67 (m, 8H), 3.23–2.92 (m, 5H), 2.63–2.50 (m, 1H), 1.93–1.64 (m, 12H). ^{13}C NMR (DMSO-d_6): δ 173.6 (2C), 165.2 (2C), 164.6, 159.1, 158.9, 119.1, 66.51 (2C), 52.6, 52.5, 44.8, 43.7, 41.3, 30.2 (2C), 30.1 (2C), 26.2 (2C), 26.1 (2C). ESI-MS m/z: 468.3 $[M + H]^+$.

(4-((4-(2-Aminopyrimidin-5-yl)-6-morpholino-1,3,5-triazin-2-yl)(methyl)amino)piperidin-1-yl)(cyclohexyl) methanone (**B15**): white solid, yield 53%. mp 181–182 °C. ^{1}H NMR ($CDCl_3$) δ 9.19 (s, 2H), 5.55 (s, 2H), 5.05–4.75 (m, 2H), 4.07–4.00 (m, 1H), 3.82–3.70 (m, 8H), 3.29–2.99 (m, 4H), 2.62–2.51 (m, 2H), 1.80–1.75 (m, 8H), 1.51–1.46 (m, 4H), 1.43–1.25 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 173.7 (2C), 165.2 (2C), 164.6, 159.1, 159.0, 119.1, 66.51 (2C), 54.0, 52.5, 44.7, 43.7, 42.3, 41.1, 38.7, 29.7, 26.1, 25.7 (2C), 18.5, 17.2, 13.0. ESI-MS m/z: 482.3 $[M + H]^+$.

(4-((4-(2-Aminopyrimidin-5-yl)-6-morpholino-1,3,5-triazin-2-yl)(methyl)amino)piperidin-1-yl)(tetrahydro-2H-pyran-4-yl)methanone (**B16**): white solid, yield 67%. mp 237–239 °C. ^{1}H NMR ($CDCl_3$) δ 9.18 (s, 2H), 5.44 (s, 2H), 5.16–4.70 (m, 2H), 4.04–4.00 (m, 3H), 3.97–3.63 (m, 8H), 3.48–3.44 (m, 2H), 3.18–3.01 (m, 4H), 2.80–2.64 (s, 2H), 2.06–1.79 (m, 4H), 1.70–1.45 (m, 4H, CH_2). ^{13}C NMR (DMSO-d_6): δ 172.6, 165.2 (2C), 164.6, 159.1, 159.0, 119.1, 66.8 (4C), 66.5 (2C), 52.6 (2C), 44.7, 43.7, 36.8, 29.5, 29.5. ESI-MS m/z: 506.3 $[M + Na]^+$.

(4-((4-(2-Aminopyrimidin-5-yl)-6-morpholino-1,3,5-triazin-2-yl)(cyclopropyl)amino)piperidin-1-yl)(cyclopropyl)methanone (**B17**): white solid, yield 46%. mp: >250 °C. ^{1}H NMR ($CDCl_3$) δ 9.19 (s, 2H), 5.53 (s, 2H), 4.77–4.60 (m, 1H), 4.59–4.25 (m, 2H), 4.00–3.78 (m, 8H), 3.19–3.15 (m, 1H), 2.65–2.53 (m, 2H), 2.19–2.02 (m, 2H), 2.02–1.79 (m, 3H), 1.09–0.85 (m, 4H), 0.80–0.73 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 171.8, 167.1, 167.0, 164.6, 164.0, 159.1 (2C), 121.31, 66.8 (2C), 57.4 (2C), 45.7, 43.6 (2C), 42.4, 11.0 (2C), 9.3, 7.3 (2C). ESI-MS m/z: 466.3 $[M + H]^+$.

(4-((4-(2-Aminopyrimidin-5-yl)-6-morpholino-1,3,5-triazin-2-yl)(cyclopropyl)amino)piperidin-1-yl)(cyclopentyl)methanone (**B18**): white solid, yield 63%. mp 233–236 °C. Anal. calcd for $C_{25}H_{35}N_9O_2$: C, 60.83; H, 7.15; N, 25.54; found: C, 60.86; H, 7.13; N, 25.57%; ^{1}H NMR ($CDCl_3$) δ 9.18 (s, 2H), 5.52 (s, 2H), 4.81 (d, J = 8.7 Hz, 1H), 4.43 (s, 1H), 4.09 (d, J = 8.7 Hz, 1H), 3.80–3.73 (m, 8H), 3.11–2.85 (m, 2H), 2.60–2.50 (m, 2H), 2.18–1.97 (m, 2H), 1.95–1.74 (m, 8H), 1.66–1.51 (m, 2H), 0.92 (m, 2H), 0.79–0.66 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 174.4, 167.1, 167.0, 164.6, 164.1, 159.1 (2C), 121.1, 66.8 (2C), 58.3, 57.5, 45.6, 43.6, 42.2, 41.1 (2C), 31.2, 30.3, 30.1, 26.1, 26.0, 18.4, 9.3, 9.2. ESI-MS m/z: 494.4 $[M + H]^+$.

4-((4-(2-Aminopyrimidin-5-yl)-6-morpholino-1,3,5-triazin-2-yl)(cyclopropyl)amino)piperidin-1-yl)(tetrahydro-2H-pyran-4-yl)methanone (**B19**): white solid, yield 58%. mp 149–151 °C. Anal. calcd for $C_{25}H_{35}N_9O_3$: C, 58.92; H, 6.92; N, 24.74; found: C, 58.96; H, 6.89; N, 24.78%; ^{1}H NMR ($CDCl_3$) δ 9.17 (s, 2H), 5.52 (s, 2H), 4.81 (d, J = 8.7 Hz, 1H), 4.43–4.35 (m, 1H), 4.03–4.00 (m, 2H), 3.80–3.75 (m, 8H), 3.48–3.44 (m, 2H), 3.15–3.11 (m, 1H), 2.80–2.74 (m, 1H), 2.63–2.49 (m, 2H), 2.21–1.83 (m, 7H), 1.63–1.50 (m, 2H), 0.95–0.84 (m, 2H), 0.79–0.66 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 172.7, 167.1, 167.0, 164.6, 164.2, 159.1 (2C), 121.1, 67.3 (2C), 66.8, 58.2, 57.4, 45.5, 43.6, 42.1, 37.7 (2C), 29.3, 29.1, 27.3, 18.4 (2C), 9.3 (2C). ESI-MS m/z: 510.3 $(M + H)^+$.

Antiproliferative assays by MTT method

VS-5584 was purchased from Shanghai Biochempartner Company (Purity: 99%, high-performance liquid chromatography), and 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl-*2H*-tetrazolium bromide (MTT) was purchased from Sigma (St. Louis, MO, USA). The cellular chemosensitivity was determined by using a modified MTT method assay in human cell lines HCT-116 and MCF-7, which was similar to the procedures described.[16] In brief, HCT-116, MCF-7 cells in 200 µL culture medium were seeded into 96-well microplates at 3000–5000 cells/well, respectively, and cultured in Dulbecco's Modified Eagle Medium with 10% fetal bovine serum (FBS), or RPMI-1640 with 10% calf serum, incubated at 37 °C for 24 h prior to drug exposure. Cell numbers were titrated to keep control cells growing in the exponential phase throughout the 72 h incubation period. Cells were treated with final concentrations of 10.0, 5.0, 1.0, and 0.5 µM of tested compounds simultaneously and incubated for 72 h and then 20 µL of MTT solution (5 mg/mL in PBS) was added to each well at lucifugal condition and incubated for 4 h at 37 °C. The formed purple formazan crystals were pelleted at the bottom of the well, separated from the supernatant, and dissolved in 200 µL of DMSO. The optical density at 570 nm was determined by

Varioskan Flash Multimode Reader (Thermo scientific). Three separate experiments with triplicate data were performed to obtain mean cell viability. The IC_{50} value was calculated according to the inhibition ratios.

PI3K enzymatic activity assay

PI3K and mTOR enzymatic assay was carried out as described in Wang et al.[18]

Molecular modeling

The PI3Kα (PDB code: 5UK8) was used in the molecular modeling, and the docking procedure was performed as described in Hei et al.[17] The protein-ligand complex crystal structure was chosen as the template, to elucidate the binding mode of **B18**. Protein structure was downloaded from Protein Data Bank (PDB 5UK8). The molecular docking procedure was performed within SYBYLX 2.0 software. The EGFR enzyme was defined as a receptor and the site sphere was selected based on the ligand binding location. Ligand was removed and compound **B18** was placed, other parameters were set as default. After accomplishment of the molecular docking procedure, 20 docking poses were scored and selected based on calculated energy. The best docking pose was prepared using PyMOL as showed in Figure 3.

Declaration of conflicting interests

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