Design, synthesis, and structure-activity relationship studies of 6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole derivatives as necroptosis inhibitors postprint

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Abstract

The development of necroptosis inhibitors has emerged as a promising strategy to effectively mitigate necroptosis-related inflammatory diseases, neurodegenerative diseases, and cancers. In this paper, we reported a series of 6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole derivatives as potent necroptosis inhibitors. The representative compound 26 displayed potent anti-necroptotic activity in both human and mouse cellular assays and exhibited potent inhibitory activity against receptor-interacting protein kinase 1 (RIPK1). In vivo pharmacokinetic studies were performed to determine the oral exposure of compound 26. Finally, molecular docking elucidated that compound 26 could effectively bind to the allosteric pocket of RIPK1 and serve as a type III inhibitor. Taken together, our findings highlighted that compound 26 represented a promising lead compound for future necroptosis inhibitor development.

Full Text

Preamble

Medicinal Chemistry RESEARCH ARTICLE Cite this: RSC Med. Chem., 2024, 15, Received 16th April 2024, Accepted 17th May 2024 DOI: 10.1039/d4md00265b Design, synthesis, and structure–activity relationship studies of 6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole derivatives as necroptosis inhibitors† Zechen Jin,‡ac Yang Dai,‡bc Yinchun Ji,b Xia Peng,b Wenhu Duan,acd Jing Ai*bc and Hefeng Zhang The development of necroptosis inhibitors has emerged as a promising strategy to effectively mitigate necroptosis-related inflammatory diseases, neurodegenerative diseases, and

cancers. In this paper, we reported a series of 6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole derivatives as potent necroptosis inhibitors.

The representative compound 26 displayed potent anti-necroptotic activity in both human and mouse cellular assays and exhibited potent inhibitory activity against receptor-interacting protein kinase 1 (RIPK1).

In vivo pharmacokinetic studies were performed to determine the oral exposure of compound 26. Finally, molecular docking elucidated that compound 26 could effectively bind to the allosteric pocket of RIPK1 and serve as a type III inhibitor. Taken together, our findings highlighted that compound 26 represented a rsc.li/medchem promising lead compound for future necroptosis inhibitor development.

Introduction

Necroptosis is an important form of programmed cell death (PCD) that is precisely regulated by the receptor-interacting protein kinase (RIPK) family. Although necroptosis shares certain characteristics with necrosis, it is activated when apoptosis pathways are compromised. In the classic tumor necrosis factor-α (TNF-α)-mediated necroptosis signaling pathway, RIPK1 is initially ubiquitinated and plays a central role in complex I. When RIPK1 dissociates, it translocates to complex IIa/IIb to initiate PCD. With the deficiency of caspase-8 in the cytoplasm, RIPK1, RIPK3, and mixed lineage kinase domain-like protein (MLKL) join together to form necrosomes, where RIPK1 is deubiquitinated and triggers cascade phosphorylation that eventually initiates necroptosis [Figure 1: see original paper]. Although necroptosis is essential for some physiological processes such as nerve development, abnormal necroptosis can lead to various diseases. Necroptosis upregulation has been implicated in autoimmune disorders including systemic lupus erythematosus, neurodegenerative diseases such as Alzheimer's disease and stroke-induced brain injury, and several cancers including glioblastoma, lung cancer, pancreatic ductal adenocarcinoma, and ovarian carcinoma. The development of necroptosis inhibitors may offer promising therapeutic strategies for the treatment of these diseases.

The development of RIPK1-targeted necroptosis inhibitors has attracted increased attention in recent years. Based on different binding modes, current RIPK1 inhibitors are categorized into three classes: type I, II, and III [Figure 2: see original paper]. Type I RIPK1 inhibitors are ATP-competitive inhibitors that interact with the DLG-in (Asp-Leu-Gly-in) conformation of RIPK1. Multi-target kinase inhibitors such as tozasertib (1a) and pazopanib exhibit submicromolar level potency. Type II RIPK1 inhibitors interact with DLG-out RIPK1 by occupying both the hinge region pocket and the allosteric pocket, exhibiting elevated bioactivity and selectivity. Compounds 2a and 2b are orally bioavailable type II RIPK1 inhibitors with favorable selectivity profiles. A series of benzothiazole-based type II RIPK1 inhibitors were reported recently, with representative compound 2c demonstrating potent anti-necroptotic efficacy both

in vitro and in vivo. Type III inhibitors occupy the allosteric pocket of RIPK1 [Figure 3: see original paper] and feature exceptional inhibitory potency and selectivity. Nec-1 is the first type III selective RIPK1 inhibitor. GSK2982772 (4a) has progressed into phase II clinical trials for treating several autoimmune diseases. GSK3145095 (4b) has advanced into phase II clinical studies for cancer treatment (terminated). Several analogs of 4a, such as ZB-R-55 (4c), have been reported recently. GSK 547 (5) stands out as a potent inhibitor against both human and murine RIPK1 (hRIPK1 and mRIPK1). Compound 6 is another type III RIPK1 inhibitor that demonstrates strong anti-necroptotic activity against human-derived HT29 cells. In this paper, we report a series of novel 6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-based type III RIPK1 inhibitors that display remarkable anti-necroptotic activity in both human and murine cells.

Results and discussion

Chemistry

The synthetic routes for compounds 7–20 are shown in Scheme 1. First, phenolic compounds S1a–c were treated with methyl 4-bromobutanoate to obtain carboxylic acid esters S2a–c. Treatment of esters S2a–c with methylmagnesium bromide afforded intermediates S3a–c, which underwent Miyaura borylation and Suzuki coupling reactions to yield amines S5a–g. Finally, amines S5a–g were condensed with corresponding acids S7a–h to afford the target compounds 7–20. Compounds 21–28 were obtained via similar routes (Scheme 2). Phenol ethers S8a–e were obtained through nucleophilic substitution reactions. Then intermediates S8a–e underwent Suzuki coupling reactions with (2-aminopyridin-4-yl)boronic acid to give amines S9a–e. Finally, amines S9a–e were condensed with corresponding acids S7a, S11a or S11b to afford the target compounds 21–28.

Design rationale and structure–activity relationship (SAR) studies

Compound 6, which exhibits good human cellular activity (HT29 IC50 = 100 nM according to the literature), was selected as the starting point for our research. The anti-TNF-α-induced necroptosis activity of compounds was evaluated using both human I2.1 cells and murine Hepa1-6 cells. Despite demonstrating potent anti-necroptotic activity against I2.1 cells (90.0% recovery at 10 nM), compound 6 exhibited no recovery activity against Hepa1-6 cells (0% recovery at 1 M). The species selectivity of compound 6 impedes its further biological evaluation in rodents, which must be addressed.

It is noteworthy that GSK2982772, another RIPK1 inhibitor featuring the 3-benzyl-4H-1,2,4-triazole moiety as the allosteric fragment, also exhibited species selectivity between hRIPK1 and mRIPK1 (hRIPK1 IC50 = 16 nM, mRIPK1 IC50 = 2.5 M). It has been reported that such species differences might be attributed to the diminished flexibility of residues near the allosteric regions in mRIPK1. This evidence suggests that the allosteric fragment of compound 6

requires further optimization to develop novel necroptosis inhibitors that are active in both human and murine cells.

Our design rationale is illustrated in [Figure 4: see original paper]. We were drawn to the hRIPK/mRIPK inhibitor GSK 547. The 5-phenyl-4,5-dihydro-1H-pyrazol-1-yl allosteric moiety of GSK 547 appears critical for its mRIPK1 inhibitory activity. We adopted a cyclization design strategy and incorporated the allosteric structural feature of GSK 547 into compound 6. As a result, the 6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole derivative 7 was designed. Encouragingly, compound 7 showed a marked increase in anti-necroptotic activity for murine Hepa1-6 cells (86.6% recovery at 1 M) while retaining potent efficacy for human I2.1 cells.

Following this breakthrough, we conducted systematic SAR studies on compound 7. Compounds achieving over 75% recovery rate in I2.1 cells at 10 nM were further tested to determine their anti-necroptotic activities in Hepa1-6 cells.

Initially, we explored the terminal phenyl ring at the R3-position and synthesized compounds 8–10. Introduction of a fluorine substituent at the ortho (compound 8), meta (compound 9), or para (compound 10) position of the phenyl ring led to a slight decrease in activity. This result suggested that additional substituents on the phenyl ring were unfavorable. Replacing the phenyl ring with cycloalkyls (compounds 11 and 12) maintained activity, indicating that the R3 group interacts with the RIPK1 protein primarily through hydrophobic interactions rather than π–π stacking. Considering both activity and molecular weight, the unsubstituted phenyl was selected as the optimal substitution pattern for the R3 group.

Next, we introduced a fluorine substituent at the 7 -position of the 6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole moiety and obtained cis–trans isomers 13 and 14 . Both compounds 13 and 14 showed enhanced activity in Hepa1-6 cells, suggesting that the 7 -fluoro might contribute to additional van der Waals interactions with mRIPK1. The cis-isomer 13 exhibited over 100-fold improved potency in Hepa1-6 cells compared to compound 7. Consequently, the cis-substituted fluorine at the 7 -position was chosen as the preferred substitution pattern for this segment.

We next explored the SAR of the pyridine–phenyl moiety (compounds 15–20, Table 1). Introduction of a halogen atom (15 and 16) or methyl group (17) at the R2-position resulted in a slight loss of activity. This indicated that the R2-position might be close to the pocket surface and could not accommodate additional substituents. Replacing the pyridine ring with pyridazine led to decreased potency (18). At the R1-position, replacing the methyl group of compound 7 with a chlorine substituent (19) or removing the methyl group (20) diminished activity in Hepa1-6 cells. The R1-methyl group was presumed to restrain the appropriate dihedral angle of the pyridine–phenyl moiety.

We also investigated the SAR of the R4 group . Replacing the R4 group of

compound 7 with methyl (21) or cyanopropyl (24) resulted in decreased potency, which might be due to the reduced solubility of these compounds. Compounds 22 and 23 with hydrophilic groups as R4 substituents exhibited elevated potency in both I2.1 and Hepa1-6 cells. Given the potential metabolic liability of the hydroxyl group, the 2-(tetrahydro-2H-pyran-4-yl)ethyl group of compound 23 was identified as the optimal substituent for the R4 group.

Ultimately, we combined the advantageous substitution patterns from compounds 7, 14, and 23 and performed appropriate derivatizations to synthesize compounds 25–28 with defined absolute configurations. Additionally, we tested their RIPK1 inhibitory activities at a concentration of 1 M . The results indicated that compounds 25 and 27 with "R,R" chiral centers had no RIPK1 inhibitory effect. This consequence highlighted the critical impact of absolute configuration on this series of compounds: the 5 -(R) configuration should have impeded the extension of the 5 -phenyl into the hydrophobic pocket of RIPK1. In contrast, the "S,S"-configured compounds 26 and 28 showed satisfactory RIPK1 inhibitory activity and cellular recovery efficacy. Compound 26, which features a morpholine fragment at the R4-substituent, exhibited superior activity in I2.1 cells. Therefore, compound 26 was selected for further studies.

Pharmacokinetic properties of compound 26

We evaluated the pharmacokinetic properties of compound 26 in SD rats (N = 3) at a dose of 3 mg kg^{-1} (p.o.). The plasma concentrations of compound 26 were monitored for 24 h. As shown in Table 4, compound 26 reached a low peak plasma concentration (Cmax) of 8.90 ng mL^{-1} at 0.67 h. The elimination half-life (T1/2) of compound 26 was determined to be 1.91 h, suggesting a moderate duration of presence in the systemic circulation. Additionally, the area under the curve (AUC), a critical measure of the drug's overall presence in plasma over time, was calculated to be 15.2 h ng mL^{-1}.

Molecular docking

Molecular docking was conducted to elucidate the binding mode of compound 26. [Figure 5: see original paper] illustrates that compound 26 effectively occupied the allosteric hydrophobic pocket of RIPK1 and formed two critical hydrogen bonds with ASP156. The 6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole ring and the 7 -fluoro substituent interacted with RIPK1 primarily through hydrophobic interactions. Additionally, the morpholine fragment formed two salt bridge interactions with ASN99 and GLU142. The pyridine–phenyl moiety adopted a cross conformation and acted as a linker connecting the allosteric segment with the solvent-exposed moiety. In summary, compound 26 is a potential type III RIPK1 inhibitor.

Conclusion

The development of necroptosis inhibitors has attracted widespread attention for their potential applications in autoimmune diseases, neurodegenerative diseases, and cancers. In this study, we started with the human-cell specific inhibitor compound 6 and employed a cyclization design strategy to synthesize 6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole derivatives as potent necroptosis inhibitors. These compounds exhibited potent anti-necroptotic activity against both human and murine cells. Systematic SAR studies led to the identification of the optimal compound 26. Compound 26 demonstrated robust anti-necroptotic activity in both human I2.1 and murine Hepa1-6 cells and significantly inhibited RIPK1 enzymatic activity. In pharmacokinetic evaluation, compound 26 displayed relatively low oral exposure. Molecular docking revealed that compound 26 served as a potential type III RIPK1 inhibitor and effectively bound to the allosteric pocket of RIPK1. In summary, compound 26 represents a promising lead compound for future development of necroptosis inhibitors.

Experimental section

Chemistry

Unless specifically stated, all reagents and solvents were obtained from commercial sources and used without further purification. Reactions were generally carried out under an argon atmosphere unless otherwise stated. NMR spectra were obtained on Varian Mercury 400 or Bruker AVANCE NMR 400, 500, 600 spectrometers. ^{1}H and ^{13}C NMR spectra were obtained at 400/600 MHz and 126/151 MHz, respectively. Chemical shifts are reported in ppm relative to tetramethylsilane or deuterated solvents (chloroform-d, DMSO-d_6, methanol-d_4, acetone-d_6) as internal standards. The observed splitting patterns are designated as "s" for singlet, "d" for doublet, "t" for triplet, "q" for quartet, and "m" for multiplet, respectively. Mass spectrometric analysis was performed using an Agilent 6110 Quadrupole LC/MS in ESI-MS mode. High-resolution mass spectrometry (HR-MS) was conducted on an Agilent G6520 Q-TOF spectrometer (ESI-MS mode). Purity assessment of all compounds (>95%) was performed using an Agilent Infinity 1260 high-performance liquid chromatography (HPLC) system.

Preparation procedure of compounds 7–28

Methyl 4-(3-bromo-4-methylphenoxy)butanoate (S2a). To a mixture of 3-bromo-4-methylphenol (1.0 g, 5.35 mmol) and cesium carbonate (16.04 mmol) in N,N-dimethylformamide (DMF, 5 mL) was added methyl 4-bromobutanoate (675 L, 5.35 mmol). The resulting mixture was stirred for 12 h. The reaction was quenched with water (20 mL) and extracted with ethyl acetate (30 mL × 3). The organic layers were combined, washed with saturated brine (30 mL × 5), dried over anhydrous sodium sulfate, and concentrated under reduced pressure to obtain intermediate S2a as a pale-yellow oil (1.53 g, 99%). ^{1}H NMR (400

MHz, chloroform-d) δ 7.12–7.06 (m, 2H), 6.76–6.72 (m, 1H), 3.96 (t, J = 6.0 Hz, 2H), 3.69 (s, 3H), 2.51 (t, J = 7.2 Hz, 2H), 2.31 (s, 3H), 2.13–2.04 (m, 2H). MS (ESI) m/z: 309.0 $[M + Na]^+$.

5-(3-Bromo-4-methylphenoxy)-2-methylpentan-2-ol (S3a). Methyl magnesium bromide (3 M in tetrahydrofuran, 5.29 mL, 15.88 mmol) was added dropwise to a solution of intermediate S2a (1.52 g, 5.29 mmol) at 0 °C under an argon atmosphere. The resulting mixture was warmed to room temperature and stirred for 5 h. After completion, the reaction was quenched with saturated ammonium chloride solution (50 mL) at 0 °C, and the aqueous phase was extracted with ethyl acetate (30 mL × 3). The organic phases were combined, washed with brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The resulting residue was purified by flash chromatography (0% to 20% ethyl acetate in petroleum ether) to afford intermediate S3a as a pale-yellow oil (1.26 g, 83%). ^{1}H NMR (400 MHz, chloroform-d) δ 7.13–7.07 (m, 2H), 6.75 (dt, J = 8.4, 2.7 Hz, 1H), 3.93 (td, J = 6.5, 2.7 Hz, 2H), 2.31 (d, J = 2.8 Hz, 3H), 1.90–1.83 (m, 2H), 1.67–1.59 (m, 2H), 1.26 (d, J = 3.3 Hz, 7H). MS (ESI) m/z: 309.0 $[M + Na]^+$.

2-Methyl-5-(4-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)pentan-2-ol (S4a). 5-(3-Bromo-4-methylphenoxy)-2-methylpentan-2-ol (S3a, 710 mg, 2.47 mmol), bis(pinacolato)diboron (753 mg, 2.97 mmol), [1,1 -bis(diphenylphosphino)ferrocene]dichloropalladium (217 mg, 0.297 mmol), and potassium acetate (728 mg, 7.42 mmol) were added to 1,4-dioxane (10 mL). The resulting mixture was degassed with argon three times and stirred at 100 °C for 10 h. The mixture was concentrated under reduced pressure, and the residue was purified by flash chromatography (0% to 20% ethyl acetate in petroleum ether) to afford intermediate S4a as a white solid (860 mg, 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.10 (d, J = 2.9 Hz, 1H), 7.07 (d, J = 8.4 Hz, 1H), 6.90 (dd, J = 8.4, 2.9 Hz, 1H), 3.89 (t, J = 6.5 Hz, 2H), 2.37 (s, 3H), 1.77–1.66 (m, 2H), 1.51–1.44 (m, 2H), 1.29 (s, 12H), 1.09 (s, 6H). MS (ESI) m/z: 357.3 $[M + Na]^+$.

5-(3-(2-Aminopyridin-4-yl)-4-methylphenoxy)-2-methylpentan-2-ol (S5a). To a mixture of S4a (131 mg, 0.393 mmol), 4-bromopyridin-2-amine (45 mg, 0.262 mmol), and sodium carbonate (83 mg, 0.786 mmol) in 1,4-dioxane (4 mL) and water (1 mL) was added [1,1 -bis(diphenylphosphino)ferrocene]dichloropalladium (19 mg, 0.0262 mmol). The mixture was degassed with argon three times and stirred at 100 °C for 3 h. After completion, the mixture was concentrated under vacuum. The residue was purified by flash chromatography (0% to 30% ethyl acetate in petroleum ether) to afford intermediate S5a as a brown solid (50 mg, 64%). ^{1}H NMR (400 MHz, chloroform-d) δ 8.09 (d, J = 5.3 Hz, 1H), 7.17 (d, J = 8.3 Hz, 1H), 6.88–6.82 (m, 1H), 6.76 (t, J = 2.1 Hz, 1H), 6.66–6.62 (m, 1H), 6.46 (s, 1H), 4.57 (s, 2H), 4.00 (t, J = 6.7 Hz, 2H), 2.21 (d, J = 1.5 Hz, 3H), 1.96–1.89 (m, 2H), 1.70–1.63 (m, 2H), 1.28 (s, 6H). MS (ESI) m/z: 301.2 $[M + H]^+$.

Ethyl 5-phenyl-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-

carboxylate (S6a). Intermediate S6a was synthesized according to the literature. ^{1}H NMR (400 MHz, chloroform-d) δ 7.39–7.30 (m, 3H), 7.11–7.06 (m, 2H), 5.48 (dd, J = 8.4, 5.2 Hz, 1H), 4.52–4.38 (m, 2H), 3.29–3.18 (m, 1H), 3.18–3.09 (m, 1H), 3.09–2.99 (m, 1H), 2.72–2.62 (m, 1H), 1.40 (t, J = 7.1 Hz, 3H). MS (ESI) m/z: 258.1 $[M + H]^+$.

5-Phenyl-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxylic acid (S7a). To a solution of compound S6a (450 mg, 1.75 mmol) in methanol (10 mL) was added 1 M lithium hydroxide (aq, 3.27 mL, 3.27 mmol). The mixture was stirred for 3 h before completion. The solvent was removed under vacuum, and the residue was dissolved in water. Then 1 M hydrochloric acid was added dropwise to adjust the pH to 1–2, and a white precipitate formed. The precipitate was filtered, washed with water (5 mL), and dried under vacuum to afford intermediate S7a as a white solid (380 mg, 95%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.12 (s, 1H), 7.44–7.33 (m, 3H), 7.27–7.21 (m, 2H), 5.57 (dd, J = 8.0, 6.0 Hz, 1H), 3.23–3.14 (m, 1H), 3.14–3.05 (m, 1H), 3.03–2.94 (m, 1H), 2.61–2.52 (m, 1H). MS (ESI) m/z: 228.2 $[M–H]^-$.

N-(4-(5-((4-Hydroxy-4-methylpentyl)oxy)-2-methylphenyl)pyridin-2-yl)-5-phenyl-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxamide (7). To a solution of compound S7a (30 mg, 0.100 mmol), HATU (38 mg, 0.100 mmol), and DIPEA (43 L, 0.250 mmol) in DMF (1 mL) was added intermediate S5a (19 mg, 0.0832 mmol). The resulting mixture was stirred at room temperature for 12 h. After completion, the reaction was quenched with water, and the solution was extracted with ethyl acetate (30 mL × 3). The organic layers were combined, washed with saturated brine (20 mL × 5), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by preparative thin layer chromatography to obtain the title compound as a white solid (23 mg, 54%). ^{1}H NMR (400 MHz, acetone-d_6) δ 9.58 (s, 1H), 8.39 (d, J = 5.1 Hz, 1H), 8.33 (s, 1H), 7.44–7.29 (m, 5H), 7.23 (d, J = 8.4 Hz, 1H), 7.16 (dd, J = 5.1, 1.6 Hz, 1H), 6.92 (dd, J = 8.4, 2.7 Hz, 1H), 6.85 (d, J = 2.8 Hz, 1H), 5.67 (dd, J = 8.3, 5.9 Hz, 1H), 4.02 (t, J = 6.6 Hz, 2H), 3.45–3.33 (m, 1H), 3.25 (s, 1H), 3.23–3.17 (m, 1H), 3.16–3.07 (m, 1H), 2.76–2.70 (m, 1H), 2.23 (s, 3H), 1.93–1.83 (m, 2H), 1.64–1.56 (m, 2H), 1.19 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.7, 160.6, 157.2, 156.9, 151.1, 150.7, 148.3, 139.5, 139.3, 131.8, 128.9, 128.4, 126.8, 126.1, 120.7, 114.9, 114.6, 113.7, 68.5, 68.3, 61.1, 35.4, 29.3, 24.0, 20.6, 19.0. HRMS: calcd for $C_{30}H_{34}N_5O_3$ $[M + H]^+$, 512.2662; found, 512.2657.

Intermediates S6g, S6h, S10a, and S10b were synthesized according to the literature.

(R,R)-Ethyl-7-fluoro-5-phenyl-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxylate (S10a), (S,S)-ethyl-7-fluoro-5-phenyl-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxylate (S10b), trans-ethyl-7-fluoro-5-phenyl-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxylate (S6g). ^{1}H NMR (400 MHz, chloroform-d) δ 7.45–7.37 (m, 3H), 7.17–7.13 (m, 2H), 6.10 (ddd, J = 55.4, 6.4, 1.3 Hz, 1H), 5.75 (td, J = 6.7, 3.1

Hz, 1H), 4.59–4.41 (m, 2H), 3.50–3.35 (m, 1H), 3.14–2.95 (m, 1H), 1.44 (t, J = 7.1 Hz, 4H). MS (ESI) m/z: 276.3 $[M + H]^+$.

cis-Ethyl-7-fluoro-5-phenyl-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxylate (S6h). ^{1}H NMR (400 MHz, chloroform-d) δ 7.42–7.34 (m, 3H), 7.23 (dd, J = 7.5, 2.0 Hz, 2H), 6.02 (ddd, J = 55.7, 7.1, 1.6 Hz, 1H), 5.50 (ddd, J = 8.8, 6.2, 2.7 Hz, 1H), 4.52–4.40 (m, 2H), 3.72–3.55 (m, 1H), 3.03–2.87 (m, 1H), 1.42 (t, J = 7.1 Hz, 3H). MS (ESI) m/z: 276.3 $[M + H]^+$.

Compounds 8–28 were synthesized via similar routes to compound 7.

5-(2-Fluorophenyl)-N-(4-(5-((4-hydroxy-4-methylpentyl)oxy)-2-methylphenyl)pyridin-2-yl)-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxamide (8). Purified by preparative thin layer chromatography (dichloromethane:methanol = 20:1, V:V) as a white solid, yield 58%. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.38 (d, J = 5.1 Hz, 1H), 8.27 (s, 1H), 7.48–7.39 (m, 1H), 7.28–7.16 (m, 5H), 6.92 (dd, J = 8.4, 2.7 Hz, 1H), 6.82 (d, J = 2.7 Hz, 1H), 5.86 (dd, J = 8.6, 5.8 Hz, 1H), 4.01 (t, J = 6.4 Hz, 2H), 3.42–3.36 (m, 1H), 3.28–3.11 (m, 2H), 2.82–2.72 (m, 1H), 2.24 (s, 3H), 1.93–1.81 (m, 2H), 1.67–1.62 (m, 2H), 1.23 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.8, 160.7, 160.0 (^{1}Jc–f = 247.0 Hz), 157.2, 157.0, 150.9 (^{2}Jc–f = 58.0 Hz), 148.3, 139.5, 131.8, 130.7 (^{3}Jc–f = 8.4 Hz), 128.9 (^{4}Jc–f = 3.0 Hz), 126.1, 126.0 (^{3}Jc–f = 12.5 Hz), 124.9, 120.7, 116.0, 115.9, 114.9, 114.6, 113.8, 68.5, 68.3, 56.1, 34.0, 29.3, 24.0, 20.6, 19.0. HRMS: calcd for $C_{30}H_{33}FN_5O_3$ $[M + H]^+$, 530.2567; found, 530.2563.

5-(3-Fluorophenyl)-N-(4-(5-((4-hydroxy-4-methylpentyl)oxy)-2-methylphenyl)pyridin-2-yl)-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxamide (9). Purified by preparative thin layer chromatography (dichloromethane:methanol = 20:1, V:V) as a white solid, yield 56%. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.38 (d, J = 5.2 Hz, 1H), 8.28 (s, 1H), 7.51–7.41 (m, 1H), 7.23 (d, J = 8.4 Hz, 1H), 7.18 (d, J = 5.2 Hz, 1H), 7.17–7.06 (m, 3H), 6.92 (dd, J = 8.4, 2.8 Hz, 1H), 6.83 (d, J = 2.7 Hz, 1H), 5.69–5.62 (m, 1H), 4.01 (t, J = 6.4 Hz, 2H), 3.30–3.07 (m, 3H), 2.78–2.68 (m, 1H), 2.25 (s, 3H), 1.92–1.82 (m, 2H), 1.69–1.60 (m, 2H), 1.23 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.9, 162.4 (^{1}Jc–f = 244.0 Hz), 160.7, 157.1 (^{2}Jc–f = 32.0 Hz), 151.1, 150.7, 148.3, 142.0 (^{3}Jc–f = 7.1 Hz), 139.5, 131.8, 130.9 (^{3}Jc–f = 8.0 Hz), 126.1, 123.0, 120.7, 115.3, 115.2, 114.9, 114.6, 113.8, 113.7 (^{2}Jc–f = 21.0 Hz), 68.5, 68.3, 60.4, 35.2, 29.3, 24.0, 20.6, 19.0. HRMS: calcd for $C_{30}H_{33}FN_5O_3$ $[M + H]^+$, 530.2567; found, 530.2560.

5-(4-Fluorophenyl)-N-(4-(5-((4-hydroxy-4-methylpentyl)oxy)-2-methylphenyl)pyridin-2-yl)-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxamide (10). Purified by preparative thin layer chromatography (dichloromethane:methanol = 20:1, V:V) as a white solid, yield 55%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.90 (s, 1H), 8.42 (d, J = 5.1 Hz, 1H), 8.10 (d, J = 1.3 Hz, 1H), 7.38 (dd, J = 8.7, 5.4 Hz, 2H), 7.30–7.19 (m, 4H), 6.93 (dd, J = 8.4, 2.7 Hz, 1H), 6.80 (d, J = 2.7 Hz, 1H), 5.67 (t, J = 7.2 Hz, 1H), 4.19

(s, 1H), 3.97 (t, J = 6.6 Hz, 2H), 3.26–3.01 (m, 4H), 2.19 (s, 3H), 1.80–1.70 (m, 2H), 1.52–1.43 (m, 2H), 1.10 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.6, 162.0 (^{1}Jc–f = 244.0 Hz), 160.6, 157.1 (^{2}Jc–f = 31.0 Hz), 151.1, 150.7, 148.3, 139.5, 135.4, 131.8, 129.1 (^{3}Jc–f = 8.1 Hz), 126.1, 120.7, 115.8, 115.6, 114.9, 114.6, 113.7, 68.5, 68.3, 60.4, 35.3, 29.3, 24.0, 20.6, 19.0. HRMS: calcd for $C_{30}H_{33}FN_5O_3$ $[M + H]^+$, 530.2567; found, 530.2562.

5-Cyclohexyl-N-(4-(5-((4-hydroxy-4-methylpentyl)oxy)-2-methylphenyl)pyridin-2-yl)-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxamide (11). Purified by preparative thin layer chromatography (dichloromethane:methanol = 20:1, V:V) as a white solid, yield 52%. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.39 (d, J = 5.1 Hz, 1H), 8.30 (s, 1H), 7.23 (d, J = 8.4 Hz, 1H), 7.18 (dd, J = 5.2, 1.4 Hz, 1H), 6.91 (dd, J = 8.5, 2.7 Hz, 1H), 6.83 (d, J = 2.7 Hz, 1H), 4.43–4.34 (m, 1H), 4.00 (t, J = 6.4 Hz, 2H), 3.02–2.93 (m, 2H), 2.89–2.80 (m, 2H), 2.66–2.53 (m, 1H), 2.25 (s, 3H), 2.03–1.75 (m, 8H), 1.75–1.60 (m, 4H), 1.51–1.41 (m, 2H), 1.23 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.2, 160.0, 157.3, 156.9, 151.1, 150.7, 148.3, 139.6, 131.8, 126.1, 120.6, 114.9, 114.6, 113.7, 68.5, 68.3, 62.3, 41.1, 29.3, 28.2, 27.7, 27.1, 25.7, 25.4, 24.0, 20.5, 19.0. HRMS: calcd for $C_{30}H_{40}N_5O_3$ $[M + H]^+$, 518.3131; found, 518.3124.

5-Cyclopentyl-N-(4-(5-((4-hydroxy-4-methylpentyl)oxy)-2-methylphenyl)pyridin-2-yl)-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxamide (12). Purified by preparative thin layer chromatography (dichloromethane:methanol = 20:1, V:V) as a white solid, yield 70%. ^{1}H NMR (400 MHz, acetone-d_6) δ 9.59 (s, 1H), 8.40 (d, J = 5.1 Hz, 1H), 8.35 (d, J = 1.4 Hz, 1H), 7.24 (d, J = 8.4 Hz, 1H), 7.16 (dd, J = 5.2, 1.6 Hz, 1H), 6.92 (dd, J = 8.4, 2.8 Hz, 1H), 6.86 (d, J = 2.7 Hz, 1H), 4.43 (td, J = 8.0, 4.8 Hz, 1H), 4.03 (t, J = 6.6 Hz, 2H), 3.30 (s, 1H), 3.00–2.91 (m, 3H), 2.58–2.47 (m, 1H), 2.36–2.26 (m, 1H), 2.24 (s, 3H), 2.03–1.96 (m, 1H), 1.93–1.84 (m, 2H), 1.81–1.72 (m, 1H), 1.68–1.45 (m, 8H), 1.19 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.0, 159.9, 157.3, 156.9, 151.1, 150.7, 148.3, 139.6, 131.8, 126.2, 120.6, 114.9, 114.6, 113.6, 68.5, 68.3, 61.7, 43.7, 30.2, 29.3, 28.8, 28.0, 25.0, 24.6, 24.0, 20.3, 19.0. HRMS: calcd for $C_{29}H_{38}N_5O_3$ $[M + H]^+$, 504.2975; found, 504.2966.

trans-7-Fluoro-N-(4-(5-((4-hydroxy-4-methylpentyl)oxy)-2-methylphenyl)pyridin-2-yl)-5-phenyl-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxamide (13). Purified by preparative thin layer chromatography (dichloromethane:methanol = 20:1, V:V) as a white solid, yield 42%. ^{1}H NMR (400 MHz, chloroform-d) δ 9.56 (s, 1H), 8.43–8.40 (m, 1H), 8.37 (d, J = 5.1 Hz, 1H), 7.47–7.41 (m, 3H), 7.23–7.17 (m, 3H), 7.08 (dd, J = 5.2, 1.5 Hz, 1H), 6.88 (dd, J = 8.3, 2.8 Hz, 1H), 6.84 (d, J = 2.7 Hz, 1H), 6.13 (dd, ^{2}Jh–f = 55.4 Hz, J = 6.2 Hz, 1H), 5.78 (td, J = 6.6, 3.1 Hz, 1H), 4.01 (t, J = 6.3 Hz, 2H), 3.52–3.38 (m, 1H), 3.17–3.01 (m, 2H), 2.83 (d, J = 0.6 Hz, 3H), 2.27 (s, 3H), 1.96–1.86 (m, 2H), 1.70–1.66 (m, 2H), 1.28 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.7, 158.7 (^{2}Jc–f = 22 Hz), 156.9, 151.1, 150.6, 148.3, 139.5, 137.3, 131.8, 128.9, 128.8, 127.3, 126.1, 120.8, 114.9, 114.6, 114.1, 83.4 (^{1}Jc–f = 178.0 Hz), 68.5, 68.3, 60.4, 44.1 (^{2}Jc–f = 23.0 Hz), 38.2, 29.3, 24.0, 19.0. HRMS: calcd for $C_{30}H_{33}FN_5O_3$ [M +

H]$^+$, 530.2567; found, 530.2559.

cis-7-Fluoro-N-(4-(5-((4-hydroxy-4-methylpentyl)oxy)-2-methylphenyl)pyridin-2-yl)-5-phenyl-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxamide (14). Purified by preparative thin layer chromatography (dichloromethane:methanol = 20:1, V:V) as a white solid, yield 38%. ^{1}H NMR (400 MHz, chloroform-d) δ 9.53 (s, 1H), 8.38 (s, 1H), 8.34 (d, J = 4.8 Hz, 1H), 7.39 (s, 3H), 7.17 (d, J = 8.1 Hz, 1H), 7.05 (d, J = 4.7 Hz, 1H), 6.85 (d, J = 8.1 Hz, 1H), 6.81 (s, 1H), 6.05 (dd, ^{2}Jh–f = 55.9 Hz, J = 7.0 Hz, 1H), 5.51 (s, 1H), 4.02–3.96 (m, 2H), 3.75–3.58 (m, 1H), 3.07–2.91 (m, 1H), 2.23 (s, 3H), 1.92–1.83 (m, 2H), 1.68–1.63 (m, 2H), 1.25 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.8, 158.7 (^{2}Jc–f = 23.0 Hz), 156.9, 151.1, 150.6, 148.3, 139.5, 138.3, 131.8, 129.0, 128.7, 126.7, 126.1, 120.8, 114.9, 114.6, 114.1, 83.2 (^{1}Jc–f = 178.0 Hz), 68.5, 68.3, 60.1, 43.0 (^{2}Jc–f = 22.0 Hz), 29.3, 24.0, 19.0. HRMS: calcd for $C_{30}H_{33}FN_5O_3$ [M + H]$^+$, 530.2567; found, 530.2564.

N-(5-Fluoro-4-(5-((4-hydroxy-4-methylpentyl)oxy)-2-methylphenyl)pyridin-2-yl)-5-phenyl-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxamide (15). Purified by preparative thin layer chromatography (dichloromethane:methanol = 20:1, V:V) as a white solid, yield 55%. ^{1}H NMR (400 MHz, acetone-d_6) δ 9.64 (s, 1H), 8.33 (d, J = 1.4 Hz, 1H), 8.30 (d, J = 5.6 Hz, 1H), 7.43–7.33 (m, 3H), 7.32–7.24 (m, 3H), 6.96 (dd, J = 8.4, 2.7 Hz, 1H), 6.88 (d, J = 2.7 Hz, 1H), 5.65 (dd, J = 8.3, 5.9 Hz, 1H), 4.01 (t, J = 6.6 Hz, 2H), 3.40–3.28 (m, 2H), 3.25–3.14 (m, 1H), 3.14–3.03 (m, 1H), 2.73 (s, 1H), 2.15 (s, 3H), 1.91–1.82 (m, 2H), 1.62–1.56 (m, 2H), 1.18 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.7, 160.6, 157.1, 156.8, 153.1 (^{1}Jc–f = 249.0 Hz), 147.1, 139.3, 138.1 (^{3}Jc–f = 16.0 Hz), 136.2 (^{2}Jc–f = 27.0 Hz), 133.6, 131.4, 128.9, 128.4, 127.1, 126.7, 115.6, 115.3, 115.1, 68.5, 68.4, 61.1, 35.4, 29.3, 23.9, 20.6, 18.4. HRMS: calcd for $C_{30}H_{33}FN_5O_3$ [M + H]$^+$, 530.2567; found, 530.2566.

N-(5-Chloro-4-(5-((4-hydroxy-4-methylpentyl)oxy)-2-methylphenyl)pyridin-2-yl)-5-phenyl-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxamide (16). Purified by preparative thin layer chromatography (dichloromethane:methanol = 20:1, V:V) as a white solid, yield 52%. ^{1}H NMR (400 MHz, acetone-d_6) δ 9.64 (s, 1H), 8.44 (s, 1H), 8.27 (s, 1H), 7.44–7.34 (m, 3H), 7.30 (d, J = 7.3 Hz, 2H), 7.25 (d, J = 8.6 Hz, 1H), 6.95 (dd, J = 8.4, 2.6 Hz, 1H), 6.78 (s, 1H), 5.66 (t, J = 7.3 Hz, 1H), 4.01 (t, J = 6.6 Hz, 2H), 3.39 (s, 1H), 3.27–3.17 (m, 2H), 3.16–3.09 (m, 1H), 1.91–1.84 (m, 2H), 1.62–1.57 (m, 2H), 1.18 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.7, 160.5, 157.3, 156.7, 149.5, 149.4, 147.5, 139.3, 137.2, 131.2, 128.9, 128.4, 126.8, 126.4, 125.3, 115.2, 115.0, 114.3, 68.5, 68.3, 61.1, 35.4, 29.3, 23.9, 20.6, 18.2. HRMS: calcd for $C_{30}H_{33}ClN_5O_3$ [M + H]$^+$, 546.2272; found, 546.2263.

N-(4-(5-((4-Hydroxy-4-methylpentyl)oxy)-2-methylphenyl)-5-methylpyridin-2-yl)-5-phenyl-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxamide (17). Purified by preparative thin layer chromatography (dichloromethane:methanol = 20:1, V:V) as a white solid, yield 43%. ^{1}H NMR (400 MHz, acetone-d_6) δ 9.52 (s, 1H), 8.28 (s, 1H), 8.11 (s, 1H), 7.45–7.38 (m,

3H), 7.35–7.29 (m, 2H), 7.25 (d, J = 8.4 Hz, 1H), 6.92 (dd, J = 8.4, 2.8 Hz, 1H), 6.72 (d, J = 2.7 Hz, 1H), 5.67 (dd, J = 8.3, 5.9 Hz, 1H), 4.02 (t, J = 6.6 Hz, 2H), 3.44–3.34 (m, 1H), 3.27–3.18 (m, 2H), 3.18–3.08 (m, 1H), 2.81 (s, 3H), 2.75 (s, 1H), 2.02 (s, 3H), 1.91–1.85 (m, 2H), 1.64–1.59 (m, 2H), 1.20 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.6, 160.7, 157.0, 156.7, 150.9, 148.9, 148.7, 139.3, 131.2, 128.9, 128.4, 127.4, 126.7, 125.9, 114.3, 113.9, 113.3, 68.5, 68.2, 61.0, 35.4, 29.3, 23.9, 20.6, 18.3, 15.8. HRMS: calcd for $C_{31}H_{36}N_5O_3$ [M + H]$^+$, 526.2818; found, 526.2813.

N-(5-(5-((4-Hydroxy-4-methylpentyl)oxy)-2-methylphenyl)pyridazin-3-yl)-5-phenyl-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxamide (18). Purified by preparative thin layer chromatography (dichloromethane:methanol = 20:1, V:V) as a white solid, yield 28%. ^{1}H NMR (400 MHz, acetone-d_6) δ 10.13 (s, 1H), 9.70 (d, J = 2.5 Hz, 1H), 8.33 (d, J = 2.5 Hz, 1H), 7.46–7.35 (m, 3H), 7.32–7.23 (m, 3H), 7.06 (d, J = 2.6 Hz, 1H), 6.96 (d, J = 8.7 Hz, 1H), 5.66 (t, J = 7.1 Hz, 1H), 4.04 (t, J = 6.6 Hz, 2H), 3.43–3.31 (m, 1H), 3.30 (s, 1H), 3.26–3.17 (m, 1H), 3.16–3.06 (m, 1H), 2.31 (s, 3H), 1.92–1.85 (m, 2H), 1.64–1.58 (m, 2H), 1.19 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.9, 161.0, 160.4, 158.8, 156.9, 142.4, 139.4, 138.2, 137.7, 131.9, 128.9, 128.4, 127.2, 126.8, 115.4, 115.3, 115.2, 68.5, 68.3, 61.1, 35.5, 29.3, 24.0, 20.5, 19.0. HRMS: calcd for $C_{29}H_{33}N_6O_3$ [M + H]$^+$, 513.2614; found, 513.2605.

N-(4-(2-Chloro-5-((4-hydroxy-4-methylpentyl)oxy)phenyl)pyridin-2-yl)-5-phenyl-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxamide (19). Purified by preparative thin layer chromatography (dichloromethane:methanol = 20:1, V:V) as a white solid, yield 49%. ^{1}H NMR (400 MHz, acetone-d_6) δ 9.61 (s, 1H), 8.47–8.39 (m, 2H), 7.46 (d, J = 8.4 Hz, 1H), 7.43–7.36 (m, 3H), 7.33–7.30 (m, 2H), 7.25 (dd, J = 5.3, 1.4 Hz, 1H), 7.07–7.02 (m, 2H), 5.67 (dd, J = 8.3, 6.0 Hz, 1H), 4.08 (t, J = 6.6 Hz, 2H), 3.44–3.28 (m, 2H), 3.26 (s, 1H), 3.23–3.07 (m, 2H), 1.94–1.85 (m, 2H), 1.65–1.57 (m, 2H), 1.19 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.7, 160.6, 157.8, 157.2, 150.7, 148.5, 148.4, 139.3, 138.2, 131.0, 128.9, 128.4, 126.8, 121.8, 120.8, 116.7, 116.5, 113.9, 68.8, 68.5, 61.1, 35.4, 29.3, 23.8, 20.6. HRMS: calcd for $C_{29}H_{31}ClN_5O_3$ [M + H]$^+$, 532.2115; found, 532.2109.

N-(4-(3-((4-Hydroxy-4-methylpentyl)oxy)phenyl)pyridin-2-yl)-5-phenyl-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxamide (20). Purified by preparative thin layer chromatography (dichloromethane:methanol = 20:1, V:V) as a white solid, yield 58%. ^{1}H NMR (400 MHz, acetone-d_6) δ 9.59 (s, 1H), 8.64 (s, 1H), 8.41 (d, J = 5.3 Hz, 1H), 7.50–7.38 (m, 5H), 7.37–7.30 (m, 4H), 7.08 (d, J = 8.3 Hz, 1H), 5.69 (t, J = 7.3 Hz, 1H), 4.13 (t, J = 6.5 Hz, 2H), 3.45–3.33 (m, 1H), 3.29 (d, J = 1.5 Hz, 1H), 3.25–3.09 (m, 2H), 2.81–2.69 (m, 1H), 1.99–1.88 (m, 2H), 1.70–1.61 (m, 2H), 1.22 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.7, 160.6, 159.3, 157.2, 151.3, 149.5, 148.9, 139.3, 138.8, 130.5, 128.9, 128.4, 126.8, 118.9, 118.2, 115.3, 112.9, 111.1, 68.5, 68.4, 61.1, 35.4, 29.3, 23.9, 20.6. HRMS: calcd for $C_{29}H_{32}N_5O_3$ [M + H]$^+$, 498.2505; found, 498.2500.

N-(4-(5-Methoxy-2-methylphenyl)pyridin-2-yl)-5-phenyl-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxamide (21). Purified by preparative thin layer chromatography (dichloromethane:methanol = 20:1, V:V) as a white solid, yield 53%. ^{1}H NMR (400 MHz, acetone-d_6) δ 9.58 (s, 1H), 8.39 (d, J = 5.1 Hz, 1H), 8.32 (d, J = 1.4 Hz, 1H), 7.44–7.35 (m, 3H), 7.34–7.29 (m, 2H), 7.25 (d, J = 8.5 Hz, 1H), 7.15 (dd, J = 5.2, 1.5 Hz, 1H), 6.92 (dd, J = 8.5, 2.8 Hz, 1H), 6.85 (d, J = 2.8 Hz, 1H), 5.69–5.64 (m, 1H), 3.81 (s, 3H), 3.44–3.33 (m, 1H), 3.27–3.17 (m, 1H), 3.16–3.05 (m, 1H), 2.80 (s, 1H), 2.79–2.69 (m, 1H), 2.23 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.7, 160.6, 157.5, 157.2, 151.1, 150.7, 148.3, 139.6, 139.3, 131.7, 128.9, 128.4, 126.8, 126.3, 120.7, 114.3, 114.1, 113.7, 61.1, 55.2, 35.4, 20.6, 19.0. HRMS: calcd for $C_{25}H_{24}N_5O_2$ $[M + H]^+$, 426.1930; found, 426.1925.

N-(4-(5-(2-Hydroxy-2-methylpropoxy)-2-methylphenyl)pyridin-2-yl)-5-phenyl-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxamide (22). Purified by preparative thin layer chromatography (dichloromethane:methanol = 20:1, V:V) as a white solid, yield 45%. ^{1}H NMR (400 MHz, acetone-d_6) δ 9.58 (s, 1H), 8.39 (d, J = 5.1 Hz, 1H), 8.32 (s, 1H), 7.45–7.34 (m, 3H), 7.33–7.28 (m, 2H), 7.24 (d, J = 8.4 Hz, 1H), 7.16 (dd, J = 5.1, 1.6 Hz, 1H), 6.95 (dd, J = 8.5, 2.8 Hz, 1H), 6.88 (d, J = 2.6 Hz, 1H), 5.74–5.63 (m, 1H), 3.83 (s, 2H), 3.72 (s, 1H), 3.42–3.32 (m, 1H), 3.28–3.18 (m, 1H), 3.18–3.07 (m, 1H), 2.79–2.74 (m, 1H), 2.23 (s, 3H), 1.28 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.7, 160.6, 157.2, 151.1, 150.7, 148.3, 139.5, 139.3, 131.8, 128.9, 128.4, 126.8, 126.5, 126.2, 120.6, 115.1, 114.8, 113.7, 76.3, 68.6, 61.1, 35.4, 26.6, 20.6, 19.0. HRMS: calcd for $C_{28}H_{30}N_5O_3$ $[M + H]^+$, 484.2349; found, 484.2345.

N-(4-(2-Methyl-5-(2-(tetrahydro-2H-pyran-4-yl)ethoxy)phenyl)pyridin-2-yl)-5-phenyl-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxamide (23). Purified by preparative thin layer chromatography (dichloromethane:methanol = 20:1, V:V) as a white solid, yield 38%. ^{1}H NMR (400 MHz, acetone-d_6) δ 9.58 (s, 1H), 8.39 (d, J = 5.1 Hz, 1H), 8.32 (s, 1H), 7.45–7.34 (m, 3H), 7.34–7.29 (m, 2H), 7.24 (d, J = 8.4 Hz, 1H), 7.15 (dd, J = 5.2, 1.5 Hz, 1H), 6.92 (dd, J = 8.3, 2.8 Hz, 1H), 6.86 (d, J = 2.7 Hz, 1H), 5.66 (dd, J = 8.3, 6.0 Hz, 1H), 4.08 (t, J = 6.4 Hz, 2H), 3.92–3.81 (m, 2H), 3.43–3.26 (m, 3H), 3.26–3.16 (m, 1H), 3.16–3.07 (m, 1H), 2.79–2.67 (m, 1H), 2.23 (s, 3H), 1.75–1.63 (m, 4H), 1.32–1.25 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.7, 160.6, 157.2, 156.8, 151.1, 150.7, 148.3, 139.5, 139.3, 131.7, 128.9, 128.4, 126.8, 126.2, 120.7, 114.9, 114.7, 113.7, 67.0, 65.1, 61.1, 35.6, 35.4, 32.6, 31.5, 20.6, 19.0. HRMS: calcd for $C_{31}H_{34}N_5O_3$ $[M + H]^+$, 524.2662; found, 524.2660.

N-(4-(5-(3-Cyanopropoxy)-2-methylphenyl)pyridin-2-yl)-5-phenyl-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxamide (24). Purified by preparative thin layer chromatography (dichloromethane:methanol = 20:1, V:V) as a white solid, yield 53%. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.35 (d, J = 5.2 Hz, 1H), 8.25 (s, 1H), 7.43–7.33 (m, 3H), 7.29–7.20 (m, 3H), 7.18–7.12 (m, 1H), 6.93 (dd, J = 8.3, 2.7 Hz, 1H), 6.84 (d, J = 2.7 Hz, 1H), 5.60 (t, J = 7.2 Hz, 1H), 4.08 (t, J = 5.9 Hz, 2H), 3.27 (s, 1H), 3.18 (dd, J =

9.4, 4.4 Hz, 1H), 3.14–3.05 (m, 1H), 2.76–2.67 (m, 1H), 2.64 (t, J = 7.1 Hz, 2H), 2.22 (s, 3H), 2.16–2.05 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.7, 160.6, 157.2, 156.5, 151.0, 150.7, 148.3, 139.6, 139.3, 131.8, 128.9, 128.4, 126.8, 120.6, 120.3, 115.0, 114.7, 113.7, 65.9, 61.1, 35.4, 24.7, 20.6, 19.0, 13.3. HRMS: calcd for $C_{28}H_{27}N_6O_2$ $[M + H]^+$, 479.2195; found, 479.2193.

(R,R)-7-Fluoro-N-(4-(2-methyl-5-(2-morpholinoethoxy)phenyl)pyridin-2-yl)-5-phenyl-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxamide (25). Purified by preparative thin layer chromatography (dichloromethane:methanol = 15:1, V:V) as a white solid, yield 40%. ^{1}H NMR (600 MHz, methanol-d_4) δ 8.37 (d, J = 5.2 Hz, 1H), 8.25 (s, 1H), 7.46–7.37 (m, 3H), 7.33–7.29 (m, 2H), 7.23 (d, J = 8.4 Hz, 1H), 7.19–7.15 (m, 1H), 6.95–6.91 (m, 1H), 6.85 (d, J = 2.7 Hz, 1H), 6.15 (dd, ^{2}Jh–f = 56.3 Hz, J = 7.1 Hz, 1H), 5.70–5.64 (m, 1H), 4.15 (t, J = 5.5 Hz, 2H), 3.84–3.74 (m, 1H), 3.71 (t, J = 4.7 Hz, 4H), 2.90–2.83 (m, 1H), 2.81 (t, J = 5.4 Hz, 2H), 2.60 (t, J = 4.7 Hz, 4H), 2.23 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.8, 158.7 (^{2}Jc–f = 22.0 Hz), 156.9, 156.7, 151.0, 150.6, 148.3, 139.5, 138.3, 131.8, 129.0, 128.7, 126.7, 126.4, 120.8, 114.9, 114.8, 114.1, 83.2 (^{1}Jc–f = 178.0 Hz), 66.2, 65.5, 60.1, 57.0, 53.6, 43.0 (^{2}Jc–f = 22.0 Hz), 19.0. HRMS: calcd for $C_{30}H_{32}FN_6O_3$ $[M + H]^+$, 543.2520; found, 543.2518.

(S,S)-7-Fluoro-N-(4-(2-methyl-5-(2-morpholinoethoxy)phenyl)pyridin-2-yl)-5-phenyl-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxamide (26). Purified by preparative thin layer chromatography (dichloromethane:methanol = 15:1, V:V) as a white solid, yield 43%. ^{1}H NMR (600 MHz, methanol-d_4) δ 8.37 (d, J = 5.2 Hz, 1H), 8.25 (s, 1H), 7.46–7.37 (m, 3H), 7.33–7.29 (m, 2H), 7.23 (d, J = 8.4 Hz, 1H), 7.19–7.15 (m, 1H), 6.95–6.91 (m, 1H), 6.85 (d, J = 2.7 Hz, 1H), 6.15 (dd, ^{2}Jh–f = 56.3 Hz, J = 7.1 Hz, 1H), 5.70–5.64 (m, 1H), 4.15 (t, J = 5.5 Hz, 2H), 3.84–3.74 (m, 1H), 3.71 (t, J = 4.7 Hz, 4H), 2.90–2.83 (m, 1H), 2.81 (t, J = 5.4 Hz, 2H), 2.60 (t, J = 4.7 Hz, 4H), 2.23 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.8, 158.7 (^{2}Jc–f = 22.0 Hz), 156.9, 156.7, 151.0, 150.6, 148.3, 139.5, 138.3, 131.8, 129.0, 128.7, 126.7, 126.4, 120.8, 114.9, 114.8, 114.1, 83.2 (^{1}Jc–f = 178.0 Hz), 66.2, 65.5, 60.1, 57.0, 53.6, 43.0 (^{2}Jc–f = 22.0 Hz), 19.0. HRMS: calcd for $C_{30}H_{32}FN_6O_3$ $[M + H]^+$, 543.2520; found, 543.2515.

(R,R)-7-Fluoro-N-(4-(2-methyl-5-(2-(tetrahydro-2H-pyran-4-yl)ethoxy)phenyl)pyridin-2-yl)-5-phenyl-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxamide (27). Purified by preparative thin layer chromatography (dichloromethane:methanol = 15:1, V:V) as a white solid, yield 40%. ^{1}H NMR (600 MHz, methanol-d_4) δ 8.38 (d, J = 5.1 Hz, 1H), 8.26 (s, 1H), 7.46–7.39 (m, 3H), 7.33 (d, J = 7.4 Hz, 2H), 7.23 (d, J = 8.5 Hz, 1H), 7.18 (d, J = 5.0 Hz, 1H), 6.91 (d, J = 8.5 Hz, 1H), 6.82 (s, 1H), 6.16 (dd, ^{2}Jh–f = 56.2 Hz, J = 7.1 Hz, 1H), 5.69 (d, J = 8.3 Hz, 1H), 4.06 (t, J = 6.4 Hz, 2H), 3.97–3.90 (m, 2H), 3.86–3.75 (m, 1H), 3.43 (t, J = 11.8 Hz, 2H), 2.87 (dd, J = 26.6, 15.2 Hz, 1H), 2.24 (s, 3H), 1.83 (s, 1H), 1.76–1.68 (m, 4H), 1.40–1.28 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.8, 158.7 (^{2}Jc–f = 22.0 Hz), 156.9, 156.9, 151.1, 150.6, 148.3, 139.5, 138.2,

131.8, 129.0, 128.7, 126.7, 126.2, 120.8, 114.9, 114.7, 114.1, 83.2 (^{1}Jc–f = 178.0 Hz), 67.0, 65.1, 60.1, 43.0 (^{2}Jc–f = 22.0 Hz), 35.6, 32.6, 31.5, 19.0. HRMS: calcd for $C_{31}H_{33}FN_5O_3$ $[M + H]^+$, 542.2567; found, 542.2560.

(S,S)-7-Fluoro-N-(4-(2-methyl-5-(2-(tetrahydro-2H-pyran-4-yl)ethoxy)phenyl)pyridin-2-yl)-5-phenyl-6,7-dihydro-5H-pyrrolo[1,2-b][1,2,4]triazole-2-carboxamide (28). Purified by preparative thin layer chromatography (dichloromethane:methanol = 15:1, V:V) as a white solid, yield 39%. ^{1}H NMR (600 MHz, methanol-d_4) δ 8.38 (d, J = 5.1 Hz, 1H), 8.26 (s, 1H), 7.46–7.39 (m, 3H), 7.33 (d, J = 7.4 Hz, 2H), 7.23 (d, J = 8.5 Hz, 1H), 7.18 (d, J = 5.0 Hz, 1H), 6.91 (d, J = 8.5 Hz, 1H), 6.82 (s, 1H), 6.16 (dd, ^{2}Jh–f = 56.2 Hz, J = 7.1 Hz, 1H), 5.69 (d, J = 8.3 Hz, 1H), 4.06 (t, J = 6.4 Hz, 2H), 3.97–3.90 (m, 2H), 3.86–3.75 (m, 1H), 3.43 (t, J = 11.8 Hz, 2H), 2.87 (dd, J = 26.6, 15.2 Hz, 1H), 2.24 (s, 3H), 1.83 (s, 1H), 1.76–1.68 (m, 4H), 1.40–1.28 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.8, 158.7 (^{2}Jc–f = 22.0 Hz), 156.9, 156.9, 151.1, 150.6, 148.3, 139.5, 138.2, 131.8, 129.0, 128.7, 126.7, 126.2, 120.8, 114.9, 114.7, 114.1, 83.2 (^{1}Jc–f = 178.0 Hz), 67.0, 65.1, 60.1, 43.0 (^{2}Jc–f = 22.0 Hz), 35.6, 32.6, 31.5, 19.0. HRMS: calcd for $C_{31}H_{33}FN_5O_3$ $[M + H]^+$, 542.2567; found, 542.2565.

Biology

Kinase inhibition assay. The RIPK1 inhibitory activities of compounds were evaluated using the ADP-Glo Assay Kit (Promega, United States) according to the manufacturer's instructions. A SpectraMax Paradigm reader (MD) was used to record luminescence. Inhibition% = ((specific signal (DMSO control) – specific signal (inhibitor))/(specific signal (DMSO control))) × 100%.

Necroptosis induction and cell viability assays. I2.1 and Hepa1-6 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were seeded in 96-well plates with growth media at low density and incubated overnight. For I2.1 cells, designated concentrations of the tested compounds were added to each well. After 1 h, cells were stimulated with TNF-α (20 ng mL^{-1}) and further incubated for 24 h. For Hepa1-6 cells, on the following day, cells were treated with TNF-α (20 ng mL^{-1}) and BV6 (10 M) to induce necroptosis, along with different concentrations of the tested compounds for 24 h. Z-VAD (10 M) was added 1 h prior to the above treatments. Finally, the effect of the tested compounds on I2.1 and Hepa1-6 cell viability was determined using a cell counting kit (CCK-8) (Dojindo, Japan) assay.

Pharmacokinetic evaluation. Compound 26 was dissolved in DMSO/0.5% HMPC (5/95, v/v) and administered orally to Sprague Dawley (SD) rats (male, N = 3). Blood samples were collected at 0.25, 0.5, 1, 2, 4, 8, and 24 h after p.o. administration, and the concentrations of compound 26 in serum were determined by LC-MS/MS. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Shanghai Institute of Materia Medica and approved by the Animal Ethics Committee of Shanghai Institute of Materia Medica (approval no. 2023-01-YY-26).

Molecular docking study. The docking studies were carried out with Maestro 11.0 using the crystal structure (PDB code: 6NYH) obtained from the RCSB Protein Data Bank as the receptor. Both bond orders and hydrogen atoms were optimized, and water molecules were removed. The docking box was set to 14 Å × 14 Å × 14 Å and centered at the native ligand. Compound 26 was prepared with LigPrep using the OPLS force field. Compound 26 was then docked into the well-defined docking grids with standard precision (SP) mode. [Figure 3: see original paper] was generated with PyMOL version 1.3.

Author contributions

Z. J. and Y. D. contributed equally to this article. Z. J. and Y. D.: investigation, methodology, writing – original draft. Y. J.: conceptualization, supervision, writing – review & editing. H. Z. and J. A.: conceptualization, investigation, supervision, writing – review & editing. W. D.: investigation, methodology.

Conflicts of interest

The authors declare that they have no known competing financial interests.

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Note: Figure translations are in progress. See original paper for figures.