Design And Synthesis Of Novel Pyrazolopyrimidine-Based Derivatives As Reversible BTK Inhibitors With Potent Antiproliferative Activity In Mantle Cell Lymphoma

Fansheng Ran

Nantong University

Yang Liu

Shandong University

Guisen Zhao (✉ guisenzhao@sdu.edu.cn)

Shandong University <https://orcid.org/0000-0002-5391-7993>

Research Article

Keywords: BTK inhibitors, Mantle cell lymphoma, Apoptosis, Mitochondrial membrane potential, Reactive oxygen species

Posted Date: December 3rd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1123045/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Medicinal Chemistry Research on February 23rd, 2022. See the published version at <https://doi.org/10.1007/s00044-022-02861-7>.

Abstract

Development of Bruton's tyrosine kinase (BTK) inhibitors is of great value and significance in the treatment of B-cell malignancies and autoimmune diseases. Herein, a novel class of pyrazolopyrimidine-based BTK inhibitors were designed and evaluated in mantle cell lymphoma (MCL) cell lines. We demonstrated that target compounds had made great progress in improvement of antiproliferative activity compared to lead compound. Compounds **13c**, **13g**, **13h**, **13l**, **13n** and **13o** demonstrated effectively antiproliferative activity in MCL cells lines with single-digit micromolar potency. Furthermore, compound **13l** specifically disturbed mitochondrial membrane potential and increased reactive oxygen species level in Z138 cells in a dose-dependent manner. **13l** induced cell apoptosis through the caspase 3- mediated apoptotic pathway in Z138 cells. Overall, this study provides valuable lead compounds for developing antitumor agents.

1. Introduction

Mantle cell lymphoma (MCL) is a rare and incurable B-cell lymphoma defined by the (11;14)(q13;q32) translocation and resultant overexpression of cyclin D1.¹⁻² It appears that the incidence of MCL in Asian countries is lower than that in western countries.³ MCL is most commonly treated with combination chemo-immunotherapy at diagnosis because of the poor prognosis. Long-term follow-up on chemo-immunotherapy studies has shown durable remissions in some patients; however, long-term toxicities remain a serious concern with chemotherapy. Developing novel treatment strategies is important for patients with MCL. In recent years, the therapeutic options based on MCL pathogenesis have made a breakthrough, especially in blocking B cell receptor (BCR) signaling pathway.⁴

Bruton's tyrosine kinase (BTK), a member of Tec family kinase, is expressed in B-lineage cells and myeloid cells, and is a key component of BCR signaling pathway, which regulates the survival, activation, proliferation, differentiation and maturation of B cells.⁵⁻⁶ In BCR signaling pathway, BTK is phosphorylated and activated by SYK and LYN kinases. Activated BTK leads to activation of multiple signaling networks including RAS/RAF/MEK/ERK, PI3K/AKT/mTOR, and nuclear factor kappa B (NF- κ B) pathways, which regulate the activation, survival, and proliferation of B cells.⁷⁻⁹ It has been reported that BTK is overexpressed and aberrantly active in the pathogenesis of various B cell hematological malignancies, including MCL, chronic lymphocytic leukemia (CLL), and waldenstrom's macroglobulinemia (WM).¹⁰⁻¹² BTK is considered as a promising target for the treatment of B-cell malignancies.

During recent decade, various BTK inhibitors have been developed. Based on the binding modes with the BTK catalytic domains, reported inhibitors can be classified into covalent and non-covalent inhibitors. Most covalent inhibitors have Michael acceptor groups, forming a covalent bond with the cysteine 481 (Cys481) residue, whereas non-covalent BTK inhibitors interact with BTK via hydrogen bonds and hydrophobic interactions.¹³ Covalent BTK inhibitors, ibrutinib (2013), acalabrutinib (2017), zanubrutinib (2019), tirabrutinib (2020), and orelabrutinib (2020) have been approved to treat B-cell malignancies in

the US, Japan and China.⁸ Despite the remarkable potency and efficacy of covalent BTK inhibitors against B-cell malignancies in the clinics, a subset of patients loses the initial treatment responses or relapse after the therapy. The mutation of Cys481 to serine (C481S) commonly occurs in patients receiving BTK inhibitor treatment, resulting in disruption of covalent binding and representing the main cause of acquired resistance for covalent BTK inhibitors.¹⁴⁻¹⁵

The ability of these non-covalent BTK inhibitors in overcoming the acquired resistance associated with covalent BTK inhibitors has been proved in multiple clinical studies. Fenebrutinib (GDC-0853, **6**), a highly selective reversible BTK inhibitor, is undergoing a phase 1 trial (NCT01991184) which includes relapsed/refractory NHL or CLL patients bearing BTK^{C481S} mutation. ARQ 531 (MK-1026, **7**), and LOXO-305 (**8**) are in phase 2 (NCT04728893) and phase 3 (NCT04662255) for treating relapsed/refractory B cell malignancies, respectively. Compared to covalent BTK inhibitors, the development of non-covalent BTK inhibitors has been relatively limited. No reversible BTK inhibitors have been approved for clinical applications as of now. In addition, the non-covalent binding with the target decreases the toxicity and potential risks associated with covalent inhibitors, such as allergic reactions resulting from haptenization of serum proteins by the Michael acceptors.^{5, 16} Therefore, development of reversible BTK inhibitors has important clinical value and significance.

In previous studies, a reversible BTK inhibitor **9** exhibited moderate inhibitory activity against BTK (IC_{50} = 0.23 μ M) and low antiproliferative activity in MCL cells.¹⁷ In this work, we performed further structural modifications for compound **9** to improve its antiproliferative activity. It has been reported that optimization of the linker and terminal groups in the BTK inhibitors stands a potential strategy to improve its anti-tumor activity.¹⁷⁻¹⁹ Based on these, we retained the backbone and hydrophobic groups of lead compound **9**, extended the linker and introduced diverse substituents (single, multiple, electron-withdrawing, or electron-donating) on the terminal benzene ring for additional interactions with the amino acid residues around the solvent region. In this way, a series of 3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine-based derivatives are designed (**Figure 2**).

2. Results And Discussion

2.1. Chemistry

The synthetic routes of these compounds are illustrated in Scheme 1. Condensation of various substituted amines (**10a-10v**) and bromoacetyl bromide afforded compounds **11a-11v**. *N*-alkylation of compound **12**¹⁷ with compounds **11a-11v** gave us the target compounds **13a-13v**.

2.2. *In vitro* BTK kinase activity

The newly synthesized compounds were evaluated for their activity against BTK via a kinase profiler radiometric protein kinase assay. Compounds with BTK residual activity < 20% at 10 μ M were further chosen to test the IC_{50} values. As shown in Table 1, introduction of nitro or halogen group at R₁ is more

beneficial to improve BTK inhibitory activity than trifluoromethyl group (**13a** vs **13b**, **13c** and **13d**). Extending the length of linker did not increase the BTK inhibitory activities (**13e-13g**). Electron-withdrawing group (**13j-13l**) on the terminal phenyl group showed more potent inhibitory activity against BTK than electron-donating group (**13i**), especially compound **13j**, the best candidate from the designed series, demonstrated comparable BTK inhibition potency to the lead **9** (IC_{50} values = 0.35 μ M and 0.23 μ M, respectively). Compounds **13l** and **13o** contained nitro group and dicyano group also displayed favorable BTK kinase activity with IC_{50} of 1.1 and 1.98 μ M, respectively.

Table 1. The inhibitory effects of series **13** on BTK activity

Code	n	R ₁	R ₂	R ₃	BTK residual activity at 10μM (%) or IC ₅₀ (μM)
9	-	-	-	-	0.23
13a	0	CF ₃	H	H	93%
13b	0	NO ₂	H	H	39%
13c	0	Br	H	H	48%
13d	0	Cl	H	H	65%
13e	1	F	H	H	61%
13f	2	H	F	H	62%
13g	0	H	F	H	47%
13h	0	H	Br	H	51%
13i	0	H	Me	H	47%
13j	0	H	CN	H	0.35
13k	0	H	CF ₃	H	39%
13l	0	H	NO ₂	H	1.10
13m	0	Cl	F	H	53%
13n	0	Cl	CN	H	36%
13o	0	CN	CN	H	1.98
13p	0	CF ₃	CN	H	90%
13q	0	CF ₃	Br	H	86%
13r	0	H	H	2-Br	90%
13s	0	Cl	H	2-F	88%
13t	0	F	F	2-F	37%
13u	0	Br	H	2-Me	127%
13v	0	Me	H	5-Me	105%

2.3. Antiproliferative activity in MCL cell lines

In order to further study the structure-activity relationship, we performed the antiproliferation assay in MCL cell lines (Mino, Jeko-1, Z138 and Maver-1). As listed in Table 2, the antiproliferative activities of

most compounds **13** were significantly improved compared with lead compound **9**. Similar to our findings in BTK activity assay, extension of linker did not enhance their antiproliferative activities (**13g** vs **13e** and **13f**). Compounds **13j-13l** contained electron-withdrawing group on the terminal phenyl group also exhibited more potent growth inhibition activity than that of compound **13i** with electron-donating group. The introduction of electron-donating group (**13u** and **13v**) was not conducive to biological activity. The nitro group occupying the R₂ position indicated better antiproliferative activity than the R₁ position (**13l** vs **13b**). Except for **13q**, compounds **13m-13q** with multiple substituents at R₁ and R₂ positions showed potent antiproliferative activity against MCL cells. Meaningfully, **13c**, **13g**, **13h**, **13l**, **13n** and **13o** demonstrated improved antiproliferative activity with IC₅₀ values at the single-digit micromolar levels. BTK-independent mechanisms might be involved for the compounds with potent antiproliferative activities while limited BTK inhibition.

Table 2. The antiproliferative activities of compounds **13** in MCL cell lines.

Code	Cell viability assay, IC ₅₀ /μM			
	Mino	Jeko-1	Z138	Maver-1
9	50.3	>60	>60	>60
13a	16.1	29.7	22.2	22.7
13b	14.2	11.3	10.7	12.5
13c	7.6	10.0	7.0	10.5
13d	15.1	15.5	9.6	18.0
13e	5.0	39.7	30.5	7.6
13f	> 60	> 60	54.3	> 60
13g	7.2	5.4	4.2	6.5
13h	8.4	10.1	8.2	10.7
13i	22.5	>60	>60	>60
13j	9.7	12.8	7.7	21.2
13k	20.7	13.7	22.2	21.1
13l	5.4	4.3	2.5	6.1
13m	10.4	11.3	8.2	12.5
13n	7.6	3.5	4.3	10.0
13o	7.0	5.2	6.6	7.7
13p	12.7	10.3	10.6	10.8
13q	> 60	> 60	> 60	> 60
13r	16.1	29.7	22.2	22.7
13s	> 60	> 60	51.3	> 60
13t	16.9	13.9	10.4	13.8
13u	> 60	> 60	> 60	> 60
13v	50.4	>60	>60	>60

2.4. Cell apoptosis assay

Compound **13l** with excellent antiproliferative activity was selected for further studies on its apoptosis inductive effect on Z138 and Jeko-1 cells. The results were shown in Figure 3A and 3B. After 24 h of

treatment, dose-dependent apoptosis effects were observed in Z138 and Jeko-1 cells. After the treatment at 20 μ M, 42.8% and 36.6% of apoptotic cells were detected in Z138 and Jeko-1 cells, respectively. The cleavage level of caspase-3 in Z138 cells after **13l** treatment were also measured. Impressively, as shown in Figure 3C, **13l** induced the cleavage of caspase-3 in a dose-dependent manner, indicating that **13l** induced cell apoptosis through the caspase 3- mediated apoptotic pathway.

2.5. Effect on mitochondrial membrane potential

Mitochondrial membrane potential($\Delta\Psi_m$) loss is an early event in apoptosis. We evaluated the effect of **13l** on $\Delta\Psi_m$ in MCL via a JC-1 fluorescence probe, which selectively enters mitochondria and changes own color from red to green as a sign for $\Delta\Psi_m$ decreases. As shown in Figure 4A, the red fluorescence intensity gradually decreased and green fluorescence gradually increased as the concentrations of **13l** increased, indicating a gradually attenuation of $\Delta\Psi_m$ level in Z138 cells.

2.6. Regulation of reactive oxygen species levels

Reactive oxygen species (ROS) accumulation has been reported to cause cellular apoptosis by the mitochondria-dependent pathway.²⁰ It has been reported that BTK has a role in negatively regulating ROS levels, and ibrutinib can enhance ROS production in CLL.^{21–22} Then we used the DCFH-DA probe to evaluate the effect of **13l** on ROS level in Z138 cells. As shown in Figure 4B, the green fluorescence intensity gradually increased as the concentrations of **13l** increased. **13l** enhanced ROS production in Z138 cells in a dose-dependent manner, which may be an important component in the potency of the antiproliferative activity.

2.7. Induction autophagy in MCL cells

Autophagy is considered a mechanism of cell death induced by death programs. Muqbil et al. reported that BTK inhibitors treatment caused autophagic cell death of Hodgkin's lymphoma cells.²³ Therefore, we investigated the induction of autophagy by **13l** in Z138 cells. As shown in Figure 3C, the western blotting results showed that expression of LC3B, a critical component in regulating the autophagy, was significantly increased in a dose-dependent manner after **13l** treatment of Z138 cells.

3. Conclusion

In this study, we outlined the design, synthesis and biological evaluation of novel pyrazolopyrimidine-based derivatives as reversible BTK inhibitors for treating MCL. Compared to lead compound **9**, most target compounds had made great progress in antiproliferative activity. Compounds **13c**, **13g**, **13h**, **13l**, **13n** and **13o** displayed favorable antiproliferative activity in MCL cells lines with single-micromolar IC₅₀ values. Especially, compound **13l** specifically disturbed mitochondrial membrane potential and induced cell apoptosis through the caspase 3- mediated apoptotic pathway in Z138 cells. Furthermore, the upregulated ROS levels and autophagy induction may be the possible mechanisms for its potent

antiproliferative activity. In summary, this study provides valuable lead candidates for developing anti-lymphoma agents.

4. Experimental Section

4.1. Chemistry

All chemical solvents, catalysts, and starting materials were purchased from commercial suppliers and used without further purification. Reaction progress was examined by SGF254 plates under UV (254 nm). Compounds were purified using silica gel chromatography (60 Å, 200-300 mesh). Melting points of target compounds were determined by a Büchi capillary melting point apparatus without correction. ¹H NMR and ¹³C NMR spectra of the compounds were recorded using the Bruker Avance DRX-400 Spectrometer at 400 MHz and 100 MHz, respectively. Chemical shifts (δ) are given in ppm relative to tetramethylsilane (TMS, 0.0 ppm) in CDCl₃ or DMSO-*d*₆; *J* values are given in Hz.

4.2. General synthesis of target compounds 13

Various substituted amines **10a-10v** (2 mmol) and NaHCO₃ (7.4 mmol) were added to 1/1 ethyl acetate/water mixture (5 mL). Subsequently, bromoacetyl bromide (4.6 mmol) was added dropwise to the mixture under ice bath conditions. After dropping, the mixture was stirred at room temperature for 1 h. Progress of the reaction was detected by TLC. Upon completion, the organic layer was separated and the water layer was extracted. The combined organic layer phases were dried and concentrated to obtain **11a-11v**. Then compounds **11** (0.6 mmol) was added to a mixture of compound **12** (0.5 mmol) and K₂CO₃ (0.75 mmol) in *N,N*-dimethylformamide (5 mL). The mixture was stirred at room temperature for 5 h. Upon completion, the reaction solution was quenched by pouring it into water (50 mL). The suspension was filtered, and the cake was purified by column chromatography (methanol/dichloromethane, 1/200 - 1/50) to obtain target compounds **13**.

2-(4-Amino-3-(4-phenoxyphenyl)-1 H -pyrazolo[3,4-*d*]pyrimidin-1-yl)- N -(3-(trifluoromethyl) phenyl) acetamide (**13a**)

White solid, yield 73%, mp:208-212°C, ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.73 (s, 1H), 8.20 (s, 1H), 8.02 (s, 1H), 7.69 (d, *J* = 7.1 Hz, 1H), 7.62 (d, *J* = 7.5 Hz, 2H), 7.55 – 7.47 (m, 1H), 7.37 (s, 3H), 7.25 – 6.47 (m, 7H), 5.21 (s, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 166.33, 158.69, 157.69, 156.73, 156.42, 155.71, 144.25, 139.80, 130.62, 130.48, 130.03 (q, *J* = 31 Hz), 128.22, 124.50 (q, *J* = 271 Hz), 124.31, 123.24, 120.46 (q, *J* = 3 Hz), 119.54, 119.43, 115.73 (q, *J* = 3 Hz), 97.76, 50.15. HRMS(ESI) *m/z* calcd for C₂₆H₁₉F₃N₆O₂ [M + H]⁺ 505.1594, found 505.1587.

2-(4-Amino-3-(4-phenoxyphenyl)-1 H -pyrazolo[3,4-*d*]pyrimidin-1-yl)- N -(3-nitrophenyl) acetamide (**13b**)

White solid, yield 71%, mp:236-240°C, ^1H NMR (400 MHz, DMSO- d_6) δ 10.96 (s, 1H), 8.61 (s, 1H), 8.26 (s, 1H), 7.97 – 7.89 (m, 2H), 7.72 – 7.61 (m, 3H), 7.44 (t, J = 7.9 Hz, 2H), 7.35 – 6.87 (m, 7H), 5.29 (s, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 166.51, 158.67, 157.68, 156.70, 156.44, 155.69, 148.44, 144.28, 140.11, 130.86, 130.63, 130.48, 128.17, 125.65, 124.32, 119.53, 119.43, 118.67, 113.79, 97.74, 50.16. HRMS(ESI) m/z calcd for $\text{C}_{25}\text{H}_{19}\text{N}_7\text{O}_4$ $[\text{M} + \text{H}]^+$ 482.1571, found 482.1568.

2-(4-Amino-3-(4-phenoxyphenyl)-1 H -pyrazolo[3,4- d]pyrimidin-1-yl)- N -(3-bromophenyl) acetamide (13c)

White solid, yield 71%, mp:188-190°C, ^1H NMR (400 MHz, DMSO- d_6) δ 10.64 (s, 1H), 8.26 (s, 1H), 7.93 (s, 1H), 7.68 (d, J = 8.6 Hz, 2H), 7.58 – 7.39 (m, 4H), 7.37 - 7.25 (m, 3H), 7.22 – 7.11 (m, 5H), 5.24 (s, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 166.06, 158.67, 157.66, 156.72, 156.41, 155.66, 144.19, 140.60, 131.39, 130.63, 130.47, 128.21, 126.72, 124.32, 122.09, 122.00, 119.53, 119.43, 118.42, 97.71, 50.12. HRMS(ESI) m/z calcd for $\text{C}_{25}\text{H}_{19}\text{BrN}_6\text{O}_2$ $[\text{M} + \text{H}]^+$ 515.0826, found 515.0821.

2-(4-Amino-3-(4-phenoxyphenyl)-1 H -pyrazolo[3,4- d]pyrimidin-1-yl)- N -(3-chlorophenyl) acetamide (13d)

White solid, yield 83%, mp:236-238°C, ^1H NMR (400 MHz, DMSO- d_6) δ 10.58 (s, 1H), 8.26 (s, 1H), 7.65 (dd, J = 27.0, 5.5 Hz, 4H), 7.41 (d, J = 18.7 Hz, 4H), 7.32 – 6.43 (m, 7H), 5.23 (s, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 165.80, 158.67, 157.66, 156.72, 156.39, 155.64, 144.15, 138.03, 130.62, 130.47, 129.26, 128.23, 127.64, 124.31, 121.21, 119.62, 119.53, 119.42, 97.72, 50.12. HRMS(ESI) m/z calcd for $\text{C}_{25}\text{H}_{19}\text{ClN}_6\text{O}_2$ $[\text{M} + \text{H}]^+$ 471.1331, found 471.1328.

2-(4-Amino-3-(4-phenoxyphenyl)-1 H -pyrazolo[3,4- d]pyrimidin-1-yl)- N -(3-fluorobenzyl) acetamide (13e)

White solid, yield 77%, mp:229-232°C, ^1H NMR (400 MHz, DMSO- d_6) δ 8.75 (t, J = 5.6 Hz, 1H), 8.26 (s, 1H), 7.67 (d, J = 8.4 Hz, 2H), 7.44 (t, J = 7.8 Hz, 2H), 7.37 (q, J = 7.4 Hz, 1H), 7.25 – 6.68 (m, 10H), 5.07 (s, 2H), 4.34 (d, J = 5.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 167.17, 162.74 (d, J = 242 Hz), 158.65, 157.62, 156.74, 156.30, 155.50, 144.12, 142.60 (d, J = 7.2 Hz), 130.67 (d, J = 9 Hz), 130.62, 130.47, 128.30, 124.30, 123.58 (d, J = 2.5 Hz), 119.51, 119.42, 114.24 (d, J = 22 Hz), 114.02 (d, J = 22 Hz), 97.81, 49.70, 42.15. HRMS(ESI) m/z calcd for $\text{C}_{26}\text{H}_{21}\text{FN}_6\text{O}_2$ $[\text{M} + \text{H}]^+$ 469.1783, found 469.1780.

2-(4-Amino-3-(4-phenoxyphenyl)-1 H -pyrazolo[3,4- d]pyrimidin-1-yl)- N -(4-fluorophenethyl) acetamide (13f)

White solid, yield 79%, mp:186-190°C, ^1H NMR (400 MHz, DMSO- d_6) δ 8.38 - 8.33 (m, 1H), 8.25 (s, 1H), 7.66 (d, J = 8.4 Hz, 2H), 7.44 (t, J = 7.7 Hz, 2H), 7.28 – 7.23 (m, 2H), 7.22 – 6.8 (m, 9H), 4.96 (s, 2H), 3.32 - 3.27 (m, 2H), 2.72 (t, J = 6.3 Hz, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 166.78, 161.31 (d, J = 240 Hz), 158.62, 157.61, 156.74, 156.30, 155.49, 144.01, 135.92 (d, J = 3 Hz), 130.95 (d, J = 8 Hz), 130.62, 130.47, 128.32, 124.30, 119.50, 119.41, 115.42 (d, J = 21 Hz), 97.72, 49.04, 40.87, 34.48. HRMS(ESI) m/z calcd for $\text{C}_{27}\text{H}_{23}\text{FN}_6\text{O}_2$ $[\text{M} + \text{H}]^+$ 483.1940, found 483.1936.

2-(4-Amino-3-(4-phenoxyphenyl)-1 H -pyrazolo[3,4- d]pyrimidin-1-yl)- N -(4-fluorophenyl) acetamide (13g)

White solid, yield 79%, mp:216-218°C, ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.49 (s, 1H), 8.26 (s, 1H), 7.68 (d, *J* = 8.4 Hz, 2H), 7.62 - 7.59 (m, 2H), 7.44 (t, *J* = 7.7 Hz, 2H), 7.33 – 6.62 (m, 9H), 5.22 (s, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 170.82, 158.67, 158.61 (d, *J* = 238 Hz), 157.65, 156.72, 156.38, 155.63, 144.12, 135.48 (d, *J* = 2.6 Hz), 130.62, 130.47, 128.24, 124.31, 121.42 (d, *J* = 7.8 Hz), 119.52, 119.42, 115.92 (d, *J* = 22.2 Hz), 97.73, 50.05. HRMS(ESI) *m/z* calcd for C₂₅H₁₉FN₆O₂ [M + H]⁺ 455.1626, found 455.1617.

2-(4-Amino-3-(4-phenoxyphenyl)-1 H -pyrazolo[3,4- d]pyrimidin-1-yl)- N -(4-bromophenyl) acetamide (13h)

White solid, yield 80%, mp:238-240°C, ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.71 (s, 1H), 8.25 (s, 1H), 7.68 (d, *J* = 8.6 Hz, 2H), 7.59 - 7.54 (m, 3H), 7.51 (d, *J* = 8.8 Hz, 2H), 7.44 (t, *J* = 7.9 Hz, 2H), 7.25 – 7.06 (m, 6H), 5.24 (s, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 165.86, 158.65, 157.64, 156.71, 156.38, 155.63, 144.12, 138.58, 132.09, 130.63, 130.47, 128.22, 124.31, 121.55, 119.52, 119.42, 115.58, 97.69, 50.11. HRMS(ESI) *m/z* calcd for C₂₅H₁₉BrN₆O₂ [M + H]⁺ 515.0826, found 515.0819.

2-(4-Amino-3-(4-phenoxyphenyl)-1 H -pyrazolo[3,4- d]pyrimidin-1-yl)- N -(4-methylbenzyl) acetamide (13i)

White solid, yield 78%, mp:222-226°C, ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.33 (s, 1H), 8.38 (d, *J* = 2.0 Hz, 1H), 8.26 (s, 1H), 8.09 (dd, *J* = 8.5, 2.4 Hz, 1H), 7.47 - 7.43 (m, 4H), 7.29 – 6.93 (m, 9H), 5.21 (s, 2H), 2.25 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 165.34, 158.66, 157.65, 156.73, 156.36, 155.64, 144.08, 136.59, 133.00, 130.62, 130.47, 129.69, 128.28, 124.31, 119.65, 119.52, 119.43, 97.74, 50.09, 20.91. HRMS(ESI) *m/z* calcd for C₂₆H₂₂N₆O₂ [M + H]⁺ 451.1877, found 451.1871.

2-(4-Amino-3-(4-phenoxyphenyl)-1 H -pyrazolo[3,4- d]pyrimidin-1-yl)- N -(4-cyanophenyl) acetamide (12j)

White solid, yield 69%, mp:228-230°C, ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.92 (s, 1H), 8.26 (s, 1H), 7.90 - 7.72 (m, 5H), 7.68 (d, *J* = 8.4 Hz, 2H), 7.44 (t, *J* = 7.8 Hz, 2H), 7.32 – 7.05 (m, 6H), 5.29 (s, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 166.54, 158.67, 157.67, 156.71, 156.43, 155.64, 144.24, 143.23, 133.90, 130.63, 130.47, 128.16, 124.32, 119.72, 119.53, 119.42, 105.87, 97.71, 50.23. HRMS(ESI) *m/z* calcd for C₂₆H₁₉N₇O₂ [M + H]⁺ 462.1673, found 462.1668.

2-(4-Amino-3-(4-phenoxyphenyl)-1 H -pyrazolo[3,4- d]pyrimidin-1-yl)- N -(4-(trifluoromethyl) phenyl)acetamide (13k)

White solid, yield 67%, mp:228-230°C, ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.83 (s, 1H), 8.26 (s, 1H), 7.79 (d, *J* = 8.6 Hz, 2H), 7.75 – 7.66 (m, 4H), 7.44 (t, *J* = 7.9 Hz, 2H), 7.24 – 7.08 (m, 7H), 5.28 (s, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 166.34, 158.69, 157.70, 156.72, 156.42, 155.68, 144.23, 142.61, 130.61, 130.47, 128.22, 126.67 (q, *J* = 3 Hz), 124.78 (q, *J* = 269 Hz), 124.31, 124.12 (q, *J* = 32 Hz), 119.63, 119.54, 119.43, 97.76, 50.21. HRMS(ESI) *m/z* calcd for C₂₆H₁₉F₃N₆O₂ [M - H]⁻ 503.1454, found 503.1449.

2-(4-Amino-3-(4-phenoxyphenyl)-1 H -pyrazolo[3,4- d]pyrimidin-1-yl)- N -(4-nitrophenyl) acetamide (13l)

White solid, yield 63%, mp:198-202°C, ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.10 (s, 1H), 8.35 – 8.21 (m, 4H), 7.83 (d, *J* = 9.0 Hz, 2H), 7.68 (d, *J* = 8.5 Hz, 2H), 7.44 (t, *J* = 7.6 Hz, 2H), 7.24 – 7.09 (m, 6H), 5.33 (s, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 166.71, 158.67, 157.69, 156.70, 156.45, 155.67, 145.13, 144.28, 142.97, 130.62, 130.47, 128.16, 125.57, 124.32, 119.54, 119.47, 119.42, 97.73, 50.28. HRMS(ESI) *m/z* calcd for C₂₅H₁₉N₇O₄ [M + H]⁺ 482.1571, found 482.1567.

2-(4-Amino-3-(4-phenoxyphenyl)-1 H -pyrazolo[3,4- d]pyrimidin-1-yl)- N -(3-chloro-4-fluorophenyl)acetamide (13m)

White solid, yield 74%, mp:226-230°C, ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.68 (s, 1H), 8.26 (s, 1H), 7.89 (dd, *J* = 6.8, 2.1 Hz, 1H), 7.68 (d, *J* = 8.5 Hz, 2H), 7.51 – 7.36 (m, 5H), 7.23 – 6.92 (m, 6H), 5.23 (s, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 165.97, 158.66, 157.68, 156.71, 156.40, 155.68, 153.74 (d, *J* = 242 Hz), 144.22, 136.28 (d, *J* = 3 Hz), 130.61, 130.46, 128.21, 124.30, 121.13, 120.02 (d, *J* = 7 Hz), 119.70 (d, *J* = 18 Hz), 119.52, 119.42, 117.55 (d, *J* = 21 Hz), 97.74, 50.07. HRMS(ESI) *m/z* calcd for C₂₅H₁₈ClFN₆O₂ [M + H]⁺ 489.1237, found 489.1232.

2-(4-Amino-3-(4-phenoxyphenyl)-1 H -pyrazolo[3,4- d]pyrimidin-1-yl)- N -(3-chloro-4-cyanophenyl)acetamide (13n)

White solid, yield 69%, mp:219-222°C, ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.10 (s, 1H), 8.26 (s, 1H), 8.02 (d, *J* = 1.8 Hz, 1H), 7.94 (d, *J* = 8.6 Hz, 1H), 7.68 (d, *J* = 8.6 Hz, 2H), 7.61 (dd, *J* = 8.7, 1.8 Hz, 1H), 7.44 (t, *J* = 7.9 Hz, 2H), 7.27 – 6.99 (m, 7H), 5.30 (s, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 166.96, 158.68, 157.72, 156.71, 156.46, 155.71, 144.34, 144.28, 136.58, 135.83, 130.62, 130.47, 128.15, 124.33, 119.70, 119.54, 119.42, 118.37, 116.62, 106.37, 97.75, 50.28. HRMS(ESI) *m/z* calcd for C₂₆H₁₈ClN₇O₂ [M + H]⁺ 496.1283, found 496.1276.

2-(4-Amino-3-(4-phenoxyphenyl)-1 H -pyrazolo[3,4- d]pyrimidin-1-yl)- N -(3,4-dicyanophenyl) acetamide (13o)

White solid, yield 72%, mp:228-230°C, ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.25 (s, 1H), 8.28 – 8.23 (m, 2H), 8.08 (d, *J* = 8.7 Hz, 1H), 8.00 – 7.95 (m, 1H), 7.68 (d, *J* = 8.6 Hz, 2H), 7.44 (t, *J* = 7.9 Hz, 2H), 7.30 – 6.62 (m, 7H), 5.32 (s, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 167.13, 158.67, 157.71, 156.68, 156.46, 155.70, 144.38, 143.45, 135.66, 130.61, 130.46, 128.11, 124.32, 123.64, 123.55, 119.53, 119.41, 116.53, 116.23, 116.09, 108.77, 97.74, 50.27. HRMS(ESI) *m/z* calcd for C₂₇H₁₈N₈O₂ [M + H]⁺ 487.1625, found 487.1618.

2-(4-Amino-3-(4-phenoxyphenyl)-1 H -pyrazolo[3,4- d]pyrimidin-1-yl)- N -(4-cyano-3-(trifluoromethyl)phenyl)acetamide (13p)

White solid, yield 76%, mp:228-230°C, ^1H NMR (400 MHz, DMSO- d_6) δ 11.27 (s, 1H), 8.27 (s, 2H), 8.13 (d, J = 8.5 Hz, 1H), 7.95 (d, J = 8.4 Hz, 1H), 7.68 (d, J = 8.6 Hz, 2H), 7.44 (t, J = 7.9 Hz, 2H), 7.29 – 6.67 (m, 7H), 5.33 (s, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 167.17, 158.58, 157.72, 156.69, 156.34, 155.66, 144.42, 143.55, 137.16, 132.30 (q, J = 31 Hz), 130.63, 130.47, 128.09, 124.33, 122.85 (q, J = 272 Hz), 122.64, 119.55, 119.42, 117.01 (q, J = 5 Hz), 116.16, 102.54 (q, J = 2 Hz), 97.72, 50.29. HRMS(ESI) m/z calcd for $\text{C}_{27}\text{H}_{18}\text{F}_3\text{N}_7\text{O}_2$ $[\text{M} + \text{H}]^+$ 530.1547, found 530.1541.

2-(4-Amino-3-(4-phenoxyphenyl)-1 H -pyrazolo[3,4- d]pyrimidin-1-yl)- N -(4-bromo-3-(trifluoromethyl)phenyl)acetamide (13q)

White solid, yield 72%, mp:184-187°C, ^1H NMR (400 MHz, DMSO- d_6) δ 10.83 (s, 1H), 8.20 (s, 1H), 8.11 (s, 1H), 7.77 (d, J = 8.6 Hz, 1H), 7.67 - 7.61 (m, 3H), 7.37 (t, J = 7.4 Hz, 2H), 7.27 – 6.64 (m, 7H), 5.21 (s, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 166.46, 158.68, 157.70, 156.71, 156.44, 155.70, 144.29, 138.94, 136.14, 130.61, 130.47, 129.10 (q, J = 31 Hz), 128.18, 124.55, 124.31, 123.21 (q, J = 268 Hz), 119.54, 119.42, 118.63 (q, J = 6 Hz), 112.41, 97.75, 50.17. HRMS(ESI) m/z calcd for $\text{C}_{26}\text{H}_{18}\text{BrF}_3\text{N}_6\text{O}_2$ $[\text{M} + \text{H}]^+$ 583.0699, found 4583.0693.

2-(4-Amino-3-(4-phenoxyphenyl)-1 H -pyrazolo[3,4- d]pyrimidin-1-yl)- N -(2-bromophenyl) acetamide (13r)

White solid, yield 82%, mp:184-186°C, ^1H NMR (400 MHz, DMSO- d_6) δ 10.64 (s, 1H), 8.26 (s, 1H), 7.93 (s, 1H), 7.68 (d, J = 8.6 Hz, 2H), 7.52 – 7.41 (m, 3H), 7.32 – 7.11 (m, 9H), 5.24 (s, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 166.06, 158.67, 157.66, 156.72, 156.41, 155.66, 144.19, 140.60, 131.39, 130.63, 130.47, 128.21, 126.72, 124.32, 122.09, 122.00, 119.53, 119.43, 118.42, 97.71, 50.12. HRMS(ESI) m/z calcd for $\text{C}_{25}\text{H}_{19}\text{BrN}_6\text{O}_2$ $[\text{M} + \text{H}]^+$ 515.0826, found 515.0823.

2-(4-Amino-3-(4-phenoxyphenyl)-1 H -pyrazolo[3,4- d]pyrimidin-1-yl)- N -(3-chloro-2-fluorophenyl)acetamide (13s)

White solid, yield 69%, mp:200-204°C, ^1H NMR (400 MHz, DMSO- d_6) δ 10.34 (s, 1H), 8.20 (s, 1H), 7.79 (t, J = 7.4 Hz, 1H), 7.62 (d, J = 8.2 Hz, 2H), 7.37 (t, J = 7.6 Hz, 2H), 7.27 (t, J = 7.2 Hz, 1H), 7.21 - 6.81 (m, 8H), 5.27 (s, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 166.43, 158.68, 157.69, 156.72, 156.42, 155.67, 149.68 (d, J = 245 Hz), 144.25, 130.62, 130.48, 128.22, 127.71 (d, J = 12 Hz), 126.25, 125.55 (d, J = 5 Hz), 124.31, 123.04, 120.38 (d, J = 16 Hz), 119.53, 119.43, 97.75, 49.89. HRMS(ESI) m/z calcd for $\text{C}_{25}\text{H}_{18}\text{ClFN}_6\text{O}_2$ $[\text{M} + \text{H}]^+$ 489.1237, found 489.1232.

2-(4-Amino-3-(4-phenoxyphenyl)-1 H -pyrazolo[3,4- d]pyrimidin-1-yl)- N -(2,3,4-trifluorophenyl)acetamide (13t)

White solid, yield 78%, mp:224-226°C, ^1H NMR (400 MHz, DMSO- d_6) δ 10.46 (s, 1H), 8.26 (s, 1H), 7.71 – 7.58 (m, 3H), 7.44 (t, J = 7.9 Hz, 2H), 7.34-7.27 (m, 1H), 7.22 – 6.79 (m, 7H), 5.31 (s, 2H). ^{13}C NMR (100

MHz, DMSO-*d*₆) δ 166.46, 158.67, 157.69, 156.72, 156.42, 155.67, 147.50 (d, *J* = 241 Hz), 144.26, 143.68 (d, *J* = 238 Hz), 139.66 (d, *J* = 232 Hz), 130.62, 130.48, 128.20, 124.31, 124.01 (d, *J* = 5.7 Hz), 119.53, 119.42, 119.01 (d, *J* = 8 Hz), 112.37 (dd, *J* = 17.7, 3.6 Hz), 97.75, 49.75. HRMS(ESI) *m/z* calcd for C₂₅H₁₇F₃N₆O₂ [M + H]⁺ 491.1438, found 491.1432.

2-(4-Amino-3-(4-phenoxyphenyl)-1 H -pyrazolo[3,4- d]pyrimidin-1-yl)- N -(3-bromo-2-methylphenyl)acetamide (13u)

White solid, yield 74%, mp:264-268°C, ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.99 (s, 1H), 8.29 (s, 1H), 7.68 (d, *J* = 8.5 Hz, 2H), 7.45 (q, *J* = 7.7 Hz, 3H), 7.37 (d, *J* = 8.8 Hz, 1H), 7.27 – 6.90 (m, 8H), 5.28 (s, 2H), 2.30 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 166.09, 158.63, 157.66, 156.70, 156.34, 155.59, 144.21, 137.55, 132.68, 130.63, 130.48, 130.12, 128.23, 127.86, 125.56, 125.25, 124.32, 119.53, 119.42, 97.78, 49.83, 18.68. HRMS(ESI) *m/z* calcd for C₂₆H₂₁BrN₆O₂ [M + H]⁺ 529.0982, found 529.0976.

2-(4-Amino-3-(4-phenoxyphenyl)-1 H -pyrazolo[3,4- d]pyrimidin-1-yl)- N -(3,5-dimethylphenyl) acetamide (13v)

White solid, yield 81%, mp:250-254°C, ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.30 (s, 1H), 8.27 (s, 1H), 7.68 (d, *J* = 8.5 Hz, 2H), 7.44 (t, *J* = 7.9 Hz, 2H), 7.31 – 6.72 (m, 9H), 6.72 (s, 1H), 5.20 (s, 2H), 2.22 (s, 6H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 165.47, 158.65, 157.62, 156.73, 156.35, 155.67, 144.07, 138.95, 138.27, 130.63, 130.46, 128.27, 125.55, 124.30, 119.51, 119.43, 117.39, 97.70, 50.11, 21.55. HRMS(ESI) *m/z* calcd for C₂₇H₂₄N₆O₂ [M + H]⁺ 465.2034, found 465.2031.

4.3. *In vitro* BTK inhibition activity assay

The *in vitro* BTK inhibition effects of target compounds were tested via a KinaseProfiler radiometric protein kinase assay following the previously described protocol.²⁴

4.4. Cell antiproliferation assay

MCL cell lines Mino, Jeko-1, Z138 and Maver-1 were purchased from the American Type Culture Collection (ATCC). The antiproliferative activity of target compounds were tested using the CellTiter-Glo luminescent cell viability assay according to the previously published protocol.²⁵

4.5. Cell apoptosis assay

Apoptosis was quantified by Annexin V/Propidium Iodide (PI)- binding assay following the previously described protocol.²⁶ In short, Z138 Cells were plated in 6-well plates with **13l** at concentrations of 10 μM or 20 μM and incubated for 24 h. After washing with cold phosphate buffered saline (PBS) and resuspension in 100 μL binding buffer, 2 μL annexin V-FITC and 5 μL PI were added to the treated cells. The samples were subsequently gently vortexed and incubated for 15 min in the dark at room temperature. Then 200 μL binding buffer was added to the samples. Samples were analyzed by flow cytometry on a FACScan flow cytometer. Results were analyzed by the Flowjo software.

4.6. Mitochondrial membrane potential

2×10^5 Z138 cells per well were plated in 6-well plates and treated with compound **13l** at concentrations of 2.5 μ M, 10 μ M and 20 μ M for 24 h. The cells were collected, washed with PBS three times, followed by adding JC-1 binding solution, and then incubated at 37°C for 20 min in the dark, and washed twice with PBS. Then, the effects of compound **13l** on mitochondrial membrane potential were taken under a confocal laser scanning microscope (Leica TCS SP8).

4.7. ROS assay

The regulatory effect of **13l** on ROS level in Z138 cells was determined through DCFH-DA probe according to the protocol published earlier.¹⁹

4.8. Western blotting

Z138 cells were cultured with specified concentrations of **12l** for 72 h. Then the apoptosis marker and autophagy protein levels of treated cells were detected by western blotting according to the previously reported experimental procedures.¹⁹

Declarations

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 82073708).

References

1. Cinar M, Hamedani F, Mo Z, Cinar B, Amin HM, Alkan S (2013) Bruton tyrosine kinase is commonly overexpressed in mantle cell lymphoma and its attenuation by Ibrutinib induces apoptosis. *Leuk Res* 37(10):1271–1277. <https://doi.org/10.1016/j.leukres.2013.07.028>
2. Liu Y, Yin YZ, Zhang Z, Li CJ, Zhang H, Zhang DG, Jiang CY, Nomie K, Zhang L, Wang ML, Zhao GS (2017) Structural optimization elaborates novel potent Akt inhibitors with promising anticancer activity. *Eur J Med Chem* 138:543–551. <https://doi.org/10.1016/j.ejmech.2017.06.067>
3. Jain P, Wang M (2019) Mantle cell lymphoma: 2019 update on the diagnosis, pathogenesis, prognostication, and management. *Am J Hematol* 94(6):710–725. <https://doi.org/10.1002/ajh.25487>
4. Vogt N, Dai BY, Erdmann T, Berdel WE, Lenz G (2017) The molecular pathogenesis of mantle cell lymphoma. *Leuk lymphoma* 58(7):1530–1537. <https://doi.org/10.1080/10428194.2016.1248965>
5. Qiu H, Ali Z, Bender A, Caldwell R, Chen YY, Fang ZZ, Gardberg A, Glaser N, Goettsche A, Goutopoulos A, Grenningloh R, Hanschke B, Head J, Johnson T, Jones C, Jones R, Kulkarni S, Maurer C, Morandi F, Neagu C, Poetzsch S, Potnick J, Schmidt R, Roe K, Viacava Follis A, Wing C, Zhu XH, Sherer B (2021)

- Discovery of potent and selective reversible Bruton's tyrosine kinase inhibitors. *Bioorg Med Chem* 40:116163. <https://doi.org/10.1016/j.bmc.2021.116163>
6. Pal Singh S, Dammeijer F, Hendriks RW (2018) Role of Bruton's tyrosine kinase in B cells and malignancies. *Mol cancer* 17(1):57. <https://doi.org/10.1186/s12943-018-0779-z>
 7. Zhang Z, Zhang DG, Liu Y, Yang DZ, Ran FS, Wang ML, Zhao GS (2018) Targeting Bruton's tyrosine kinase for the treatment of B cell associated malignancies and autoimmune diseases: Preclinical and clinical developments of small molecule inhibitors. *Archiv der Pharmazie* 351(7):e1700369. <https://doi.org/10.1002/ardp.201700369>
 8. Liu XJ, Xu L, Pang XJ, Yuan XY, Yu GX, Li YR, Guan YF, Zhang YB, Song J, Zhang QR, Zhang SY (2021) Progress in the development of small molecular inhibitors of the Bruton's tyrosine kinase (BTK) as a promising cancer therapy. *Bioorg Med Chem* 47:116358. <https://doi.org/10.1016/j.bmc.2021.116358>
 9. Zhai Z, Li RD, Bai XY, Ning XL, Lin ZQ, Zhao XY, Jin Y, Yin YX (2019) Design, synthesis and biological evaluation of novel dithiocarbamate-substituted diphenylaminopyrimidine derivatives as BTK inhibitors. *Bioorg Med Chem* 27(18):4124–4142. <https://doi.org/10.1016/j.bmc.2019.07.043>
 10. Zheng N, Pan J, Hao Q, Li YX, Zhou WC (2018) Design, synthesis and biological evaluation of novel 3-substituted pyrazolopyrimidine derivatives as potent Bruton's tyrosine kinase (BTK) inhibitors. *Bioorg Med Chem* 26(8):2165–2172. <https://doi.org/10.1016/j.bmc.2018.03.017>
 11. McDonald C, Xanthopoulos C, Kostareli E (2021) The role of BTK in the immune system and disease. *Immunology* <https://doi.org/10.1111/imm.13416>
 12. Owen C, Berinstein NL, Christofides A, Sehn LH (2019) Review of Bruton tyrosine kinase inhibitors for the treatment of relapsed or refractory mantle cell lymphoma. *Curr Oncol* 26(2):e233–e240. <https://doi.org/10.3747/co.26.4345>
 13. Liang CY, Tian DN, Ren XD, Ding SJ, Jia MY, Xin MH, Thareja S (2018) The development of Bruton's tyrosine kinase (BTK) inhibitors from 2012 to 2017: A mini-review. *Eur J Med Chem* 151:315–326. <https://doi.org/10.1016/j.ejmech.2018.03.062>
 14. Woyach JA, Furman RR, Liu TM, Ozer HG, Zapatka M, Ruppert AS, Xue L, Li DH, Steggerda SM, Versele M, Dave SS, Zhang J, Yilmaz AS, Jaglowski SM, Blum KA, Lozanski A, Lozanski G, James DF, Barrientos JC, Lichter P, Stilgenbauer S, Buggy JJ, Chang BY, Johnson AJ, Byrd JC (2014) Resistance mechanisms for the Bruton's tyrosine kinase inhibitor ibrutinib. *N Engl J Med* 370(24):2286–2294. <https://doi.org/10.1056/NEJMoa1400029>
 15. Bond DA, Woyach JA (2019) Targeting BTK in CLL: Beyond Ibrutinib. *Curr Hematol Malig Rep* 14(3):197–205. <https://doi.org/10.1007/s11899-019-00512-0>
 16. Yao X, Sun XY, Jin SY, Yang L, Xu HJ, Rao Y (2019) Discovery of 4-Aminoquinoline-3-carboxamide Derivatives as Potent Reversible Bruton's Tyrosine Kinase Inhibitors for the Treatment of Rheumatoid Arthritis. *J Med Chem* 62(14):6561–6574. <https://doi.org/10.1021/acs.jmedchem.9b00329>
 17. Ran FS, Liu Y, Liu MX, Zhang DG, Wang P, Dong JZ, Tang WD, Zhao GS (2019) Discovery of pyrazolopyrimidine derivatives as potent BTK inhibitors with effective anticancer activity in MCL.

Bioorg Chem 89:102943. <https://doi.org/10.1016/j.bioorg.2019.102943>

18. Ran FS, Liu Y, Yu SP, Guo KW, Tang WD, Chen X, Zhao GS (2020) Design and synthesis of novel 1-substituted 3-(6-phenoxy pyridin-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine analogs as selective BTK inhibitors for the treatment of mantle cell lymphoma. *Bioorg Chem* 94:103367. <https://doi.org/10.1016/j.bioorg.2019.103367>
19. Ran FS, Liu Y, Chen X, Zhuo HJ, Xu CQ, Li YX, Duan XM, Zhao GS (2021) Design and synthesis of novel substituted benzyl pyrrolopyrimidine derivatives as selective BTK inhibitors for treating mantle cell lymphoma. *Bioorg Chem* 112:104968. <https://doi.org/10.1016/j.bioorg.2021.104968>
20. Sinha K, Das J, Pal PB, Sil PC (2013) Oxidative stress: the mitochondria-dependent and mitochondria-independent pathways of apoptosis. *Arch Toxicol* 87(7):1157–1180. <https://doi.org/10.1007/s00204-013-1034-4>
21. Honda F, Kano H, Kanegane H, Nonoyama S, Kim ES, Lee SK, Takagi M, Mizutani S, Morio T (2012) The kinase Btk negatively regulates the production of reactive oxygen species and stimulation-induced apoptosis in human neutrophils. *Nat Immunol* 13(4):369–378. <https://doi.org/10.1038/ni.2234>
22. Galicia-Vazquez G, Smith S, Aloyz R (2018) Del11q-positive CLL lymphocytes exhibit altered glutamine metabolism and differential response to GLS1 and glucose metabolism inhibition. *Blood Cancer J* 8:13. <https://doi.org/10.1038/s41408-017-0039-2>
23. Muqbil I, Chaker M, Aboukameel A, Mohammad RM, Azmi AS, Ramchandren R (2019) Pre-clinical anti-tumor activity of Bruton's Tyrosine Kinase inhibitor in Hodgkin's Lymphoma cellular and subcutaneous tumor model. *Heliyon* 5(8):e02290. <https://doi.org/10.1016/j.heliyon.2019.e02290>
24. Ran FS, Liu Y, Zhang DG, Liu MX, Zhao GS (2019) Discovery of novel pyrazole derivatives as potential anticancer agents in MCL. *Bioorg Med Chem Lett* 29(9):1060–1064. <https://doi.org/10.1016/j.bmcl.2019.03.005>
25. Yu SP, Liu Y, Zhang Z, Zhang JY, Zhao GS (2019) Design, synthesis and biological evaluation of novel 2,3-indolinedione derivatives against mantle cell lymphoma. *Bioorg Med Chem* 27(15):3319–3327. <https://doi.org/10.1016/j.bmc.2019.06.009>
26. Liu Y, Zhang Z, Ran FS, Guo KW, Chen X, Zhao GS (2020) Extensive investigation of benzylic N-containing substituents on the pyrrolopyrimidine skeleton as Akt inhibitors with potent anticancer activity. *Bioorg Chem* 97:103671. <https://doi.org/10.1016/j.bioorg.2020.103671>

Scheme

Scheme 1 is available in the Supplemental Files section

Figures

Figure 1

Structures of representative BTK inhibitors.

Figure 2

Structures of the lead compound 9 and designed compounds

Figure 3

A) Induction of apoptosis in Z138 cells after 24 h treatment with compound 13l. B) Induction of apoptosis in Jeko-1 cell after 24 h treatment with compound 13l. C) Effects of 13l on cleaved caspase 3 and LC3B. Significance is indicated as *P < 0.05 and **P < 0.01 compared with the DMSO control.

Figure 4

A) Changes of intracellular mitochondrial membrane potential in Z138 cells after 13I exposure for 24 h. Living cells with high $\Delta\Psi_m$ show strong red fluorescence, whereas the apoptotic cells with low or no mitochondrial membrane potential reveal green fluorescence. B) Changes of ROS levels in Z138 cells after 13I exposure for 24 h. In cells, the DCFH-DA probe was hydrolyzed into DCFH, which reacted with ROS to produce DCF with green fluorescence. The green fluorescence intensity was positively correlated with the ROS level

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Scheme1.png](#)
- [Supportinginformation.docx](#)