Novel pyrrolopyrimidine analogues as potent dipeptidyl peptidase IV inhibitors based on pharmacokinetic property-driven optimization postprint

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Date: 2017-11-17T00:00:00+00:00

Abstract

We previously reported a highly potent DPP-IV inhibitor 6 with low in vivo efficacy. While trying to maintain consistent in vitro and in vivo biological activity, we initiated a pharmacokinetic property-driven optimization to improve the metabolic stability and permeability of inhibitor 6. A simple scaffold replacement of thienopyrimidine with pyrrolopyrimidine (21a) led to significantly improved metabolic stability (4% vs. 65% remaining). Further modification of the pyrrolopyrimidine scaffold to produce compound 21j resulted in much better oral bioavailability than 6. Importantly, compound 21j exhibits greater in vivo efficacy than does 6 and Alogliptin and is worthy of further development. (C) 2012 Elsevier Masson SAS. All rights reserved.

Full Text

Preamble

Novel Pyrrolopyrimidine Analogues as Potent Dipeptidyl Peptidase IV Inhibitors Based on Pharmacokinetic Property-Driven Optimization

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Abstract

We previously reported a highly potent DPP-IV inhibitor, compound 6, which exhibited low in vivo efficacy. In an effort to maintain consistent in vitro and in vivo biological activity, we initiated a pharmacokinetic property-driven optimization campaign to improve the metabolic stability and permeability of inhibitor 6. A simple scaffold replacement of thienopyrimidine with pyrrolopyrimidine (21a) led to significantly improved metabolic stability (65% vs. 4% remaining after incubation). Further modification of the pyrrolopyrimidine scaffold to produce compound 21j resulted in much better oral bioavailability than compound 6. Importantly, compound 21j demonstrates greater in vivo efficacy than both compound 6 and alogliptin, making it worthy of further development.

Keywords: DPP-IV inhibitor; type 2 diabetes; PK-driven optimization; pyrrolopyrimidine analogues; oral glucose tolerance test

1. Introduction

With more than 220 million people affected worldwide, diabetes has emerged as a major epidemic in this century. Type 2 diabetes (T2D), formerly referred to as non-insulin-dependent or adult-onset diabetes, results from the body’ s ineffective use of insulin and accounts for 90% of all diabetes cases. The growing number of deaths attributable to diabetes reflects the insufficient glycemic control achieved by past and current treatments [1]. Since 2004, dipeptidyl peptidase IV (DPP-IV) inhibitors have demonstrated effectiveness and safety in controlling blood glucose, offering good patient compliance and reduced risks of hypoglycemia and other side effects [2-4]. To date, sitagliptin (1) [5-6], vildagliptin (2) [7-9], saxagliptin (3) [10], alogliptin (4) [11], and linagliptin (5) [12] have been approved for market in many countries (Figure 1 [Figure 1: see original paper]).

We previously reported a novel series of DPP-IV inhibitors [13]. Compound 6 (IC50 = 0.33 nM) was observed to be 10 times more potent against DPP-IV than alogliptin and showed selectivity for DPP-IV over other enzymes within the dipeptidyl peptidase family. However, the in vivo efficacy of compound 6 was similar to that of alogliptin, leading us to hypothesize that insufficient in vivo activity might be due to poor pharmacokinetic properties, particularly low oral bioavailability.

In this study, we conducted stability studies of thienopyrimidine analogues followed by scaffold modification to identify the source of the disconnect between in vitro and in vivo activities. All reported thienopyrimidine analogues suffered from severe hepatic clearance in metabolic stability studies. Surprisingly, a simple scaffold replacement with the isosteric pyrrolopyrimidine significantly improved the percentage remaining from 4% to 65%. However, compound 21a exhibited poor oral bioavailability (5.7%) due to low absorption caused by poor permeability. We therefore attached various substituents to the ring of compound 21a to increase permeability and reduce transporter-mediated efflux, generating a new series of highly potent and selective DPP-IV inhibitors exemplified by compound 21j. This compound achieves a good balance of activity, selectivity, PK properties, and in vivo efficacy. Herein, we report the step-by-step PK-driven optimization process that led to the identification of this novel anti-diabetic preclinical drug candidate.

2. Chemistry

The synthesis of compounds 21a–d is outlined in Scheme 1. 6-Methyluracil (11) was readily transformed to 12 via standard nitration. Compound 13 was then obtained through condensation of 12 with N,N-dimethylformamide dimethyl acetal, and subsequent treatment of 13 with zinc powder in acetic acid (AcOH) afforded intermediate 17c in 20.3% overall yield [14]. Commercially available 6-aminopyrimidine-2,4(1H,3H)-dione (14) reacted with 1-chloro-2-propanone to give compound 17b in 80% yield. Compound 17a was synthesized by heating commercially available ethyl 2-cyano-4,4-diethoxybutanoate (15) with urea at 80 °C, followed by acidification with concentrated HCl to afford the diol compound 17a in 92.1% yield [15]. The 2,4-dichloropyrrolopyrimidines 18a–c were readily prepared from 17a–c by chlorination with POCl3.

Selective hydrolysis with sodium hydroxide (NaOH) provided the chloropyrrolopyrimidines 19a–c [16]. Compound 19d was obtained under the same conditions from N-methyl 2,4-dichloropyrrolopyrimidine 18d, which was synthesized by classical methylation [17]. Compounds 19a–c were N-protected with tert-butyloxycarbonyl (Boc) groups [18] to give 19a -c . Alkylation of 19a -c and 19d with 2-cyanobenzyl bromide provided 20a–d [19]. Finally, compounds 21a–d were generated from 20a–d by chloride displacement with (R)-3-aminopiperidine in yields ranging from 67.3% to 80% [13].

The syntheses of compounds 21e–i and 21j–m are outlined in Schemes 2 and

3, respectively. The bromo compounds 21e, 21f, and 21j were obtained by bromination of the corresponding N-protected pyrrolopyrimidines 22d, 22f, and 22j [20]. Intermediates 22f and 22j also served as precursors for aryl-substituted pyrrolopyrimidines. The desired aryl compounds 21g–i and 21k–m were prepared using conventional Suzuki coupling conditions in yields of 30–45% [21].

3.1. Metabolic Stability Study on Thienopyrimidine Analogues

The highly potent inhibitor 6 (IC50 = 0.33 nM), which is 10 times more potent than alogliptin, displayed a similar anti-diabetic effect to alogliptin in an oral glucose tolerance test (OGTT) [13]. We hypothesized that the disconnect between in vitro and in vivo activity was due to poor pharmacokinetic (PK) properties, specifically the low oral bioavailability of compound 6 (23.3% in rats). To better understand its PK profile, we examined the hepatic first-pass effect. As expected, compound 6 showed 93% turnover after 30 minutes of incubation in pooled rat liver microsomes (RLM) (Table 1). Modifications including addition of a methyl group, ring rotation, and substitution with trifluoromethyl did not significantly alter the hepatic first-pass effect. All reported thienopyrimidine analogues (compounds 7–10, Table 1) suffered from severe metabolic instability, leading us to conclude that the thienyl ring was a labile functional group requiring replacement to improve metabolic stability.

3.2. Improvement of Metabolic Stability

To reduce hepatic first-pass metabolism, we considered replacing the thienyl ring with a more stable isostere. We therefore replaced the thienopyrimidine scaffold with pyrrolopyrimidine. Notably, compound 21a exhibited good metabolic stability, with the percentage remaining in RLM improving to 65.07% after 30 minutes of incubation (Table 2).

However, further evaluation of its PK properties revealed that compound 21a had significantly lower oral bioavailability (5.7%). Since oral bioavailability depends on both hepatic first-pass effect and absorption, we realized that compound 21a likely suffered from poor absorption. While the pyrrolopyrimidine scaffold successfully avoided the hepatic first-pass effect, it introduced absorption problems.

3.3. Improvement of Absorption Profile

The cascade of events determining oral bioavailability is well established [22-25]. Permeability is directly related to drug absorption and oral bioavailability [26-29]. We used a Caco-2 permeability bidirectional assay to measure the permeability of compound 21a (Table 2), which showed notably low permeability from the apical (A) to basolateral (B) side. Additionally, high transporter-mediated efflux from B to A resulted in a high efflux ratio of 19.0, leading to poor absorption.

To date, no direct structure-function relationships or pharmacophore models describe the structural features responsible for transporter substrate recognition. We therefore adopted several simple strategies based on literature precedents [30-33] to improve absorption and reduce transporter-mediated efflux, which led to the synthesis of a series of pyrrolopyrimidine analogues.

Addition of substituents to the ring proved effective: incorporation of a 2-methyl group (compound 21b) and a 2-bromo group (compound 21f) immediately reduced the efflux ratio to 2.15 and 2.08, respectively. These data suggest that creating steric hindrance may reduce compound interactions with transporters. Incorporating alkyl groups could also increase lipophilicity, thereby improving permeability to some extent.

Encouraged by the results with compound 21f, we continued to add aromatic groups (compounds 21g–i) and evaluated their absorption properties. Only compound 21i, bearing a pyridyl ring, showed desirable permeability, while analogues 21g and 21h lost permeability. Blocking the 1-nitrogen with a 1-methyl group (compound 21d) to reduce hydrogen bond donors greatly increased the efflux ratio to 13.3. However, addition of two bromo groups to 21d (compound 21e) dropped the ratio to a reasonable value (3.37) and improved permeability.

The interaction between compounds and transporters is conformation-dependent. We therefore synthesized and evaluated rotated analogues (compounds 21c and 21j–m, Table 3) for their permeability properties. The unsubstituted compound 21c showed much better permeability compared to its isomer 21a (efflux ratio of 4.20 vs. 19.0, respectively). Further modification of 21c produced compounds 21j and 21l with low efflux ratios (6.55 and 2.51, respectively).

3.4. Determination of Metabolic Stability of Key Compounds

All analogues with efflux ratios less than 10 were further evaluated for hepatic stability (Tables 2 and 3). Compounds 21e, 21c, and 21l showed potential for inducing hepatic metabolism, as the percentage remaining was less than 50%. Therefore, biological evaluations were performed on compounds 21b, 21f, 21i, and 21j.

3.5. In Vitro Biological Evaluations

Through PK-driven optimization, four compounds (21b, 21f, 21i, and 21j) were selected based on their acceptable in vitro PK properties. These compounds were subsequently evaluated for DPP-IV inhibitory activity and selectivity against DPP-8 and DPP-9, using alogliptin as a reference. Fortunately, all compounds (Table 4) were potent DPP-IV inhibitors with single-digit nanomolar IC50 values, except for compound 21b. In our studies, compounds

21f, 21i, and 21j were more active than alogliptin in vitro. The IC50 value measured for alogliptin in our study was 3.4 nM, which is consistent with the literature value (IC50 $<$ 10 nM) [11]. Additionally, all compounds exhibited more than 1000-fold selectivity for DPP-IV over DPP-8 and DPP-9. The compounds also did not inhibit the human Ether-à-go-go Related Gene (hERG) ion channel, which may be associated with cardiovascular risk [34-35].

Compound 21j was identified as the most balanced inhibitor among the synthesized analogues, combining favorable in vitro PK properties with potent activity.

3.6. In Vivo Pharmacokinetic and Pharmacodynamic Evaluations

Based on its in vitro PK data, potency, selectivity, and lack of hERG inhibition, compound 21j was advanced to in vivo studies. In rats, 21j demonstrated 41% oral bioavailability with a half-life of approximately 2 hours, similar to alogliptin in rats (BA = 45%) [36]. Considering the results obtained with compound 6 and alogliptin, 21j might exhibit even better properties in dogs or monkeys.

In subsequent OGTT studies in mice, compound 21j effectively reduced glucose excursion. The data presented in Figure 2 [Figure 2: see original paper] clearly demonstrate that compound 21j is superior to both compound 6 and alogliptin in C57BL/6J lean mice at a dosage of 3 mg/kg. An in-depth evaluation in ob/ob mice revealed that compound 21j significantly reduced glucose excursion in a dose-dependent manner, with 27.9% inhibition at 1 mg/kg, 51.9% inhibition at 3 mg/kg, and 72.7% inhibition at 10 mg/kg.

Compound 21j is a potent DPP-IV inhibitor (IC50 = 1.40 nM) that exhibits excellent selectivity over DPP-8 and DPP-9. Additionally, this compound shows good oral bioavailability and reduces glucose excursion in a dose-dependent manner in T2D model mice. Compared with compound 6 and alogliptin, compound 21j demonstrates superior in vivo efficacy.

4. Conclusions

In summary, further pharmacokinetic evaluation of our recently reported DPP-IV inhibitors revealed comprehensive hepatic instability within the thienopyrimidine scaffold. Scaffold replacement with pyrrolopyrimidine (compound 21a) improved metabolic stability from 6.6% to 65% remaining parent compound in RLM, but resulted in poor absorption. Structural modification of the pyrrolyl ring led to a series of analogues with significantly improved permeability, as reflected by reduced efflux ratios (19.0 vs. 2.08–6.55). Four compounds (21b, 21f, 21i, and 21j) exhibited acceptable in vitro PK properties (percentage remaining $>$ 50%, efflux ratio $<$ 10), high in vitro activity in the nanomolar range, high selectivity over DPP-8 and DPP-9, and no hERG inhibition.

The most active compound, 21j, was identified as a promising preclinical candidate with good oral bioavailability and superior in vivo efficacy, warranting further development. Notably, our results validate that PK-driven optimization is an efficient approach for modifying highly potent compounds with poor PK properties to generate desirable preclinical drug candidates.

5.1. Chemistry

All commercially available reagents and solvents were of reagent grade and used without further purification unless otherwise noted. Reactions were monitored by TLC using Qing Dao Hai Yang GF254 silica gel plates (5 × 10 cm); spots were visualized under ultraviolet irradiation (254 nm) and by spraying with an ethanol solution of 2,4-DNP or ninhydrin, or by exposure to iodine vapor. Silica gel column chromatography was performed using silica gel (200–300 mesh) from Qing Dao Hai Yang. NMR spectra were recorded on a Bruker AVANCE 400 (400 MHz) or Bruker AVANCE 500 (500 MHz) spectrometer. Chemical shifts ( ) are reported in ppm and coupling constants (J) in hertz (Hz). Splitting patterns describe apparent multiplicities and are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). MS data were measured on an Agilent MSD-1200 ESI-MS system. Purity data were acquired on a Gilson LC/UV system using a Phenomenex C18 column (150 mm × 4.6 mm, 5 m particle size). The following gradients were used for 30 min at a flow rate of 1 mL/min at 25 °C: a 45% B isocratic gradient for compounds 21a, 21f, 21h, and 21l–m; and a 58% B isocratic gradient for compounds 21b–e, 21g, and 21i–k (A: 0.1% TFA in H2O; B: 0.1% TFA in MeOH). All test compounds were confirmed to be 95% pure.

(R)-2-((2-(3-Aminopiperidin-1-yl)-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)methyl)benzonitrile (21a)

A mixture of 20a (13.1 g, 43.4 mmol), (R)-3-aminopiperidine dihydrochloride (11.5 g, 66.0 mmol), and NaHCO3 (17.4 g, 173.6 mmol) in a sealed tube containing 300 mL of ethanol was heated at 150 °C for 6 hours. The reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated in vacuo and purified by flash chromatography to afford compound 21a as a white powder. Yield: 67.3%. ^{1}H NMR (400 MHz, CDCl3)   ppm: 7.64 (1H, d, J = 6.8 Hz), 7.45 (1H, t, J = 7.6 Hz), 7.31 (1H, t, J = 7.2 Hz), 7.07 (1H, d, J = 8.0 Hz), 6.81 (1H, d, J = 3.2 Hz), 6.64 (1H, d, J = 3.2 Hz), 5.55 (2H, AB q, J = 38.8 Hz, 16.0 Hz), 3.21–3.17 (1H, m), 3.05–3.02 (2H, m), 2.82–2.77 (2H, m), 1.94–1.90 (1H, m), 1.73–1.68 (1H, m), 1.63–1.55 (1H, m), 1.45–1.43 (1H, m). ^{13}C NMR (100 MHz, MeOD)   ppm: 22.83 (1C), 31.49 (1C), 45.41 (1C), 47.11 (1C), 51.34 (1C), 56.96 (1C), 102.17 (1C), 104.02 (1C), 110.46 (1C), 116.98 (1C), 120.19 (1C), 126.69 (1C), 127.47 (1C), 132.72 (1C), 133.01 (1C), 141.66 (1C), 147.45 (1C), 155.75 (1C), 161.00 (1C). ESI-MS calculated for (C19H20N6O) [M+H]+, 349.2; found 349.1.

(R)-2-((2-(3-Aminopiperidin-1-yl)-6-methyl-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)methyl)benzonitrile (21b)

Compound 21b was prepared using a method identical to that described for 21a to give a yellow powder. Yield: 72%. ^{1}H NMR (400 MHz, CDCl3) ppm: 7.63 (1H, d, J = 7.2 Hz), 7.44-7.40 (1H, m), 7.31-7.29 (1H, m), 7.01 (1H, d, J = 7.6 Hz), 6.28 (1H, s), 5.55 (2H, AB q, J = 24.0 Hz, 16.0 Hz), 3.15-3.12 (1H, m), 2.98-2.95 (2H, m), 2.73-2.71 (2H, m), 2.30 (3H, s), 1.91-1.89 (1H, m), 1.76-1.66 (1H, m), 1.59-1.57 (1H, m), 1.40-1.29 (1H, m). ^{13}C NMR (125 MHz, DMSO-d6) ppm: 15.33 (1C), 24.86 (1C), 30.99 (1C), 45.78 (1C), 47.83 (1C), 51.65 (1C), 57.31 (1C), 102.55 (1C), 105.02 (1C), 111.54 (1C), 118.98 (1C), 125.17 (1C), 127.01 (1C), 127.91 (1C), 132.77 (1C), 133.24 (1C), 141.89 (1C), 147.90 (1C), 155.50 (1C), 161.58 (1C). ESI-MS calculated for (C20H22N6O) [M+H]+, 363.2; found 363.2.

(R)-2-((2-(3-Aminopiperidin-1-yl)-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)benzonitrile (21c)

Compound 21c was prepared from 2-((2-chloro-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)benzonitrile (20c) using a method similar to that described for 21a to give a white powder. Yield: 75%. ^{1}H NMR (400 MHz, CDCl3) ppm: 7.66 (1H, dd, J = 7.6 Hz, 0.8 Hz), 7.45-7.41 (1H, m), 7.31 (1H, t, J = 7.6 Hz), 7.24 (1H, d, J = 2.8 Hz), 6.93 (1H, d, J = 8.0 Hz), 6.41 (1H, d, J = 2.8 Hz), 5.62 (2H, s), 3.17-3.14 (1H, m), 3.00-2.90 (2H, m), 2.81-2.76 (1H, m), 2.68-2.63 (1H, m), 1.93-1.89 (1H, m), 1.76-1.72 (1H, m), 1.63-1.55 (1H, m), 1.24-1.23 (1H, m). ^{13}C NMR (100 MHz, MeOD) ppm: 22.39 (1C), 29.83 (1C), 45.05 (1C), 47.17 (1C), 51.35 (1C), 55.05 (1C), 102.53 (1C), 110.44 (1C), 115.25 (1C), 117.03 (1C), 126.69 (1C), 127.56 (1C), 128.54 (1C), 132.78 (1C), 133.11 (1C), 141.55 (1C), 143.28 (1C), 153.94 (1C), 155.58 (1C). ESI-MS calculated for (C19H20N6O) [M+H]+, 349.2; found 349.1.

(R)-2-((2-(3-Aminopiperidin-1-yl)-7-methyl-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)methyl)benzonitrile (21d)

Compound 21d was prepared using a method identical to that described for 21a to give a yellow powder. Yield: 80%. ^{1}H NMR (400 MHz, CDCl3) ppm: 7.66 (1H, dd, J = 7.6 Hz, 0.8 Hz), 7.48-7.44 (1H, m), 7.33-7.30 (1H, m), 7.02 (1H, d, J = 8.0 Hz), 6.74 (1H, d, J = 3.2 Hz), 6.62 (1H, d, J = 3.6 Hz), 5.57 (2H, s), 3.72 (3H, s), 3.17-3.14 (1H, m), 3.03-3.00 (1H, m), 2.97-2.91 (1H, m), 2.79-2.73 (1H, m), 2.63-2.57 (1H, m), 1.95-1.90 (1H, m), 1.77-1.72 (1H, m), 1.69-1.62 (2H, m). ^{13}C NMR (100 MHz, MeOD) ppm: 23.01 (1C), 29.98 (1C), 32.26 (1C), 45.42 (1C), 47.11 (1C), 51.33 (1C), 57.80 (1C), 101.55 (1C), 103.91 (1C), 110.49 (1C), 116.93 (1C), 124.32 (1C), 126.62 (1C), 127.44 (1C), 132.71 (1C), 132.97 (1C), 141.66 (1C), 147.00 (1C), 155.86 (1C), 160.87 (1C). ESI-MS calculated for (C20H22N6O) [M+H]+, 363.2; found 363.2.

(R)-2-((2-(3-Aminopiperidin-1-yl)-5,6-dibromo-7-methyl-4-oxo-4H-pyrrolo[2,3-d]pyrimidin-3(7H)-yl)methyl)benzonitrile (21e)

Compound 21e was prepared using a method identical to that described for 21f to give a white powder. Yield: 88%. ^{1}H NMR (400 MHz, CDCl3) ppm: 7.65 (1H, d, J = 7.6 Hz), 7.49 (1H, t, J = 7.2 Hz), 7.33 (1H, t, J = 7.6 Hz), 7.07 (1H, d, J = 8.0 Hz), 5.50 (2H, s), 3.71 (3H, s), 3.21-3.19 (1H, m), 3.08-3.05 (1H, m), 2.97-2.92 (1H, m), 2.79-2.74 (1H, m), 2.62-2.57 (1H, m), 1.96-1.92 (1H, m), 1.77-1.72 (1H, m), 1.69-1.63 (2H, m). ^{13}C NMR (100 MHz, MeOD) ppm: 23.06 (1C), 33.22 (1C), 32.35 (1C), 45.97 (1C), 47.07 (1C), 51.10 (1C), 57.66 (1C), 92.53 (1C), 102.81 (1C), 109.12 (1C), 110.45 (1C), 116.97 (1C), 126.92 (1C), 127.67 (1C), 132.91 (1C), 133.16 (1C), 141.29 (1C), 146.73 (1C), 156.85 (1C), 158.30 (1C). ESI-MS calculated for (C20H20Br2N6O) [M+H]+, 521.0; found 521.0.

(R)-2-((2-(3-Aminopiperidin-1-yl)-6-bromo-4-oxo-4H-pyrrolo[2,3-d]pyrimidin-3(7H)-yl)methyl)benzonitrile (21f)

Compound 22a (100 mg) was dissolved in DCM (20 mL) and treated with NBS (31 mg). The mixture was stirred for 4 hours at room temperature, and the solvent was removed under reduced pressure to give a crude residue, which was purified by silica gel chromatography (DCM) to afford (R)-tert-butyl(1-(6-bromo-3-(2-cyanobenzyl)-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-2-yl)piperidin-3-yl)carbamate (22f). To a solution of 22f in DCM, TFA was added at 0 °C. After stirring at room temperature for 3 hours, the solution was treated with saturated NaHCO3 to pH 6-7. Removal of the solvent under reduced pressure gave a crude residue, which was purified by silica gel chromatography (DCM/MeOH) to afford compound 21f as a grey powder. Yield: 92%. ^{1}H NMR (400 MHz, MeOD) ppm: 7.72 (1H, d, J = 7.2 Hz), 7.58 (1H, t, J = 7.6 Hz), 7.42 (1H, t, J = 7.6 Hz), 7.18 (1H, d, J = 8.0 Hz), 6.84 (1H, s), 5.53 (2H, AB q, J = 34.4 Hz, 15.2 Hz), 3.54-3.44 (2H, m), 3.15-3.06 (2H, m), 2.92-2.87 (1H, m), 2.66-2.09 (1H, m), 1.88-1.84 (1H, m), 1.80-1.71 (1H, m), 1.65-1.55 (1H, m). ^{13}C NMR (100 MHz, DMSO-d6) ppm: 22.26 (1C), 27.93 (1C), 46.03 (1C), 46.94 (1C), 51.89 (1C), 52.80 (1C), 103.02 (1C), 104.65 (1C), 105.96 (1C), 110.61 (1C), 117.80 (1C), 127.67 (1C), 128.19 (1C), 133.40 (1C), 133.81 (1C), 142.11 (1C), 147.63 (1C), 156.22 (1C), 158.05 (1C). ESI-MS calculated for (C19H27BrN6O) [M+H]+, 427.1; found 427.0.

Compounds 21g–i and 21k–m were synthesized via Suzuki coupling reactions. Tert-butyloxycarbonyl (Boc) deprotection was conducted according to the general procedure described in the supplementary materials.

(R)-2-((2-(3-Aminopiperidin-1-yl)-4-oxo-6-(4-(trifluoromethyl)phenyl)-4H-pyrrolo[2,3-d]pyrimidin-3(7H)-yl)methyl)benzonitrile (21g)

Yellow powder. Yield: 40.7%. ^{1}H NMR (400 MHz, MeOD) ppm: 7.87-7.81 (3H, m), 7.74-7.67 (3H, m), 7.43 (1H, t, J = 7.6 Hz), 7.22 (1H, d, J = 8.0 Hz), 6.96 (1H, s), 5.56 (2H, AB q, J = 34.8 Hz, 15.2 Hz), 3.61-3.58 (1H, m), 3.51-3.49 (1H, m), 3.19-3.14 (2H, m), 2.98-2.92 (1H, m), 2.15-2.13 (1H, m), 1.92-1.81 (1H, m), 1.80-1.78 (1H, m), 1.66-1.64 (1H, m). ^{13}C NMR (100 MHz, MeOD) ppm: 21.84 (1C), 27.73 (1C), 46.06 (1C), 47.06 (1C), 51.42 (1C), 52.25 (1C), 100.85 (1C), 105.69 (1C), 110.51 (1C), 117.12 (1C), 122.93 (1C), 123.65 (1C), 124.63 (2C), 125.46 (1C), 125.49 (1C), 127.56 (1C), 127.71 (1C), 132.86 (1C), 133.07 (1C), 133.66 (1C), 141.31 (1C), 148.68 (2C), 155.85 (1C), 160.56 (1C). ESI-MS calculated for (C26H23F3N6O) [M+H]+, 493.2; found 493.2.

(R)-2-((2-(3-Aminopiperidin-1-yl)-6-(4-fluorophenyl)-4-oxo-4H-pyrrolo[2,3-d]pyrimidin-3(7H)-yl)methyl)benzonitrile (21h)

Yellow powder. Yield: 41.2%. ^{1}H NMR (400 MHz, MeOD) ppm: 7.74-7.67 (3H, m), 7.58 (1H, t, J = 7.6 Hz), 7.42 (1H, t, J = 7.6 Hz), 7.19 (1H, d, J = 8.0 Hz), 7.13 (2H, t, J = 8.8 Hz), 6.76 (1H, s), 5.56 (2H, AB q, J = 37.2 Hz, 15.6 Hz), 3.58-3.55 (1H, m), 3.51-3.45 (1H, m), 3.18-3.13 (2H, m), 2.96-2.91 (1H, m), 2.15-2.12 (1H, m), 1.91-1.82 (1H, m), 1.78-1.73 (1H, m), 1.66-1.64 (1H, m). ^{13}C NMR (100 MHz, MeOD) ppm: 21.78 (1C), 27.69 (1C), 45.89 (1C), 47.04 (1C), 51.39 (1C), 52.26 (1C), 105.54 (1C), 110.51 (1C), 115.24 (2C), 115.46 (2C), 117.13 (1C), 126.47 (1C), 127.41 (1C), 127.66 (2C), 132.83 (1C), 133.07 (1C), 133.67 (1C), 141.37 (2C), 148.20 (1C), 155.28 (1C), 160.55 (1C). ESI-MS calculated for (C25H23FN6O) [M+H]+, 442.2; found 442.2.

2-((2-((R)-3-Aminopiperidin-1-yl)-4-oxo-6-(pyridin-3-yl)-4H-pyrrolo[2,3-d]pyrimidin-3(7H)-yl)methyl)benzonitrile (21i)

Yellow powder. Yield: 30%. ^{1}H NMR (400 MHz, MeOD) ppm: 9.21 (1H, s), 8.87 (1H, d, J = 8.0 Hz), 8.69 (1H, d, J = 5.2 Hz), 8.12-8.09 (1H, m), 7.72 (1H, d, J = 7.6 Hz), 7.61 (1H, t, J = 7.2 Hz), 7.43 (1H, t, J = 8.0 Hz), 7.30-7.26 (2H, m), 5.54 (2H, AB q, J = 35.6 Hz, 15.2 Hz), 3.71-3.65 (1H, m), 3.55-3.52 (1H, m), 3.27-3.18 (2H, m), 3.03-2.97 (1H, m), 2.17-2.14 (1H, m), 1.95-1.86 (1H, m), 1.81-1.79 (1H, m), 1.69-1.64 (1H, m). ^{13}C NMR (125 MHz, DMSO-d6) ppm: 22.57 (1C), 27.89 (1C), 47.36 (1C), 47.52 (1C), 52.09 (1C), 52.51 (1C), 105.19 (1C), 106.21 (1C), 110.16 (1C), 118.31 (1C), 125.08 (1C), 127.30 (1C), 128.90 (1C), 129.18 (1C), 130.64 (1C), 134.14 (1C), 134.54 (1C), 137.48 (1C), 139.65 (1C), 141.22 (1C), 141.66 (1C), 149.86 (1C), 157.66 (1C), 160.27 (1C). ESI-MS calculated for (C24H23N7O) [M+H]+, 426.2; found 426.1.

(R)-2-((2-(3-Aminopiperidin-1-yl)-7-bromo-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)benzonitrile (21j)

Compound 21j was prepared from (R)-2-((2-(3-aminopiperidin-1-yl)-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)benzonitrile (21c) using a method similar to that described for 21f to give a white powder. Yield: 82%. ^{1}H NMR (400 MHz, MeOD) ppm: 8.05 (1H, d, J = 7.6 Hz), 7.88 (1H, t, J = 7.6 Hz), 7.74-7.71 (2H, m), 7.41 (1H, d, J = 8.0 Hz), 5.82 (2H, AB q, J = 57.2 Hz, 15.6 Hz), 3.78-3.75 (1H, m), 3.72-3.64 (1H, m), 3.46-3.38 (2H, m), 3.21-

3.18 (1H, m), 2.37-2.35 (1H, m), 2.14-2.11 (1H, m), 1.98-1.90 (2H, m). ^{13}C NMR (100 MHz, DMSO-d6) ppm: 22.35 (1C), 27.90 (1C), 45.69 (1C), 47.02 (1C), 49.05 (1C), 52.92 (1C), 90.20 (1C), 110.73 (1C), 115.77 (1C), 117.78 (1C), 127.49 (1C), 128.23 (1C), 128.31 (1C), 133.36 (1C), 133.93 (1C), 140.19 (1C), 141.93 (1C), 154.70 (1C), 154.76 (1C). ESI-MS calculated for (C19H19BrN6O) [M+H]+, 427.1; found 427.0.

2-((2-((R)-3-Aminopiperidin-1-yl)-4-oxo-7-(pyridin-3-yl)-4H-pyrrolo[3,2-d]pyrimidin-3(5H)-yl)methyl)benzonitrile (21k)

White powder. Yield: 45.2%. ^{1}H NMR (400 MHz, DMSO-d6) ppm: 9.55 (1H, s), 9.11 (1H, d, J = 8.4 Hz), 8.70 (1H, d, J = 5.6 Hz), 8.28 (1H, d, J = 3.2 Hz), 8.01 (1H, t, J = 6.4 Hz), 7.84 (1H, d, J = 7.6 Hz), 7.61 (1H, t, J = 7.6 Hz), 7.45 (1H, t, J = 7.6 Hz), 7.12 (1H, d, J = 8.0 Hz), 5.48 (2H, AB q, J = 29.6 Hz, 15.6 Hz), 3.60-3.57 (2H, m), 3.13-3.10 (2H, m), 3.00-2.90 (1H, m), 2.04-1.96 (1H, m), 1.90-1.81 (1H, m), 1.66-1.52 (2H, m). ^{13}C NMR (125 MHz, MeOD) ppm: 21.58 (1C), 21.17 (1C), 46.99 (1C), 47.09 (1C), 51.76 (1C), 51.99 (1C), 109.00 (1C), 109.21 (1C), 116.41 (1C), 117.82 (1C), 127.16 (1C), 127.99 (1C), 128.10 (1C), 128.42 (1C), 133.67 (1C), 133.73 (1C), 133.76 (1C), 137.79 (1C), 137.42 (1C), 140.46 (1C), 140.80 (1C), 142.02 (1C), 155.31 (1C), 156.01 (1C). ESI-MS calculated for (C24H23Br2N7O) [M+H]+, 426.2; found 426.2.

(R)-2-((2-(3-Aminopiperidin-1-yl)-4-oxo-7-(pyridin-4-yl)-4H-pyrrolo[3,2-d]pyrimidin-3(5H)-yl)methyl)benzonitrile (21l)

White powder. Yield: 42.5%. ^{1}H NMR (400 MHz, MeOD) ppm: 8.87-8.66 (2H, m), 8.62-8.51 (2H, m), 8.25 (1H, s), 7.64 (1H, d, J = 8.0 Hz), 7.50 (1H, t, J = 7.6 Hz), 7.34 (1H, t, J = 7.6 Hz), 7.15 (1H, d, J = 7.6 Hz), 5.51 (2H, AB q, J = 35.2 Hz, 15.6 Hz), 3.61-3.58 (1H, m), 3.43-3.39 (1H, m), 3.17-3.11 (2H, m), 2.91-2.85 (1H, m), 2.09-2.07 (1H, m), 1.84-1.81 (1H, m), 1.75-1.70 (1H, m), 1.61-1.58 (1H, m). ^{13}C NMR (125 MHz, MeOD) ppm: 23.44 (1C), 28.99 (1C), 35.29 (1C), 47.40 (1C), 53.04 (1C), 53.81 (1C), 111.77 (1C), 112.65 (1C), 118.36 (1C), 119.08 (1C), 122.75 (1C), 128.97 (1C), 129.14 (1C), 132.04 (1C), 134.23 (2C), 134.40 (2C), 141.69 (1C), 142.07 (1C), 143.37 (1C), 153.50 (1C), 156.44 (1C), 157.00 (1C). ESI-MS calculated for (C24H23Br2N7O) [M+H]+, 426.2; found 426.2.

2-((2-((R)-3-Aminopiperidin-1-yl)-4-oxo-7-(thiophen-2-yl)-4H-pyrrolo[3,2-d]pyrimidin-3(5H)-yl)methyl)benzonitrile (21m)

Yellow powder. Yield: 38.6%. ^{1}H NMR (400 MHz, DMSO-d6) ppm: 7.98 (1H, s), 7.82-7.78 (2H, m), 7.69 (1H, d, J = 4.0 Hz), 7.61-7.55 (3H, m), 7.06 (1H, d, J = 8.0 Hz), 5.46 (2H, AB q, J = 50.4 Hz, 15.6 Hz), 3.46-3.43 (1H, m), 3.36-3.33 (1H, m), 3.11-3.05 (2H, m), 2.90-2.75 (1H, m), 2.02-1.92 (1H, m), 1.88-1.76 (1H, m), 1.66-1.46 (2H, m). ^{13}C NMR (125 MHz, DMSO-d6) ppm: 30.00 (1C), 35.43 (1C), 46.66 (1C), 47.69 (1C), 52.56 (1C), 53.21 (1C), 110.76 (1C), 113.58 (1C), 116.58 (1C), 118.56 (1C), 126.67 (1C), 127.09 (1C), 128.84

(1C), 129.11 (1C), 129.93 (1C), 130.02 (1C), 134.20 (1C), 134.52 (1C), 134.59 (1C), 140.49 (1C), 142.27 (1C), 154.90 (1C), 155.90 (1C). ESI-MS calculated for ($C_{23}H_{22}N_6OS$) $[M+H]^+$, 431.2; found 431.2.

Acknowledgments. This research was supported by Grant No. 2009Z1-E871 from the Bureau of Science and Technology of Guangzhou Municipality and the Natural Science Foundation of China (NSFC 21102145).

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