Rapid Generation of a novel DPP-4 inhibitor with long-acting property SAR study and PKPD evaluation (Postprint)

Authors: Hui Xiea, Shaogao Zengb, Guicheng Zhangb, Lili Zeng b, Guifa Zhong b, Junling Zhaob, Xin Zhaoc, Hongjiang Xud, Ling Yangd, Xiquan Zhangd, Shanchun Wangd, Wenhui Huc

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

Abstract

Drug compliance is critical for the patients with chronic diseases like diabetes. In our continuous effort to find better glucose lowering agents, an exploration for long-acting DPP-4 inhibitor had been launched. Based on our previous reported compound 111 bearing a pyrrolopyrimidine scaffold, lead compound 114 (IC50 = 2.3 nM, t1/2(rat) = 5.46 h) was rapidly determined with the pharmacokinetic superiority. Further SAR study indicated that the pyrrole ring was generally tolerable for variation, in which the β-substitution gave a better DPP-4 affinity. In depth evaluation on β position of pyrrole ring brought up with highly potent compound 124 (IC50 = 0.76 nM, t1/2(rat) = 7.89 h). In vivo pharmacodynamics tests demonstrated a similar or even slightly better sustained DPP-4 inhibition of compound 114 and 124 compared with the first marketed once-weekly drug Trelagliptin in this category, indicating that improvement of DPP-4 inhibitory activity or pharmacokinetic profile might be both feasible ways to rapid generation of long-acting DPP-4 inhibitors.

Full Text

Preamble

Rapid Generation of a Novel DPP-4 Inhibitor with Long-Acting Property: SAR Study and PK/PD Evaluation

Hui Xie^a, Shaogao Zeng^b, Guicheng Zhang^b, Lili Zeng^b, Guifa Zhong^b, Junling Zhao^b, Xin Zhao^c, Hongjiang Xu^d, Ling Yang^d, Xiquan Zhang^d, Shanchun Wang^d, Wenhui Hu^a

^a The First Affiliated Hospital of Guangzhou Medical University, 151 Yanjiang Road, Guangzhou, 510120, China
^b Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Science, 190 Kaiyuan Avenue, Guangzhou, 510530, China
^c Key Laboratory of Molecular Clinical Pharmacology & The Fifth Affiliated Hospital, Guangzhou Medical University, Guangzhou, 511436, China
^d Jiangsu Chia-Tai Tianqing Pharmaceutical Co. Ltd, No. 8 Julong North Rd, Xinpu, Lianyungang, Jiangsu, 222006, China

GRAPHIC ABSTRACT
Compound 124 with IC50 of 0.76 nM and competent in vivo efficacy comparable to Trelagliptin.

ABSTRACT

Drug compliance is critical for patients with chronic diseases such as diabetes. In our continuous effort to identify better glucose-lowering agents, we launched an exploration for long-acting DPP-4 inhibitors. Based on our previously reported compound 111 bearing a pyrrolopyrimidine scaffold, lead compound 114 (IC50 = 2.3 nM, t1/2(rat) = 5.46 h) was rapidly identified with pharmacokinetic superiority. Further SAR studies indicated that the pyrrole ring was generally tolerant to variation, with β-substitution providing better DPP-4 affinity. In-depth evaluation of the β-position of the pyrrole ring yielded the highly potent compound 124 (IC50 = 0.76 nM, t1/2(rat) = 7.89 h). In vivo pharmacodynamic tests demonstrated that compounds 114 and 124 achieved similar or even slightly better sustained DPP-4 inhibition compared with the first marketed once-weekly drug Trelagliptin in this category, indicating that improvement of either DPP-4 inhibitory activity or pharmacokinetic profile might represent feasible strategies for the rapid generation of long-acting DPP-4 inhibitors.

KEYWORDS: DPP-4 inhibitor; type 2 diabetes; SAR; long-acting; in vivo efficacy

1. Introduction

Diabetes mellitus is a chronic metabolic disease characterized by hyperglycemia that has reached a global prevalence of 415 million patients, with this population expected to rise rapidly to 642 million by 2040. Type 2 diabetes (T2D), which accounts for up to 90% of diabetes cases, results from insufficient insulin response. Undiagnosed T2D and the multisystem complications caused by hyperglycemia are leading causes of patient disability and mortality. Dipeptidyl peptidase-4 (DPP-4) inhibitors represent a class of weight-neutral and well-tolerated glucose-lowering agents that function primarily by preventing cleavage of glucagon-like peptide-1 (GLP-1). GLP-1, an important incretin hormone, possesses multiple glucose-regulating functions: it stimulates insulin release in a glucose-dependent manner, increases insulin sensitivity, and reduces glucagon secretion. Currently,

DPP-4 inhibitors are recommended as add-on therapy with metformin or as first-line therapy in patients with metformin contraindications.

However, despite the availability of many drugs, poor adherence has led to unsatisfactory glycemic control in approximately half of T2D patients. Combination treatment and reduction of dosing frequency are common strategies to improve patient adherence. Consequently, long-acting glucose-lowering agents have been successively marketed, including once-weekly DPP-4 inhibitors. Trelagliptin and Omarigliptin were the first two long-acting DPP-4 inhibitors to receive marketing authorization in Japan in 2015. Clinical trials of these two drugs have demonstrated superior efficacy compared to placebo without association with severe adverse events. Although once-weekly Trelagliptin and Omarigliptin did not show significantly better glycemic control compared with regular DPP-4 inhibitors, and Omarigliptin was reported to have potential safety issues, both manufacturers abandoned their marketing plans in other countries due to high financial costs. Nonetheless, long-acting DPP-4 inhibitors remain necessary to develop given the rapidly escalating T2D patient population, providing more therapeutic options regardless of these concerns.

Previously, we reported a series of pyrrolopyrimidine analogues based on pharmacokinetic (PK) property-driven optimization, with compound 111 representing the basal scaffold. In our continuous drug discovery effort for oral, potent DPP-4 inhibitors with long-acting properties, we started from the pyrrolopyrimidine scaffold bearing a favorable PK profile. Inspired by the discovery of Trelagliptin, we simply added 5-fluoro substitution to the cyanobenzyl group to generate lead compound 114 (IC50 = 2.3 nM), which displayed a similar half-life to Trelagliptin in rat PK experiments. Expanded structure-activity relationship (SAR) studies around compound 114 indicated that β-substitution on the pyrrole ring improved DPP-4 affinity and was open to wide variation. Eventually, the thienyl-substituted compound 124 demonstrated sustained in vivo DPP-4 inhibition in pharmacodynamics (PD) assays, similar to or slightly better than Trelagliptin.

2. Chemistry

The synthesis of compounds 4a-d is outlined in Scheme 1. Compounds 1a-d were hydrolyzed with aqueous sodium hydroxide to give 2a-d, and 2a-b were converted to 2a' -b' by protection with di-tert-butyl pyrocarbonate. Selective N-alkylation of 2a'-b'and 2c-d with 2-(bromomethyl)-4-fluorobenzonitrile provided precursors 3a-d. The final compounds 4a-d were obtained by amination of the chloro-precursors 3a-d with 3-(R)-aminopiperidine.

Scheme 1. Synthesis of compounds 4a-d. Reagents: (i) NaOH, H2O, 100℃; (ii) Boc2O, TEA, DMAP, DMF, rt; (iii) 2-(bromomethyl)-4-fluorobenzonitrile, NaH, LiBr, DMF/DME; (iv) 3-(R)-aminopiperidine, NaHCO3, EtOH, 120℃.

The synthesis of compounds 7a and 7b is outlined in Scheme 2. Compounds 6a

and 6b were obtained by amination of fluoride 5 with morpholino and dimethylamine respectively. Compound 5 was obtained by protection of compound 4a with di-tert-butyl pyrocarbonate. The final compounds 7a and 7b were obtained by de-protection with HCl/MeOH solution from 6a and 6b.

Scheme 2. Synthesis of compounds 7a-b. Reagents: (i) Boc2O, TEA, DCM, rt; (ii) Morpholino or dimethylamine, CuI, K2CO3, DMSO, 120℃; (iii) HCl/MeOH, rt.

The synthesis of compounds 9a-f is outlined in Scheme 3. Compound 3a was converted to compounds 8a and 8b by bromination with N-bromosuccinimide (NBS), to compounds 8c and 8d by fluorination with selectfluor, and to compounds 8e and 8f by sulfonation with methanesulfonyl chloride and N-alkylation with 2-(bromomethyl)-4-fluorobenzonitrile respectively. The final compounds 9a-f were obtained by amination of the chloro-precursors 8a-f with 3-(R)-aminopiperidine.

Scheme 3. Synthesis of compounds 9a-f. Reagents: (i) 8a-b: NBS, DMF, rt; 8c-d: Selectfluor, ZrCl4, CH3CN, 80℃; 8e: CH3SO2Cl, TEA, DMF, rt; 8f: 2-(bromomethyl)-4-fluorobenzonitrile, NaH, DMF, 80℃; (ii) 3-(R)-aminopiperidine, NaHCO3, EtOH, 120℃.

The synthesis of compounds 12a-j is outlined in Scheme 4. Compounds 11a-j were obtained by Suzuki reaction with various phenylboronic acids from bromide 10, which was obtained by protection of compound 9a with di-tert-butyl pyrocarbonate. The final compounds 12a-j were obtained by de-protection with HCl/MeOH solution from 11a-j.

Scheme 4. Synthesis of compounds 12a-j. Reagents: (i) Boc2O, TEA, DCM, rt; (ii) Arylboronic acid, dppf.PdCl2, K2CO3, 1,4-dioxane/H2O, 100℃; (iii) HCl/MeOH, rt.

3. Results and Discussion

3.1. Rapid Generation of the Lead Compound 114

In our approach to enrich the alternatives of glucose-lowering agents, we have identified several DPP-4 inhibitors bearing different scaffolds or pharmacophores based on various medicinal chemistry strategies. The pyrrolopyrimidine scaffold was first considered in this successive exploration of long-acting DPP-4 inhibitors due to its favorable PK property within our chemotypes, represented by compound 111. Tentative variations on the cyanobenzyl group and heterocyclic ring were carried out on a small scale (Table 1). Reviewing the discovery of Trelagliptin from Alogliptin, simple 5-fluoro substitution on the cyanobenzyl group resulted in much prolonged in vivo DPP-4 inhibition. Although the contribution of the fluorine atom to the long-acting property was not illustrated, several studies have discussed the SAR on the benzyl group of xanthine-based scaffolds. Consistently, 2-cyano, 5-fluoro substitution provided the best potency. DPP-4 crystal structure with Trelagliptin revealed that the

fluorine atom lies in the S1 pocket and has potential Van der Waals interactions with Trp659, Tyr631, and Val656. Additionally, electrical attraction between fluorine (partial negative) and ligand or protein might contribute to increased DPP-4 activity.

As shown in our small-scale variations listed in Table 1, 5-fluoro substitution on the cyanobenzyl group in the pyrrolopyrimidine scaffold provided better activity (compounds 114-117 vs. compounds 112-113), consistent with published results. Substitution with large steric hindrance (compound 112) or partial positive charge (compound 113) resulted in remarkable potency decreases. Additionally, increasing the number of heteroatoms did not uniformly improve potency (compounds 116-117). Nanomolar-level in vitro activity distinguished the pyrrolopyrimidine scaffold compounds 114 and 115. Among them, compound 114 was selected as the lead based on the close distance between the heteroatom of the pyrrole ring and the carbonyl group, which might provide better absorption permeability through hydrogen bonding according to our previous experience.

3.2. Preliminary Assessment of Lead Compound 114

Preliminary assessment of compound 114 was subsequently carried out, including in vitro activity, selectivity, and in vivo PK and PD evaluation. Inhibition of DPP-4 related proteases (DPP-8, DPP-9) has been reported to be linked with multiple organ and immune system toxicities. The inhibitory IC50 values of compound 114 against DPP-8/9 were determined to be over 300 μM, more than 10-fold higher than that against DPP-4. Enzymatic kinetic assay indicated that compound 114 inhibited DPP-4 via a reversible non-covalent mechanism with a Ki of 2.9 nM, the same as Trelagliptin. The difference lay in the specific inhibitory mode in our assay: compound 114 inhibited DPP-4 in a mixed mode while Trelagliptin inhibited in a substrate-competitive manner (Fig. 3A). Hepatic stability was evaluated in pooled liver microsomes from different species. Although species variation existed, compound 114 generally demonstrated good in vitro metabolic stability as expected for the pyrrolopyrimidine scaffold. The good performance in human liver microsomes indicated a potentially better PK profile in humans (Fig. 3B, Table 3). In PK experiments, we were pleased to find that compound 114 had a similar oral half-life to Trelagliptin (t1/2(rat): 5.46 h vs. 5.36 h, Fig. 3C, Table 4). Accordingly, compound 114 displayed similar DPP-4 inhibition to Trelagliptin in 52-hour PD tests, in both mice and rats (Fig. 3D, Table 5).

3.3. Further Optimization and SAR Study of Compound 114

Xanthine-based DPP-4 inhibitors were mainly discovered through structure-based design. The active functional sites interacting with DPP-4 were generally unambiguous and nearly identical, whether in co-complex crystal studies of Alogliptin and Trelagliptin or in docking predictions from our previous study. Aminopiperidine forms a salt bridge with Glu205 and Glu206. The cyanobenzyl group, with or without F-substitution, lies in the S1 pocket formed by Val656,

Tyr631, Trp659, Tyr666, and Val711 and interacts with Arg125. The carbonyl group and backbone NH of Tyr631 constitute an important hydrogen bond critical for activity. The unsaturated scaffold engages in π-stacking with Tyr547. Thus, the pyrrolopyrimidine scaffold is supposed to be open to structural variation, especially on the fused heterocycle.

Initially, we attempted to evaluate the effect of substitutions at the N-, α-, and β-positions of the pyrrole ring on DPP-4 activity (compounds 118-123). Substitutions were generally tolerable except for N-substitution, which decreased activity to double-digit IC50 values in compounds 122 and 123. Single β-substitution or α,β-substitution maintained good DPP-4 affinity, with β-substitution being slightly better (compound 118/120 vs. compound 119/121). Hence, extended variation at the β-position was undertaken, leading to compounds 124 to 133. We were pleased to find that the β-position could tolerate a wide range of variations with good activity at single-digit nanomolar levels regardless of steric hindrance or polarity of the substituent group. All β-substituted compounds were examined for metabolic stability in both rat liver microsomes (RLM) and human liver microsomes (HLM). Except for two compounds with poorer stability in HLM than in RLM (compounds 125, 130), the remaining compounds in Table 3 were not expected to suffer from severe hepatic clearance and were advanced to in vivo pharmacokinetic studies. During PK screening, compound 124 displayed a longer half-life than Trelagliptin in rats and was selected for in vivo PD evaluation (Tables 4 & 5).

3.4. PK/PD Evaluation of Compound 124

Compound 124 is a potent DPP-4 inhibitor with an IC50 value of 0.76 nM and good selectivity against DPP-8/9. Its hepatic stability is generally acceptable, with calculated half-lives of 47 min and 160 min in RLM and HLM incubations, respectively. Moreover, in pharmacokinetic studies, compound 124 showed a prolonged half-life compared with Trelagliptin in the same batch (t1/2: 7.89 h vs. 5.13 h, Fig. 4A, Table 4). The 52-hour in vivo PD tests in both rats and mice demonstrated that compound 124 achieved similar or slightly better DPP-4 inhibition than Trelagliptin, both in total DPP-4 inhibition and at the final sampling point at 52 hours (Fig. 4B, Table 5).

3.5. Potential Factors Contributing to the Long-Acting Properties of Pyrrolopyrimidine-Based DPP-4 Inhibitors

Patient adherence is very important for managing chronic diseases, especially T2D. Although the advantages remain under controversy, the development of long-acting DPP-4 inhibitors should continue, at least for newly diagnosed T2D patients with metformin contraindications and ineffective lifestyle modifications.

Firstly, we hypothesized that slow binding or dissociation between the compound and DPP-4 enzyme might be a possible mechanism for long-acting effects. Preliminary drug-target binding kinetics evaluations were conducted on

typical once-weekly drugs Omarigliptin and Trelagliptin, and regular once-daily Sitagliptin and Alogliptin. However, while the dissociation rate constant (koff) data between these two types of drugs showed differences in our research, they were not sufficiently distinguishable. This conclusion also coincided with later published evaluations of Trelagliptin.

Thus, we turned our attention back to the traditional PK-PD strategy. During optimization, it was quite challenging to determine which specific PK parameters to improve. In our initial pharmacokinetic comparison between once-weekly and once-daily DPP-4 inhibitors in rats, we noticed longer half-life and lower clearance in once-weekly Trelagliptin and Omarigliptin. However, the differences in half-life and clearance were not sufficient to sustain week-long efficacy. Therefore, we had to mainly rely on in vivo measures to screen this batch of compounds. Considering that β-substituted pyrrolopyrimidine compounds maintained excellent in vitro DPP-4 inhibitory activity, we advanced compounds with half-lives similar to or better than Trelagliptin to in vivo PD tests and discovered compounds 114 and 124.

Reviewing the research and development of long-acting pyrrolopyrimidine-based DPP-4 inhibitors, we affirmed that optimization of PK profile is a feasible approach to obtain long-acting compounds. Half-life is an important parameter, but it is difficult to consider it a decisive factor based on available data, and it is not a suitable parameter for high-throughput screening as it cannot be easily correlated with in vitro indicators like hepatic stability. As orally administered drugs, bioavailability does not appear to be a determinant for sustained efficacy (Table 4). Secondly, it is generally believed that the glycemic benefits of DPP-4 inhibitors are related to residual enzyme inhibition at the end of the dosing interval. In the 52-hour PD evaluations, compounds 114 and 124 both showed stronger inhibition than Trelagliptin at the last sampling point, except for the 10 mg/kg dosing group in rats (Table 5). Hence, long-duration in vitro DPP-4 inhibition tests might be a probable way to screen long-acting compounds after establishing a reliable long-duration in vitro reaction system. Finally, comprehensive evaluation of Trelagliptin suggested that potency partially contributed to in vivo efficacy due to stronger inhibition at lower concentrations. Based on this concept, compounds 114 and 124 displayed lower and better IC50 values than Trelagliptin in our assay, yet achieved similar in vivo efficacy. Although the three compounds were at the same potency level, the contribution of DPP-4 affinity could not be firmly established. We believe that higher potency is meaningful for sustained long-term efficacy.

4. Conclusion

In continuation of our discovery of long-acting DPP-4 inhibitors, the previously reported pyrrolopyrimidine scaffold-based compound 111 was selected as the hit compound due to its pharmacokinetic superiority. Inspired by the discovery of Trelagliptin, tentative variation on the pyrrole ring yielded lead compound 114 (IC50 = 2.3 nM, t1/2(rat) = 5.46 h). Considering the binding mode between

xanthine-based compounds and DPP-4, extensive lead optimization was conducted. β-Substitution on the pyrrole ring was determined to be a favorable position open to wide variation, while excellent DPP-4 inhibitory activity was maintained for β-substituted compounds, represented by compound 124 (IC50 = 0.76 nM, t1/2(rat) = 7.89 h). In vivo pharmacodynamics studies demonstrated similar or slightly better sustained efficacy compared with Trelagliptin. Reviewing the development process, improved pharmacokinetic profile was important for prolonging the efficacy of pyrrolopyrimidine or xanthine-based DPP-4 inhibitors, particularly regarding half-life and DPP-4 inhibition after long duration. Based on these preliminary data, compounds 114 and 124 will be advanced to more comprehensive evaluation, and in-depth dose-response relationship studies will be conducted to evaluate the mechanism of action of these compounds.

5. Experimental Section

5.1. Chemistry

All commercially available compounds and solvents were of reagent grade and used without further treatment unless otherwise noted. Reactions were monitored by TLC using Qing Dao Hai Yang GF254 silica gel plates (5 × 10 cm); zones were detected visually 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 on silica gel (200-300 mesh) from Qing Dao Hai Yang. NMR spectra were recorded on a Bruker NMR AVANCE 400 (400 MHz) or Bruker NMR AVANCE 500 (500 MHz). Chemical shifts (δ) were recorded 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.

5.1.1. 2-((2-Chloro-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)-4-fluorobenzonitrile (3a) NaH (60% in oil, 0.46 g, 11.5 mmol) was added to a stirred solution of 2a' (2.70 g, 10.0 mmol) in DME (40 mL) and DMF (10 mL) at 0℃. Twenty minutes later, LiBr (1.74 g, 20.0 mmol) was added, and the mixture was allowed to warm to room temperature. After 15 min, 2-(bromomethyl)-4-fluorobenzonitrile (2.46 g, 11.5 mmol) was added, and the mixture was heated at 65℃ overnight. After cooling, the mixture was poured into water (250 mL) and extracted with ethyl acetate (250 × 3 mL). The organic layer was combined, dried with anhydrous sodium sulfate, and filtered. The filtrate was concentrated in vacuo and purified by flash chromatography to yield the title compound 3a as a white powder (2.27 g, yield 75.2%). ^{1H} NMR (400 MHz, CDCl3) δ: 10.28 (s, 1H), 7.76-7.23 (m, 1H), 7.40-7.39 (m, 1H), 7.14-7.09 (m, 1H), 6.85-6.82 (m, 1H), 6.55-6.54 (m, 1H), 5.77 (s, 2H); ESI-MS calculated for (C14H8ClFN4O) [M + H]+, 302.04, found 303.0.

5.1.2. (R)-2-((2-(3-Aminopiperidin-1-yl)-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)-4-fluorobenzonitrile (4a) A mixture of 3a (3.03 g, 10.0 mmol), 3-(R)-aminopiperidine dihydrochloride (2.07 g, 12.0 mmol), and NaHCO3 (2.10 g, 25.0 mmol) in a sealed tube containing 30 mL of ethanol was heated at 120℃ overnight. The reaction mixture was subsequently cooled to room temperature and filtered. The filtrate was concentrated in vacuo and purified by flash chromatography to yield compound 4a as a white foam (2.33 g, yield 63.5%). ^{1H} NMR (400 MHz, MeOD) δ: 7.85-7.81 (m, 1H), 7.37 (s, 1H), 7.22-7.18 (m, 1H), 6.76-6.74 (d, J = 9.6 Hz, 1H), 6.40 (s, 1H), 5.60 (s, 2H), 3.26-3.23 (m, 1H), 3.06-3.03 (m, 1H), 2.86-2.75 (m, 2H), 2.66-2.61 (m, 1H), 1.96-1.94 (m, 1H), 1.78-1.75 (m, 1H), 1.63-1.60 (m, 1H), 1.29-1.24 (m, 1H); ^{13C} NMR (125 MHz, MeOD) δ: 166.32, 164.29, 155.46, 154.27, 145.45, 143.39, 135.58, 128.52, 116.14, 114.94, 114.05, 106.64, 102.45, 58.26, 53.32, 51.33, 44.85, 32.52, 22.84; ESI-MS calculated for (C19H19FN6O) [M + H]+, 366.16, found 367.1.

5.1.3. (R)-2-((2-(3-Aminopiperidin-1-yl)-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)methyl)-4-fluorobenzonitrile (4b) Compound 4b was prepared in a manner identical to that described for 4a as a yellow powder. Yield: 53.3%. ^{1H} NMR (400 MHz, CDCl3) δ: 9.65 (s, 1H), 7.64-7.63 (m, 1H), 7.45-7.42 (m, 1H), 7.31-7.29 (m, 1H), 7.03-7.01 (d, J = 6.4 Hz, 1H), 6.82-6.81 (d, J = 2.4 Hz, 1H), 6.64-6.63 (d, J = 2.4 Hz, 1H), 5.59-5.52 (dd, J = 4.4 Hz, J = 17.2 Hz, 2H), 3.16-3.14 (m, 1H), 2.98-2.93 (m, 2H), 2.75-2.71 (m, 1H), 2.65-2.61 (m, 1H), 1.92 (s, 2H), 1.74-1.71 (m, 1H), 1.64-1.59 (m, 1H), 1.24-1.22 (m, 1H); ^{13C} NMR (125 MHz, CDCl3) δ: 160.28, 156.33, 147.09, 141.79, 133.16, 132.79, 127.47, 126.87, 119.28, 117.36, 110.63, 104.45, 103.78, 58.48, 51.70, 47.35, 45.71, 33.32, 23.06; ESI-MS calculated for (C19H19FN6O) [M + H]+, 366.16, found 367.1.

5.1.4. (R)-2-((5-(3-Aminopiperidin-1-yl)-7-oxothiazolo[5,4-d]pyrimidin-6(7H)-yl)methyl)-4-fluorobenzonitrile (4c) Compound 4c was prepared in a manner identical to that described for 4a as a yellow oil. Yield: 36.4%. ^{1H} NMR (400 MHz, CDCl3) δ: 8.48 (s, 1H), 7.68-7.67 (m, 1H), 7.43-7.41 (m, 1H), 7.09 (s, 1H), 5.74 (s, 2H), 4.41-4.37 (m, 1H), 3.79-3.63 (m, 2H), 3.09-3.03 (m, 1H), 2.87 (s, 1H), 1.97 (s, 2H), 1.83-1.76 (m, 1H), 1.49-1.47 (m, 1H), 1.24-1.22 (m, 1H); ^{13C} NMR (125 MHz, CDCl3) δ: 167.08, 163.47, 156.81, 152.55, 147.56, 143.78, 133.64, 119.05, 115.88, 114.63, 114.25, 106.82, 50.90, 50.13, 42.75, 37.10, 36.43, 23.07; ESI-MS calculated for (C18H17FN6OS) [M + H]+, 384.12, found 385.1.

5.1.5. (R)-2-((2-(3-Aminopiperidin-1-yl)-7-methyl-6-oxo-6,7-dihydro-1H-purin-1-yl)methyl)-4-fluorobenzonitrile (4d) Compound 4d was prepared in a manner identical to that described for 4a as a yellow oil. Yield: 38.3%. ^{1H} NMR (400 MHz, CDCl3) δ: 8.04 (s, 1H), 7.81 (s, 1H), 7.39 (s, 1H), 7.11-7.04 (m, 1H), 5.49 (s, 2H), 4.73 (s, 3H), 4.30-4.17 (m, 1H), 3.43 (s,

1H), 2.96 (s, 2H), 2.76 (s, 1H), 1.99 (s, 2H), 1.63 (s, 1H), 1.41 (m, 1H), 1.22 (s, 1H); ^{13C} NMR (125 MHz, CDCl3) δ: 167.06, 163.12, 156.84, 152.09, 147.54, 144.43, 133.68, 115.84, 115.51, 114.28, 106.85, 50.92, 50.12, 42.72, 36.48, 33.34, 23.07; ESI-MS calculated for (C19H20FN7O) [M + H]+, 381.17, found 382.2.

5.1.6. 2-((2-Chloro-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)-4-fluorobenzonitrile (5) Di-tert-butyl dicarbonate (2.62 g, 12.0 mmol) was added dropwise to a mixture of 4a (3.66 g, 10.0 mmol) and triethylamine (1.11 g, 11.0 mmol) in a round-bottom flask containing 60 mL of dichloromethane at room temperature, and the solution was stirred overnight at room temperature. The reaction mixture was poured into water (100 mL) and extracted with ethyl acetate (100 × 3 mL). The organic layer was combined, dried with anhydrous sodium sulfate, and filtered. The filtrate was concentrated in vacuo and purified by flash chromatography to yield the title compound 5 as a white foam (4.21 g, yield 90.4%). ^{1H} NMR (400 MHz, DMSO-d6) δ: 11.97 (s, 1H), 7.98-7.94 (m, 1H), 7.38-7.37 (m, 1H), 7.35-7.29 (m, 1H), 6.86-6.79 (m, 2H), 6.32-6.31 (m, 1H), 5.48-5.42 (m, 2H), 3.39-3.37 (m, 1H), 3.09-2.99 (m, 2H), 2.66-2.54 (m, 2H), 1.77 (m, 1H), 1.75 (m, 1H), 1.72-1.68 (m, 1H), 1.53-1.47 (m, 1H), 1.36-1.35 (m, 1H), 1.34 (s, 9H); ESI-MS calculated for (C24H27FN6O3) [M + H]+, 466.21, found 467.1.

5.1.7. (R)-tert-Butyl 1-(3-(2-cyano-5-morpholinobenzyl)-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-2-yl)piperidin-3-ylcarbamate (6a) A mixture of 5 (2.33 g, 5.0 mmol), morpholine (1.31 g, 15.0 mmol), CuI (2.86 g, 15.0 mmol), and K2CO3 (1.38 g, 10.0 mmol) in a sealed tube containing 30 mL of methylsulfinylmethane was heated at 120℃ under Ar2 atmosphere overnight. The reaction mixture was subsequently cooled to room temperature, poured into water (80 mL), and extracted with ethyl acetate (80 × 3 mL). The organic layer was combined, dried with anhydrous sodium sulfate, and filtered. The filtrate was concentrated in vacuo and purified by flash chromatography to yield the title compound 6a as a light-yellow solid (1.26 g, yield 47.4%). ^{1H} NMR (400 MHz, CDCl3) δ: 10.59 (s, 1H), 7.52-7.50 (m, 1H), 7.34-7.32 (m, 1H), 6.73-6.70 (m, 1H), 6.44-6.43 (m, 1H), 6.38 (s, 1H), 5.53 (s, 2H), 4.91 (s, 1H), 3.77 (s, 1H), 3.74-3.72 (m, 4H), 3.40-3.37 (m, 1H), 3.11-3.09 (m, 4H), 3.01 (s, 1H), 2.88-2.86 (m, 2H), 1.82 (s, 2H), 1.70 (s, 1H), 1.48 (m, 1H), 1.42 (s, 9H); ESI-MS calculated for (C28H35N7O4) [M + H]+, 533.28, found 534.3.

5.1.8. (R)-2-((2-(3-Aminopiperidin-1-yl)-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)-4-morpholinobenzonitrile hydrochloride (7a) Compound 6a (1.20 g, 2.2 mmol) was dissolved in 30 mL saturated HCl/MeOH solution and stirred overnight at room temperature. The reaction solution was concentrated in vacuo to give a yellow solid. The solid was suspended in 50 mL ether and filtered. The filter cake was washed with ether and dried to yield the title compound 7a as a light-yellow solid (0.91 g, yield 85.8%). ^{1H} NMR (400 MHz, DMSO-d6) δ: 12.05-11.98 (m, 1H),

8.26-8.14 (m, 3H), 7.59-7.57 (m, 1H), 7.37-7.36 (m, 1H), 6.93-6.91 (m, 1H), 6.53-6.51 (m, 1H), 6.32 (s, 1H), 5.39-5.26 (m, 2H), 3.46-3.42 (m, 4H), 3.35-3.26 (m, 1H), 3.13-3.12 (m, 2H), 3.03-3.01 (m, 4H), 2.96-2.93 (m, 2H), 2.67 (m, 1H), 1.98 (s, 1H), 1.75-1.73 (m, 1H), 1.54-1.51 (m, 2H); ^{13C} NMR (125 MHz, MeOD) δ: 155.41, 153.64, 152.64, 140.54, 134.33, 133.09, 131.18, 129.75, 118.24, 115.31, 114.55, 113.31, 97.69, 66.08, 51.88, 50.95, 50.08, 46.61, 29.74, 27.35, 22.13; ESI-MS calculated for (C23H27N7O2) [M + H]+, 433.22, found 434.2.

5.1.9. (R)-2-((2-(3-Aminopiperidin-1-yl)-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)-4-(dimethylamino)benzonitrile (7b) Compound 7b was prepared in a manner identical to that described for 7a as a yellow solid. Yield (two steps): 35.3%. ^{1H} NMR (400 MHz, CDCl3) δ: 11.08 (s, 1H), 7.44-7.42 (d, J = 8.8 Hz, 1H), 7.25-7.24 (d, J = 2.8 Hz, 1H), 6.50-6.47 (dd, J = 8.8 Hz, J = 2.4 Hz, 1H), 6.40-6.39 (d, J = 2.8 Hz, 1H), 6.40-6.39 (d, J = 2.8 Hz, 1H), 6.07-6.06 (d, J = 2.4 Hz, 1H), 5.55-5.46 (m, 2H), 3.24-3.21 (m, 1H), 3.06-3.02 (m, 1H), 2.99 (s, 2H), 2.88 (s, 1H), 2.82 (s, 6H), 2.77-2.72 (m, 2H), 1.92-1.89 (m, 1H), 1.77-1.75 (m, 1H), 1.66-1.58 (m, 1H), 1.35-1.25 (m, 1H); ^{13C} NMR (125 MHz, CDCl3+MeOD) δ: 156.14, 154.29, 152.82, 143.33, 142.39, 134.05, 128.51, 119.25, 115.17, 110.27, 108.32, 103.13, 95.33, 56.85, 51.78, 46.95, 45.74, 39.62, 29.60, 22.59; ESI-MS calculated for (C21H25N7O) [M + H]+, 391.21, found 392.2.

5.1.10. (R)-2-((2-(3-Aminopiperidin-1-yl)-7-bromo-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)-4-fluorobenzonitrile hydrochloride (9a) Compound 9a was prepared in a manner identical to that described for 4a as a yellow solid with one hydrochloride. Yield (last step): 63.2%. ^{1H} NMR (400 MHz, DMSO-d6) δ: 12.43 (s, 1H), 8.23 (s, 3H), 7.97-7.93 (m, 1H), 7.58-7.57 (d, J = 3.2 Hz, 1H), 7.35-7.30 (m, 1H), 7.00-6.98 (m, 1H), 5.52-5.33 (dd, J = 16.0 Hz, J = 63.6 Hz, 2H), 3.45-3.38 (m, 1H), 3.26 (m, 1H), 3.02 (s, 2H), 2.74 (s, 1H), 1.98-1.97 (m, 1H), 1.80 (s, 1H), 1.57 (s, 2H); ^{13C} NMR (125 MHz, CDCl3+MeOD) δ: 165.96, 163.94, 154.58, 145.87, 140.13, 136.39, 128.32, 117.10, 115.96, 115.64, 115.37, 106.99, 90.12, 52.70, 52.04, 46.86, 45.91, 27.65, 22.15; ESI-MS calculated for (C19H18BrFN6O) [M + H]+, 444.07, 446.07, found 445.1, 447.1.

5.1.11. (R)-2-((2-(3-Aminopiperidin-1-yl)-6,7-dibromo-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)-4-fluorobenzonitrile hydrochloride (9b) Compound 9b was prepared in a manner identical to that described for 4a as a yellow solid with one hydrochloride. Yield (last step): 66.2%. ^{1H} NMR (400 MHz, DMSO-d6) δ: 13.32 (s, 1H), 8.36 (s, 3H), 7.92 (s, 1H), 7.30 (s, 1H), 7.02-7.01 (m, 1H), 7.00-6.98 (m, 1H), 5.49-5.30 (dd, J = 14.4 Hz, J = 62.4 Hz, 2H), 3.38 (s, 1H), 3.23 (s, 1H), 3.00-2.98 (m, 2H), 2.73 (s, 1H), 1.97 (s, 1H), 1.79 (s, 1H), 1.56 (s, 2H); ^{13C} NMR (125 MHz, CDCl3+MeOD) δ: 165.90, 163.88, 155.15, 153.64, 145.54, 140.73, 136.41, 117.01, 116.71, 115.51, 115.33, 114.46, 106.89, 93.25, 52.56, 51.96, 46.81, 46.16,

27.67, 22.14; ESI-MS calculated for (C19H17Br2FN6O) [M + H]+, 523.98, found 525.0.

5.1.12. (R)-2-((2-(3-Aminopiperidin-1-yl)-7-fluoro-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)-4-fluorobenzonitrile hydrochloride (9c) Compound 9c was prepared in a manner identical to that described for 4a as a yellow solid with one hydrochloride. Yield (last step): 56.2%. ^{1H} NMR (400 MHz, DMSO-d6) δ: 12.35-12.34 (d, J = 2.4 Hz, 1H), 8.23 (s, 3H), 7.97-7.94 (m, 1H), 7.57-7.56 (d, J = 3.2 Hz, 1H), 7.36-7.31 (m, 1H), 7.07-6.97 (m, 1H), 5.52-5.33 (m, 2H), 3.41-3.37 (m, 1H), 3.25 (s, 1H), 3.07-3.03 (m, 2H), 2.74 (s, 1H), 2.00-1.98 (m, 1H), 1.80 (s, 1H), 1.59 (s, 2H); ^{13C} NMR (125 MHz, MeOD) δ: 166.57, 164.14, 154.99, 154.01, 144.88, 138.39, 135.67, 125.82, 116.31, 115.34, 114.82, 106.71, 105.12, 51.89, 51.19, 45.67, 33.31, 27.54, 21.29; ESI-MS calculated for (C19H18F2N6O) [M + H]+, 384.15, found 385.1.

5.1.13. (R)-2-((2-(3-Aminopiperidin-1-yl)-6,7-difluoro-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)-4-fluorobenzonitrile (9d) Compound 9d was prepared in a manner identical to that described for 4a as a white foam. Yield (last step): 58.9%. ^{1H} NMR (400 MHz, CDCl3) δ: 7.68-7.66 (m, 1H), 7.06-7.02 (m, 1H), 6.66-6.63 (m, 1H), 5.57 (s, 2H), 3.63 (s, 2H), 3.24-3.22 (m, 1H), 3.06-2.99 (m, 2H), 2.90-2.84 (m, 1H), 2.78-2.73 (m, 1H), 1.96-1.93 (m, 1H), 1.80-1.77 (m, 1H), 1.67-1.58 (m, 1H), 1.33-1.25 (m, 1H); ^{13C} NMR (125 MHz, CDCl3) δ: 166.34, 163.57, 154.98, 154.32, 145.36, 139.05, 135.90, 126.45, 116.97, 115.42, 114.21, 106.98, 105.77, 52.47, 51.72, 46.01, 34.29, 27.56, 21.39; ESI-MS calculated for (C19H17F3N6O) [M + H]+, 402.14, found 403.1.

5.1.14. 2-((2-Chloro-5-(methylsulfonyl)-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)-4-fluorobenzonitrile (8e) Methanesulfonyl chloride (2.28 g, 20.0 mmol) was added dropwise to a mixture of 3a (3.03 g, 10.0 mmol) and triethylamine (2.23 g, 22.0 mmol) in a round-bottom flask containing 60 mL of N,N-dimethylformamide at 0℃, and the solution was stirred overnight at room temperature. The reaction mixture was poured into water (100 mL) and extracted with ethyl acetate (100 × 3 mL). The organic layer was combined and washed successively with saturated sodium carbonate aqueous solution, water, and saturated salt solution, then dried with anhydrous sodium sulfate and filtered. The filtrate was concentrated in vacuo and purified by flash chromatography to yield the title compound 8e as a white solid (3.26 g, yield 85.7%). ^{1H} NMR (400 MHz, DMSO-d6) δ: 8.06-8.03 (m, 1H), 7.94-7.93 (d, J = 2.8 Hz, 1H), 7.43-7.39 (m, 1H), 7.31-7.29 (d, J = 9.6 Hz, 1H), 6.73 (s, 1H), 5.60 (s, 2H), 3.85 (s, 3H); ESI-MS calculated for (C15H10ClFN4O3S) [M + H]+, 380.01, found 381.0.

5.1.15. (R)-2-((2-(3-Aminopiperidin-1-yl)-5-(methylsulfonyl)-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)-4-fluorobenzonitrile (9e) Compound 9e was prepared in a manner identical to that described for 4a as a white solid. Yield: 67.5%. ^{1H} NMR (400 MHz, MeOD) δ: 7.85-7.83 (m, 1H), 7.80-7.79 (d, J = 3.2 Hz, 1H), 7.24-7.20 (m, 1H), 6.98-6.95 (d, J = 9.6 Hz, 1H), 6.55-6.54 (d, J = 2.8 Hz, 1H), 5.78 (s, 2H), 3.72 (s, 3H), 3.38-3.33 (m, 1H), 3.19 (s, 1H), 2.94-2.89 (m, 1H), 2.86-2.80 (m, 1H), 2.70-2.65 (m, 1H), 1.99-1.97 (m, 1H), 1.80-1.77 (m, 1H), 1.70-1.61 (m, 1H), 1.33-1.25 (m, 1H); ^{13C} NMR (125 MHz, MeOD) δ: 166.31, 164.28, 155.44, 154.26, 145.44, 143.38, 135.59, 128.53, 116.15, 115.10, 114.07, 106.61, 102.45, 58.12, 51.34, 47.03, 32.68, 32.23, 23.18; ESI-MS calculated for (C20H21FN6O3S) [M + H]+, 444.14, found 445.1.

5.1.16. (R)-2,5' -(2-(3-Aminopiperidin-1-yl)-4-oxo-3H-pyrrolo[3,2-d]pyrimidine-3,5(4H)-diyl)bis(methylene)bis(4-fluorobenzonitrile) hydrochloride (9f) Compound 9f was prepared in a manner identical to that described for 9e as a white solid with one hydrochloride. Yield (last step): 58.9%. ^{1H} NMR (400 MHz, MeOD) δ: 7.69-7.67 (m, 1H), 7.65-7.64 (m, 1H), 7.54 (s, 1H), 7.20-7.18 (m, 1H), 7.16-7.10 (m, 2H), 6.49 (s, 2H), 5.66 (s, 2H), 5.39-5.31 (m, 2H), 3.82-3.81 (m, 1H), 3.58 (s, 1H), 3.46-3.44 (m, 1H), 3.32-3.30 (m, 1H), 3.19 (s, 1H), 2.19-2.17 (m, 1H), 1.96-1.93 (m, 1H), 1.86-1.85 (m, 1H), 1.70-1.69 (m, 1H); ^{13C} NMR (125 MHz, MeOD) δ: 166.10, 165.82, 164.07, 163.78, 155.20, 153.18, 144.73, 143.33, 135.54, 133.90, 117.59, 116.32, 115.92, 115.75, 114.67, 114.48, 114.09, 106.87, 106.42, 99.83, 51.93, 51.02, 49.40, 48.49, 46.58, 27.35, 21.97; ESI-MS calculated for (C27H23F2N7O) [M + H]+, 499.19, found 500.2.

5.1.17. (R)-tert-Butyl 1-(7-bromo-3-(2-cyano-5-fluorobenzyl)-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-2-yl)piperidin-3-ylcarbamate (10) Compound 10 was prepared from 9a in a manner identical to that described for 5 as a yellow solid. Yield: 88.7%. ^{1H} NMR (400 MHz, CDCl3) δ: 10.87 (s, 1H), 7.71-7.68 (m, 1H), 7.34-7.33 (d, J = 2.8 Hz, 1H), 7.06-7.02 (m, 1H), 6.69-6.67 (d, J = 7.6 Hz, 1H), 5.56 (s, 2H), 5.08 (s, 1H), 3.77 (s, 1H), 3.37-3.33 (m, 1H), 3.09 (s, 1H), 3.03-2.96 (m, 2H), 1.84 (s, 2H), 1.56-1.53 (m, 1H), 1.41 (s, 9H), 1.27-1.26 (m, 1H); ESI-MS calculated for (C24H26BrFN6O3) [M + H]+, 544.12, 546.12, found 545.1, 547.1.

5.1.18. (R)-tert-Butyl 1-(3-(2-cyano-5-fluorobenzyl)-4-oxo-7-(thiophen-3-yl)-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-2-yl)piperidin-3-ylcarbamate (11a) A mixture of 10 (1.09 g, 2.0 mmol), thiophen-3-ylboronic acid (0.31 g, 2.4 mmol), 1,1' -Bis(diphenylphosphino)ferrocene-palladium(II)dichloride dichloromethane complex (Pd(dppf)Cl2 · DCM, 0.10 g, 0.12 mmol), and K2CO3 (0.69 g, 5.0 mmol) in a sealed tube containing 10 mL of 1,4-dioxane and 2 mL water was heated at 100℃ under Ar2 atmosphere overnight. The reaction mixture was subsequently cooled to room temperature,

poured into water (80 mL), and extracted with ethyl acetate (100 × 3 mL). The organic layer was combined and washed successively with saturated sodium carbonate aqueous solution, water, and saturated salt solution, then dried with anhydrous sodium sulfate and filtered. The filtrate was concentrated in vacuo and purified by flash chromatography to yield the title compound 11a as a white solid (0.86 g, yield 78.3%). ^{1H} NMR (400 MHz, CDCl3) δ: 11.54 (s, 1H), 7.98-7.97 (d, J = 2.0 Hz, 1H), 7.68 (s, 1H), 7.56-7.55 (m, 2H), 7.37-7.36 (m, 1H), 7.01-6.99 (m, 1H), 6.74-6.72 (m, 1H), 5.68-5.53 (m, 2H), 4.76 (s, 1H), 3.86 (m, 1H), 3.41-3.39 (m, 1H), 3.06-3.01 (m, 2H), 2.90 (s, 1H), 1.88-1.83 (m, 2H), 1.74 (s, 1H), 1.47 (s, 1H), 1.41 (s, 9H); ESI-MS calculated for (C28H29FN6O3S) [M + H]+, 548.20, found 549.2.

5.1.19. (R)-2-((2-(3-Aminopiperidin-1-yl)-4-oxo-7-(thiophen-3-yl)-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)-4-fluorobenzonitrile hydrochloride (12a) Compound 12a was prepared from 11a in a manner identical to that described for 7a as a white solid. Yield: 89.1%. ^{1H} NMR (400 MHz, DMSO-d6) δ: 12.13-12.12 (d, J = 2.8 Hz, 1H), 8.16 (s, 1H), 8.02 (d, J = 1.2 Hz, 1H), 8.01-7.96 (m, 1H), 7.86-7.85 (d, J = 2.8 Hz, 1H), 7.74-7.73 (m, 1H), 7.60-7.58 (m, 1H), 7.37-7.32 (m, 1H), 6.98-6.95 (m, 1H), 5.56-5.38 (m, 2H), 3.48-3.46 (m, 1H), 3.36 (s, 1H), 3.09-3.03 (m, 2H), 2.82 (s, 1H), 1.99 (s, 1H), 1.84 (s, 1H), 1.60-1.58 (m, 2H); ^{13C} NMR (125 MHz, DMSO-d6+MeOD) δ: 166.04, 164.02, 154.91, 154.09, 146.17, 139.64, 136.41, 134.66, 126.73, 116.23, 115.33, 112.97, 107.16, 52.85, 52.15, 46.96, 45.73, 27.84, 22.28; ESI-MS calculated for (C23H21FN6OS) [M + H]+, 448.15, found 449.1.

5.1.20. (R)-2-((2-(3-Aminopiperidin-1-yl)-4-oxo-7-(thiophen-3-yl)-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)-4-fluorobenzonitrile hydrochloride (12b) Compound 12b was prepared in a manner identical to that described for 12a as a white solid. Yield (two steps): 62.6%. ^{1H} NMR (400 MHz, DMSO-d6) δ: 12.21 (s, 1H), 8.30 (s, 3H), 7.93 (s, 1H), 7.75 (s, 1H), 7.65-7.43 (m, 1H), 7.36-7.31 (m, 2H), 7.06-6.85 (m, 2H), 5.54-5.36 (m, 2H), 3.26-3.15 (m, 2H), 3.09-3.00 (m, 2H), 2.82 (s, 1H), 1.97 (s, 1H), 1.82 (s, 1H), 1.58 (s, 2H); ^{13C} NMR (125 MHz, DMSO-d6) δ: 166.08, 164.04, 154.86, 154.24, 146.04, 139.10, 136.48, 135.73, 127.70, 125.27, 123.66, 122.87, 117.15, 116.14, 115.35, 111.83, 107.14, 52.94, 52.13, 46.83, 45.86, 27.89, 22.24; ESI-MS calculated for (C23H21FN6OS) [M + H]+, 448.15, found 449.1.

5.1.21. (R)-2-((2-(3-Aminopiperidin-1-yl)-7-(4-fluorophenyl)-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)-4-fluorobenzonitrile hydrochloride (12c) Compound 12c was prepared in a manner identical to that described for 12a as a white solid. Yield (two steps): 66.3%. ^{1H} NMR (400 MHz, DMSO-d6) δ: 12.21 (s, 1H), 8.25 (s, 3H), 8.12 (s, 2H), 7.95 (s, 1H), 7.88 (s, 1H), 7.33 (s, 1H), 7.22-7.20 (m, 2H), 6.99-6.97 (m, 1H), 5.54-5.38 (m, 2H), 3.46-3.44 (m, 2H), 3.07-3.01 (m, 2H), 2.84 (s, 1H), 1.98 (s, 1H), 1.83 (s, 1H), 1.59 (s, 2H); ^{13C} NMR (125 MHz, DMSO-d6) δ: 161.88,

160.05, 154.95, 153.99, 146.19, 139.67, 136.47, 130.90, 128.04, 126.16, 117.19, 116.66, 115.75, 115.21, 107.16, 52.93, 51.98, 46.84, 45.77, 27.89, 22.13; ESI-MS calculated for (C25H22F2N6O) [M + H]+, 460.18, found 461.2.

5.1.22. (R)-2-((2-(3-Aminopiperidin-1-yl)-7-(4-(methylsulfonyl)phenyl)-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)-4-fluorobenzonitrile hydrochloride (12d) Compound 12d was prepared in a manner identical to that described for 12a as a white solid. Yield (two steps): 64.8%. ^{1H} NMR (400 MHz, MeOD) δ: 8.47 (s, 2H), 8.06 (s, 3H), 7.94 (s, 1H), 7.76 (s, 2H), 7.68 (s, 1H), 7.34 (s, 1H), 7.13 (s, 1H), 5.73-5.64 (m, 2H), 3.73-3.65 (m, 2H), 3.31 (s, 1H), 3.26 (s, 1H), 3.26 (s, 3H), 2.26 (s, 1H), 2.00-1.92 (m, 2H), 1.77 (s, 1H); ^{13C} NMR (125 MHz, DMSO-d6+MeOD) δ: 166.22, 164.22, 154.04, 140.50, 139.73, 137.34, 135.86, 132.42, 127.34, 127.06, 126.33, 116.73, 115.46, 114.96, 106.86, 52.60, 51.83, 47.12, 45.70, 43.41, 27.79, 22.04; ESI-MS calculated for (C26H25FN6O3S) [M + H]+, 520.17, found 521.2.

5.1.23. (R)-2-((2-(3-Aminopiperidin-1-yl)-7-(2-(methylsulfonyl)phenyl)-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)-4-fluorobenzonitrile (12e) Compound 12e was prepared in a manner identical to that described for 12a as a light-yellow solid. Yield (two steps): 70.5%. ^{1H} NMR (400 MHz, MeOD) δ: 8.23-8.21 (d, J = 8.0 Hz, 1H), 7.86-7.82 (m, 1H), 7.78-7.74 (m, 1H), 7.70-7.68 (m, 2H), 7.65-7.61 (m, 1H), 7.26-7.22 (m, 1H), 7.00-6.97 (d, J = 9.2 Hz, 1H), 5.67-5.56 (m, 2H), 3.31 (s, 2H), 3.31 (s, 1H), 3.10-3.08 (m, 2H), 2.89 (s, 3H), 2.86-2.77 (m, 2H), 2.00-1.97 (m, 1H), 1.79-1.62 (m, 2H), 1.40-1.32 (m, 1H); ^{13C} NMR (125 MHz, MeOD) δ: 166.33, 164.30, 155.68, 154.39, 145.33, 145.26, 141.63, 139.81, 135.75, 134.59, 132.80, 129.71, 128.18, 127.48, 116.28, 115.26, 115.08, 114.92, 114.73, 113.56, 106.62, 55.38, 51.28, 46.95, 45.47, 41.89, 30.34, 22.26; ESI-MS calculated for (C26H25FN6O3S) [M + H]+, 520.17, found 521.2.

5.1.24. (R)-2-((2-(3-Aminopiperidin-1-yl)-7-(3-(methylsulfonyl)phenyl)-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)-4-fluorobenzonitrile (12f) Compound 12f was prepared in a manner identical to that described for 12a as a light-yellow solid. Yield (two steps): 72.8%. ^{1H} NMR (400 MHz, MeOD) δ: 8.98 (s, 1H), 8.31-8.29 (d, J = 7.6 Hz, 1H), 7.91 (s, 1H), 7.86-7.82 (m, 1H), 7.80-7.78 (d, J = 8.0 Hz, 1H), 7.66-7.62 (m, 1H), 7.24-7.19 (m, 1H), 6.91-6.89 (dd, J = 2.0 Hz, J = 8.4 Hz, 1H), 5.65-5.60 (m, 2H), 3.50-3.48 (m, 1H), 3.20 (s, 3H), 3.18 (s, 1H), 3.15-3.11 (m, 1H), 2.95-2.89 (m, 1H), 2.87-2.81 (m, 1H), 2.06-2.02 (m, 1H), 1.82-1.72 (m, 2H), 1.38-1.36 (m, 1H); ^{13C} NMR (125 MHz, MeOD) δ: 166.34, 164.31, 155.72, 154.33, 145.26, 140.89, 135.65, 135.57, 130.18, 129.22, 126.17, 124.52, 123.71, 116.26, 115.23, 115.05, 114.65, 114.38, 106.78, 57.28, 51.43, 46.98, 45.38, 43.15, 31.70, 22.99; ESI-MS calculated for (C26H25FN6O3S) [M + H]+, 520.17, found 521.2.

5.1.25. (R)-2-((2-(3-Aminopiperidin-1-yl)-7-(4-(isopropylsulfonyl)phenyl)-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)-4-fluorobenzonitrile hydrochloride (12g) Compound 12g was prepared in a manner identical to that described for 12a as a light-yellow solid. Yield (two steps): 58.5%. ^{1H} NMR (400 MHz, DMSO-d6) δ: 12.48-12.47 (d, J = 2.8 Hz, 1H), 8.43-8.41 (d, J = 8.4 Hz, 2H), 8.32 (s, 3H), 8.13-8.12 (d, J = 3.2 Hz, 1H), 7.98-7.94 (m, 1H), 7.83-7.81 (d, J = 8.4 Hz, 2H), 7.36-7.31 (m, 1H), 7.04-7.01 (m, 1H), 5.55-5.39 (m, 2H), 3.54-3.51 (m, 1H), 3.44-3.42 (m, 1H), 3.40-3.37 (m, 1H), 3.15-3.07 (m, 2H), 2.87 (s, 1H), 1.98 (s, 1H), 1.84 (s, 1H), 1.62 (s, 2H), 1.19-1.17 (d, J = 26.8 Hz, 6H); ^{13C} NMR (125 MHz, DMSO-d6) δ: 166.87, 164.54, 154.88, 154.47, 147.85, 140.35, 140.03, 136.41, 133.35, 129.35, 127.79, 126.13, 117.13, 115.97, 115.49, 114.20, 107.10, 54.69, 51.87, 49.14, 46.67, 45.98, 27.25, 22.36, 15.74; ESI-MS calculated for (C28H29FN6O3S) [M + H]+, 548.20, found 549.1.

5.1.26. (R)-4-(2-(3-Aminopiperidin-1-yl)-3-(2-cyano-5-fluorobenzyl)-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-7-yl)benzenesulfonamide hydrochloride (12h) Compound 12h was prepared in a manner identical to that described for 12a as a light-yellow solid. Yield (two steps): 60.8%. ^{1H} NMR (400 MHz, DMSO-d6) δ: 12.40 (s, 1H), 8.29 (s, 3H), 8.29-8.27 (d, J = 8.4 Hz, 2H), 8.05-8.04 (d, J = 3.2 Hz, 1H), 7.98-7.95 (m, 1H), 7.83-7.81 (d, J = 4.2 Hz, 2H), 7.37-7.36 (m, 1H), 7.34-7.32 (m, 2H), 7.04-7.01 (m, 1H), 5.55-5.38 (m, 2H), 3.51-3.49 (m, 1H), 3.38-3.36 (m, 1H), 3.14-3.04 (m, 2H), 2.83 (s, 1H), 2.01-1.98 (m, 1H), 1.84 (s, 1H), 1.60-1.59 (m, 2H); ^{13C} NMR (125 MHz, DMSO-d6) δ: 165.97, 163.95, 154.89, 154.31, 146.01, 141.15, 140.14, 137.92, 136.40, 127.24, 126.33, 126.01, 117.14, 115.95, 115.43, 114.70, 107.09, 52.80, 51.97, 46.76, 45.89, 28.05, 22.45; ESI-MS calculated for (C25H24FN7O3S) [M + H]+, 521.16, found 522.1.

5.1.27. (R)-2-((2-(3-Aminopiperidin-1-yl)-7-(3-fluoro-4-(methylsulfonyl)phenyl)-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)-4-fluorobenzonitrile hydrochloride (12i) Compound 12i was prepared in a manner identical to that described for 12a as a light-yellow solid. Yield (two steps): 60.8%. ^{1H} NMR (400 MHz, DMSO-d6) δ: 12.57-12.56 (d, J = 2.4 Hz, 1H), 8.26 (s, 3H), 8.23-8.23 (m, 2H), 8.20-8.19 (d, J = 3.2 Hz, 1H), 7.98-7.94 (m, 1H), 7.85-7.81 (m, 1H), 7.37-7.32 (m, 1H), 7.07-7.04 (m, 1H), 5.55-5.38 (m, 2H), 3.51 (s, 1H), 3.39 (s, 1H), 3.33 (s, 3H), 3.07 (s, 2H), 2.86 (s, 1H), 2.00-1.98 (m, 1H), 1.84 (s, 1H), 1.60-1.59 (m, 2H); ^{13C} NMR (125 MHz, DMSO-d6) δ: 163.94, 160.51, 158.52, 154.81, 143.07, 140.40, 136.41, 136.33, 129.74, 128.35, 124.68, 121.86, 117.11, 117.07, 115.97, 115.79, 115.37, 113.31, 107.05, 52.74, 51.95, 46.75, 46.07, 44.32, 27.77, 27.24; ESI-MS calculated for (C26H24F2N6O3S) [M + H]+, 538.16, found 539.1.

5.1.28. (R)-2-((2-(3-Aminopiperidin-1-yl)-7-(3-fluoro-4-(methylsulfonyl)phenyl)-4-oxo-4,5-dihydro-3H-pyrrolo[3,2-d]pyrimidin-3-yl)methyl)-4-fluorobenzonitrile (12j) Compound 12j was prepared in a manner identical to that described for

12a as a light-yellow solid. Yield (two steps): 63.3%. ^{1H} NMR (400 MHz, MeOD) δ: 8.96 (s, 1H), 7.92 (s, 1H), 7.84 (m, 2H), 7.77 (s, 1H), 7.24 (s, 2H), 7.04-7.02 (d, J = 9.2 Hz, 1H), 5.68-5.56 (m, 2H), 3.61-3.58 (m, 1H), 3.45 (s, 1H), 3.20 (s, 3H), 3.20-3.13 (m, 2H), 2.94 (s, 1H), 2.16-2.14 (m, 1H), 1.89-1.83 (m, 2H), 1.62-1.60 (m, 1H); ^{13C} NMR (125 MHz, MeOD) δ: 166.30, 164.27, 157.75, 155.40, 154.25, 145.02, 141.18, 138.53, 135.74, 130.08, 129.60, 127.49, 122.84, 116.39, 115.38, 115.19, 114.40, 108.59, 106.78, 53.74, 51.71, 45.64, 43.01, 35.18, 28.80, 22.20; ESI-MS calculated for (C26H24F2N6O3S) [M + H]+, 538.16, found 539.1.

5.2. In Vitro Inhibition of DPP-4, DPP-8, and DPP-9

Solutions of test compounds at varying concentrations (final concentration $ $10 mM) were prepared in dimethyl sulfoxide (DMSO) and diluted into assay buffer containing 20 mM Tris (pH 7.4), 20 mM KCl, and 0.1 mg/mL BSA. Human DPP-IV (0.1 nM final concentration) was added to the dilutions and pre-incubated for 10 minutes at ambient temperature before the reaction was initiated by addition of Gly-Pro-AMC (H-glycyl-prolyl-7-amino-4-methylcoumarin, Sigma-Aldrich, 10 µM final concentration). The total reaction mixture volume was 100 µL. Reaction kinetics were monitored (excitation at 400 nm, emission at 505 nm) for 5-10 minutes, or an endpoint was measured after 10 minutes. Inhibition constants (IC50) were calculated from enzyme progress curves using standard mathematical models.

5.3. In Vitro DPP-4 Inhibitory Kinetic Assay

Solutions of test compounds at varying concentrations (final concentration $ $10 mM) were prepared in dimethyl sulfoxide (DMSO) and diluted into assay buffer containing 20 mM Tris (pH 7.4), 20 mM KCl, and 0.1 mg/mL BSA. Human DPP-IV (0.1 nM final concentration) was added to the dilutions and pre-incubated for 10 minutes at ambient temperature before the reaction was initiated by addition of Gly-Pro-AMC (H-glycyl-prolyl-7-amino-4-methylcoumarin, Sigma-Aldrich, 10 µM final concentration). The total reaction mixture volume was 100 µL. Reaction kinetics were monitored (excitation at 400 nm, emission at 505 nm) for 5-10 minutes, or an endpoint was measured after 10 minutes. Inhibition constants (IC50) were calculated from enzyme progress curves using standard mathematical models.

5.4. In Vitro Hepatic Stability Study

Adult male SD rats (n = 4/group) were administered test compounds dissolved in distilled water at a single dose of 25 mg/kg by oral administration and 5 mg/mL by injection. Blood samples of 100-200 µL were collected from the orbit at 11 time points within 24 hours. Blood concentrations of test compounds were determined by LC-MS/MS. PK parameters were obtained using the pharmacokinetic software DAS 2.0.

5.5. In Vivo Pharmacokinetic Study

Adult male SD rats (n = 4/group) were administered test compounds dissolved in distilled water at a single dose of 25 mg/kg by oral administration and 5 mg/mL by injection. Blood samples of 100-200 µL were collected from the orbit at 11 time points within 24 hours. Blood concentrations of test compounds were determined by LC-MS/MS. PK parameters were obtained using the pharmacokinetic software DAS 2.0.

5.6. In Vivo Efficacy Study

Adult male ICR mice (at least n = 4 per group) were orally gavaged with test compounds dissolved in distilled water at a single dose of 1 mg/kg or 3 mg/kg. Blood samples of 20-25 µL were collected from the orbit at the time points indicated in Figure 6 for 24 hours, and the plasma fraction was kept frozen until DPP-IV activity measurement. Plasma DPP-IV activity was determined by the cleavage rate of Gly-Pro-AMC (H-glycyl-prolyl-7-amino-4-methylcoumarin; Sigma-Aldrich). Plasma (10 µL) was mixed with 140 µL of 150 µM Gly-Pro-AMC in assay buffer composed of 25 mM tris(hydroxymethyl)aminomethane HCl (pH 7.4), 140 mM NaCl, 10 mM KCl, and 0.1% bovine serum albumin. Fluorescence was determined using a Thermo Scientific Fluoroskan Ascent FL (excitation at 400 nm and emission at 505 nm). One unit of activity is defined as the amount of enzyme that produces 1 µM product per minute. DPP-IV relative activity in plasma was described as activity (indicated time points)/activity (initial point).

Acknowledgments

This research was supported by the National Natural Science Foundation of China (81402795) and a Grant from the Bureau of Education of Guangzhou Municipality (1201630308).

References and Notes

[1] J. da Rocha Fernandes, K. Ogurtsova, U. Linnenkamp, L. Guariguata, T. Seuring, P. Zhang, D. Cavan, L.E. Makaroff, IDF Diabetes Atlas estimates of 2014 global health expenditures on diabetes, Diabetes Res Clin Pract, 117 (2016) 48-54.
[2] B. Kreymann, G. Williams, M.A. Ghatei, S.R. Bloom, Glucagon-like peptide-1 7-36: a physiological incretin in man, Lancet, 2 (1987) 1300-1304.
[3] B. Ahren, DPP-4 inhibitors, Best Pract Res Clin Endocrinol Metab, 21 (2007) 517-533.
[4] S.E. Inzucchi, R.M. Bergenstal, J.B. Buse, M. Diamant, E. Ferrannini, M. Nauck, A.L. Peters, A. Tsapas, R. Wender, D.R. Matthews, Management of hyperglycaemia in type 2 diabetes, 2015: a patient-centred approach. Update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes, Diabetologia, 58 (2015) 429-442.

[5] J.A. Cramer, A systematic review of adherence with medications for diabetes, Diabetes Care, 27 (2004) 1218-1224.
[6] M.E. Kruk, N. Schwalbe, The relation between intermittent dosing and adherence: preliminary insights, Clin Ther, 28 (2006) 1989-1995.
[7] C.E. Grimshaw, A. Jennings, R. Kamran, H. Ueno, N. Nishigaki, T. Kosaka, A. Tani, H. Sano, Y. Kinugawa, E. Koumura, L. Shi, K. Takeuchi, Trelagliptin (SYR-472, Zafatek), Novel Once-Weekly Treatment for Type 2 Diabetes, Inhibits Dipeptidyl Peptidase-4 (DPP-4) via a Non-Covalent Mechanism, PLoS One, 11 (2016) e0157509.
[8] T. Biftu, R. Sinha-Roy, P. Chen, X. Qian, D. Feng, J.T. Kuethe, G. Scapin, Y.D. Gao, Y. Yan, D. Krueger, A. Bak, G. Eiermann, J. He, J. Cox, J. Hicks, K. Lyons, H. He, G. Salituro, S. Tong, S. Patel, G. Doss, A. Petrov, J. Wu, S.S. Xu, C. Sewall, X. Zhang, B. Zhang, N.A. Thornberry, A.E. Weber, Omarigliptin (MK-3102): a novel long-acting DPP-4 inhibitor for once-weekly treatment of type 2 diabetes, J Med Chem, 57 (2014) 3205-3212.
[9] D. Stoimenis, T. Karagiannis, A. Katsoula, E. Athanasiadou, K. Kazakos, E. Bekiari, D.R. Matthews, A. Tsapas, Once-weekly dipeptidyl peptidase-4 inhibitors for type 2 diabetes: a systematic review and meta-analysis, Expert Opin Pharmacother, 18 (2017) 843-851.
[10] I. Gantz, T. Okamoto, Y. Ito, K. Okuyama, E.A. O' Neill, K.D. Kaufman, S.S. Engel, E. Lai, G. Omarigliptin Study, A randomized, placebo- and sitagliptin-controlled trial of the safety and efficacy of omarigliptin, a once-weekly DPP-4 inhibitor, in Japanese patients with type 2 diabetes, Diabetes Obes Metab, (2017).
[11] N. Inagaki, H. Onouchi, H. Maezawa, S. Kuroda, K. Kaku, Once-weekly trelagliptin versus daily alogliptin in Japanese patients with type 2 diabetes: a randomised, double-blind, phase 3, non-inferiority study, Lancet Diabetes Endocrinol, 3 (2015) 191-197.
[12] Y. Ito, M. Mori, Y. Matsumoto, T. Okamoto, Pharmacological action and clinical results of Omarigliptin (MARIZEV(R) tablet), a novel dipeptidyl peptidase-4 inhibitor for once-weekly treatment of Type 2 diabetes, Nihon Yakurigaku Zasshi, 149 (2017) 128-137.
[13] A.J. Garber, The importance of incretin therapies for managing type 2 diabetes, Lancet Diabetes Endocrinol, 2 (2014) 95-97.
[14] H. Xie, L. Zeng, S. Zeng, X. Lu, G. Zhang, X. Zhao, N. Cheng, Z. Tu, Z. Li, H. Xu, L. Yang, X. Zhang, M. Huang, J. Zhao, W. Hu, Novel pyrrolopyrimidine analogues as potent dipeptidyl peptidase IV inhibitors based on pharmacokinetic property-driven optimization, Eur J Med Chem, 52 (2012) 205-212.
[15] J. Deng, L. Peng, G. Zhang, X. Lan, C. Li, F. Chen, Y. Zhou, Z. Lin, L. Chen, R. Dai, H. Xu, L. Yang, X. Zhang, W. Hu, The highly potent and selective dipeptidyl peptidase IV inhibitors bearing a thienopyrimidine scaffold effectively treat type 2 diabetes, Eur J Med Chem, 46 (2011) 71-76.
[16] S. Zeng, H. Xie, L.L. Zeng, X. Lu, X. Zhao, G.C. Zhang, Z.C. Tu, H.J. Xu, L. Yang, X.Q. Zhang, W. Hu, Discovery of potent dipeptidyl peptidase IV inhibitors through pharmacophore hybridization and hit-to-lead optimization,

Bioorg Med Chem, 21 (2013) 1749-1755.
[17] H. Xie, L. Zeng, S. Zeng, X. Lu, X. Zhao, G. Zhang, Z. Tu, H. Xu, L. Yang, X. Zhang, S. Wang, W. Hu, Highly potent dipeptidyl peptidase IV inhibitors derived from Alogliptin through pharmacophore hybridization and lead optimization, Eur J Med Chem, 68 (2013) 312-320.
[18] Z. Zhang, M.B. Wallace, J. Feng, J.A. Stafford, R.J. Skene, L. Shi, B. Lee, K. Aertgeerts, A. Jennings, R. Xu, D.B. Kassel, S.W. Kaldor, M. Navre, D.R. Webb, S.L. Gwaltney, Design and synthesis of pyrimidinone and pyrimidinedione inhibitors of dipeptidyl peptidase IV, J Med Chem, 54 (2011) 510-524.
[19] Y. Nishio, H. Kimura, S. Tosaki, E. Sugaru, M. Sakai, M. Horiguchi, Y. Masui, M. Ono, T. Nakagawa, H. Nakahira, Discovery of new chemotype dipeptidyl peptidase IV inhibitors having (R)-3-amino-3-methyl piperidine as a pharmacophore, Bioorg Med Chem Lett, 20 (2010) 7246-7249.
[20] G.R. Lankas, B. Leiting, R.S. Roy, G.J. Eiermann, M.G. Beconi, T. Biftu, C.C. Chan, S. Edmondson, W.P. Feeney, H. He, D.E. Ippolito, D. Kim, K.A. Lyons, H.O. Ok, R.A. Patel, A.N. Petrov, K.A. Pryor, X. Qian, L. Reigle, A. Woods, J.K. Wu, D. Zaller, X. Zhang, L. Zhu, A.E. Weber, N.A. Thornberry, Dipeptidyl peptidase IV inhibition for the treatment of type 2 diabetes: potential importance of selectivity over dipeptidyl peptidases 8 and 9, Diabetes, 54 (2005) 2988-2994.
[21] J. Feng, Z. Zhang, M.B. Wallace, J.A. Stafford, S.W. Kaldor, D.B. Kassel, M. Navre, L. Shi, R.J. Skene, T. Asakawa, K. Takeuchi, R. Xu, D.R. Webb, S.L. Gwaltney, 2nd, Discovery of alogliptin: a potent, selective, bioavailable, and efficacious inhibitor of dipeptidyl peptidase IV, J Med Chem, 50 (2007) 2297-2300.
[22] G. Schnapp, T. Klein, Y. Hoevels, R.A. Bakker, H. Nar, Comparative Analysis of Binding Kinetics and Thermodynamics of Dipeptidyl Peptidase-4 Inhibitors and Their Relationship to Structure, J Med Chem, 59 (2016) 7466-7477.

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