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Discovery of a low-systemic-exposure DGAT-1 inhibitor with a picolinoylpyrrolidine-2-carboxylic acid moiety

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ABSTRACT

A series of diacylglycerol *O*-acyltransferase 1 (DGAT-1) inhibitors with a picolinoylpyrrolidine-2-carboxylic acid moiety were designed and synthesized. Of these compounds, compound **22** exhibited excellent DGAT-1-inhibitory activity (hDGAT-1 enzyme assay, 50% inhibitory concentration [IC_{50}] = 3.5 ± 0.9 nM) and effectively reduced the intracellular triglyceride contents in 3T3-L1, HepG2 and Caco-2 cells. A preliminary study of the plasma and tissue distributions of compound **22** in mice revealed low plasma exposure and high concentrations in different segments of the intestine and liver, which may facilitate targeting DGAT-1. Furthermore, in an acute lipid challenge test, compound **22** showed a dose-dependent inhibitory effect on high-serum triglycerides in C57/KSJ mice induced by olive oil (1, 3, and 10 mg/kg, i.g.).

1. Introduction

Diacylglycerol *O*-acyltransferase (DGAT) is a key enzyme that catalyzes the final committed step in triglyceride synthesis and is highly expressed in the small intestine, adipose tissue, liver and mammary gland.[1–4] Its imbalance could lead to the excessive accumulation of triglycerides, which is frequently associated with metabolic diseases, such as obesity, insulin resistance and hepatic steatosis.[5,6] Although the DGAT family contains two isozymes DGAT-1 and DGAT-2, their sequence homology is limited.[7] A previous investigation revealed that DGAT-2 knockout (DGAT-$2^{-/-}$) mice suffer from lipopenia and die soon after birth.[8] However, DGAT-$1^{-/-}$ mice are healthy and significantly resistant to diet-induced obesity (DIO), hyperlipidemia and hepatic steatosis when fed a high-fat diet.[9–12] Thus, DGAT-1 could be a potential target for the modulation of triglycerides to treat hyperlipidemia and other metabolic disorders.

Over the past decade, an increasing number of small-molecule DGAT-1 inhibitors had been developed with variable structural types.[13–15] Most (Fig. 1) share a privileged structure with the heteroaryl-linker-acid, which is based on compounds **1** and **2** described in the early patents of Japan Tobacco/Tularik and Bayer.[16,17] Several inhibitors containing the phenylcyclohexylacetic acid functional group from compound **1** have entered into clinical trials,[18–24] and compound **3** (LCQ908) was included in a phase III study for the treatment of familial chylomicronemia syndrome (FCS) and showed high plasma exposure after oral dosing.[25] Based on compound **2** with a keto acid moiety that was originally developed by Abbott, the terminal benzothiazole group was opened, creating lead compound **8** (A-922500), which showed highly potent inhibition of DGAT-1 (50% inhibitory concentration [IC_{50}] = 7 nM) with oral efficacy.[26] This compound was extensively applied in the study of DGAT-1, but it is poorly soluble. To increase the solubility of this lead compound, researchers attempted to exchange the keto acid moiety of compound **8** with a simple amino acid while retaining the functional acid group to reduce its LogP property and avoid possible isomerization (Fig. 2, **9** and **10**).[27–31] In our efforts to discover novel potent DGAT-1 inhibitors, we designed a series of compounds based on compound **8** in which the phenyl linker was replaced by a six-membered heteroaryl group with a five-membered amino acid scaffold. Here, we report the discovery of a new low-systemic-exposure DGAT-1 inhibitor **22**, which bears a picolinoylpyrrolidine-2-carboxylic acid moiety targeting DGAT-1 specifically.[32,33] We found that this compound exhibited dose-dependent efficacy in an oral triglyceride uptake study in mice.

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[c] J. Y. and G. W. contributed equally to this work.

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0968-0896/

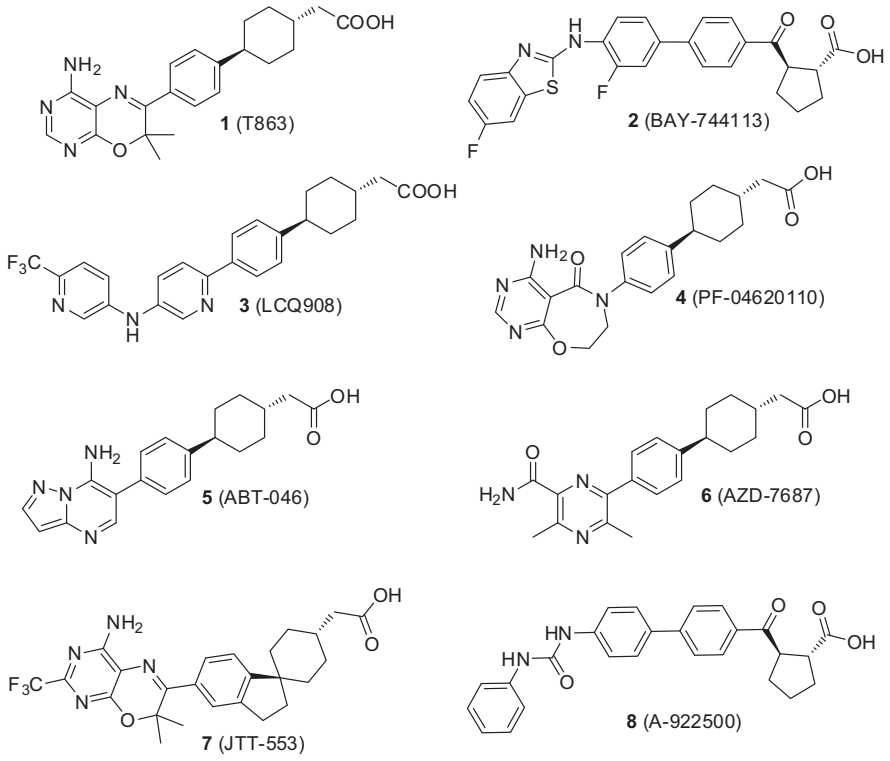


Fig. 1. Selected examples of DGAT-1 inhibitors.

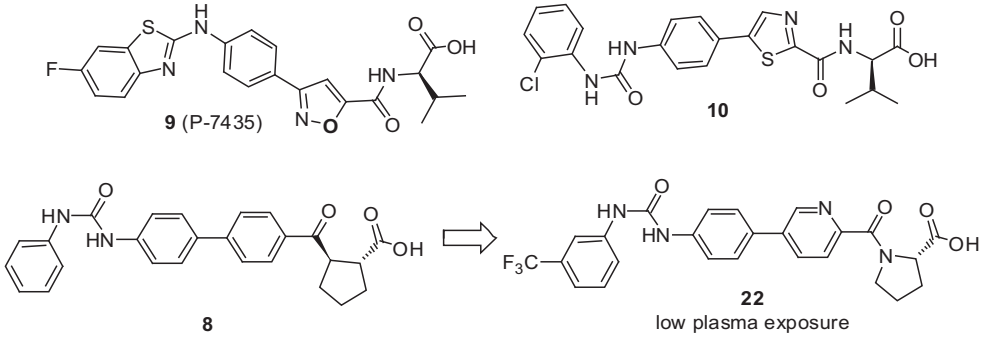


Fig. 2. The development of a low-systemic-exposure DGAT-1 inhibitor.

2. Results and discussion

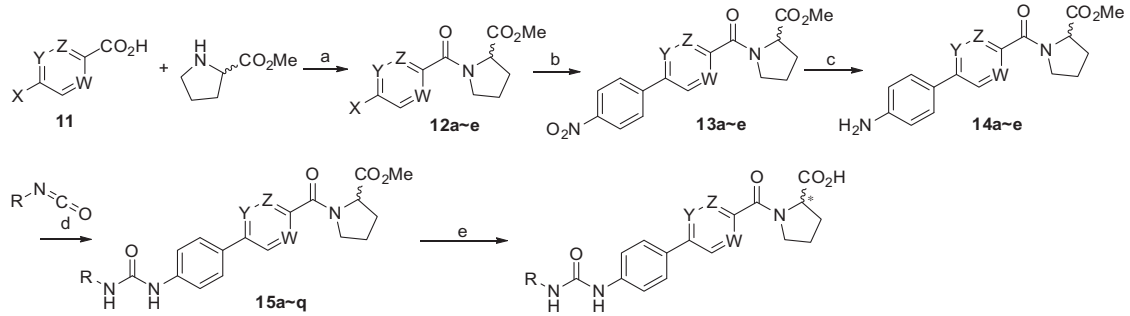
2.1. Chemistry

The condensation of methyl L/D-prolinate and halogenated heteroaromatic acids (**11a–d**) afforded halogenated heteroaromatic amides (**12a–e**), following cross-coupling with 4-nitrophenylboronic acid pinacol ester and the reduction of nitro compounds (**13a–e**) to generate the key intermediate biphenyl amines (**14a–e**). Reaction with isocyanate generated urea compounds (**15a–q**), which were hydrolyzed under basic conditions to give compounds **16–32** (Scheme 1). The addition reaction of **14b** with phenyl isothiocyanate and the subsequent hydrolysis of **33** afforded compound **34**. Compound **36** was obtained through the condensation of **14b** with *m*-(trifluoromethyl) phenylacetic acid and the hydrolysis of **35** (Scheme 2).

Amide intermediate **37** was synthesized via the condensation of 4-bromophenylacetic acid and 3-(trifluoromethyl) aniline, and then, cross-coupling with bis(pinacolato)diboron afforded the arylboronic ester **38**. The title product **40** was prepared through the Suzuki coupling of **12b** with **38** and the subsequent hydrolysis of carboxylic ester **39** (Scheme 3).

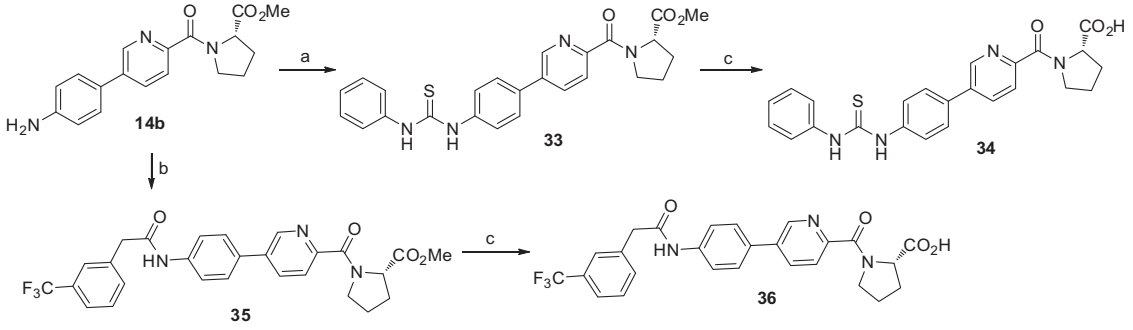
2.2. SAR study and lead generation

Initially, L-pyrrolidine-2-carboxylic acid was selected as the terminal moiety. Several different aromatic acyl linker compounds were synthesized and analyzed for their effects on DGAT-1 activity, and the results are listed in Table 1. Compound **16**, which has a benzoyl linker, caused a certain degree of hDGAT-1 inhibition. This result indicates that the keto acid moiety of compound **8** could be replaced with an *N*-acylpyrrolidine, which represents a rational optimization strategy for maintaining the functional acid group. When the benzoyl linker was replaced by 2-acyl pyridine, compound **17** displayed strong DGAT-1 inhibition activity, with an IC_{50} of 23.4 ± 3.4 nM for the human DGAT-1 enzyme. Compounds bearing 3-acyl pyridine (**18**) and 2-acyl pyrimidine (**19**) linkers exhibited significantly decreased hDGAT-1 inhibition. Changing

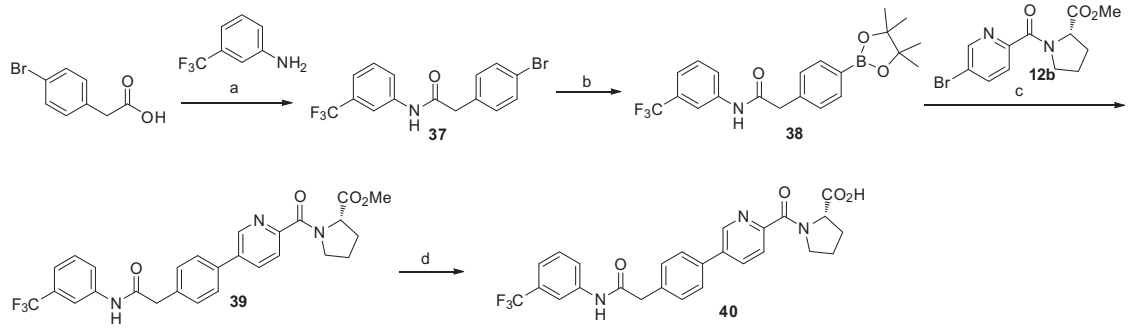


16 R=Ph, Y,Z,W=CH; *:S
17 R=Ph, Y,Z=CH, W=N; *:S
18 R=Ph, Z,W=CH, Y=N; *:S
19 R=Ph, Y=CH, Z,W=N; *:S
20 R=Ph, Y,Z=CH, W=N; *:R
21 R=2-$CF_3C_6H_4$, Y,Z=CH, W=N; *:S
22 R=3-$CF_3C_6H_4$, Y,Z=CH, W=N; *:S
23 R=4-$CF_3C_6H_4$, Y,Z=CH, W=N; *:S
24 R=2-$MeOC_6H_4$, Y,Z=CH, W=N; *:S
25 R=3-$MeOC_6H_4$, Y,Z=CH, W=N; *:S
26 R=4-$MeOC_6H_4$, Y,Z=CH, W=N; *:S
27 R=2-ClC_6H_4, Y,Z=CH, W=N; *:S
28 R=3-ClC_6H_4, Y,Z=CH, W=N; *:S
29 R=4-ClC_6H_4, Y,Z=CH, W=N; *:S
30 R=3-FC_6H_4, Y,Z=CH, W=N; *:S
31 R=*n*-Bu, Y,Z=CH, W=N; *:S
32 R=cyclohexyl, Y,Z=CH, W=N; *:S

Scheme 1. Synthesis of **16**–**32**. Reagents and conditions: (a) for **12a**: DIC, HOBT, DIPEA, DCM, room temperature (rt), 95%; for 1**2b**–**12e**: TBTU, DIPEA, DMF, rt, 88–96%; (b) 4-nitrophenylboronic acid pinacol ester, KF, $Pd(PPh_3)_4$, PhMe/EtOH/H_2O, N_2, 80 °C, 71–87%; (c) for **13a**: Fe, NH_4Cl, EtOH/H_2O, 90 °C, 70%; for **13b**–**13e**: Pd/C, $NaBH_4$, EtOH, rt, 75–82%; (d) TEA, THF, 40 °C, 88–98%; and (e) LiOH, CH_3CN/H_2O, rt, 88–98%.



Scheme 2. Synthesis of **34** and **36**. Reagents and conditions: (a) phenyl isothiocyanate, TEA, THF, 40 °C, 90%; (b) *m*-(trifluoromethyl) phenylacetic acid, TBTU, DIPEA, DMF, rt, 88%; and (c) LiOH, CH_3CN/H_2O, rt, 89–93%.

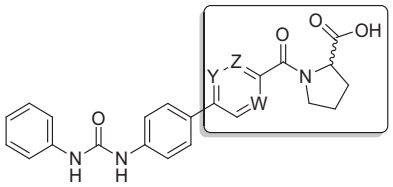


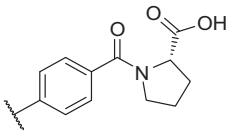
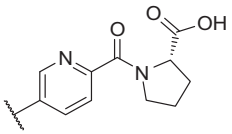
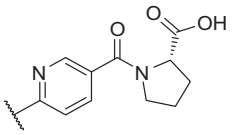
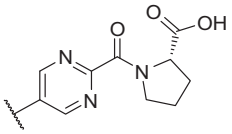
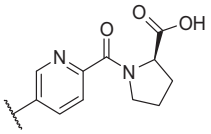
Scheme 3. Synthesis of **40**. Reagents and conditions: (a) TBTU, DIPEA, DMF, rt, 97%; (b) bis(pinacolato)diboron, $Pd(dppf)Cl_2$, KOAc, DMF, 80 °C, 71%; (c) KF, $Pd(PPh_3)_4$, PhMe/EtOH/H_2O, 62%; and (d) LiOH, CH_3CN/H_2O, rt, 93%.

L-proline into R-proline on the acid terminal resulted in a slight decline in the hDGAT-1 inhibition observed (**20**). Based on these results, the picolinoylpyrrolidine-2-carboxylic acid moiety was used as the end structure.

The substituent effect of the phenyl ring of the left urea terminal was subsequently investigated (Table 2). Introducing CF_3 at the *ortho*-, *meta*- and *para*-positions resulted in mixed results. The *ortho*-CF_3 substitution slightly decreased the activity (**21**), whereas for the *para*-CF_3 substitution, the activity was maintained (**23**). Interestingly, the *meta*-CF_3 substitution significantly improved the compound's potency, resulting in an IC_{50} value of 3.5 nM (**22**). The incorporation of the methoxy group at different positions on the benzene ring resulted in the retention of DGAT-1 inhibitory activity (compounds **24**–**26**). The introduction of Cl at different positions on the benzene ring of urea significantly enhanced hDGAT-1 inhibition, resulting in one-digit IC_{50} values in the nanomolar range (**27**–**29**). The 3-fluoro-substituted compound (**30**) exhibited decreased inhibitory activity compared with the

Table 1
Structure-activity relationships of the acyl pyrrolidine-2-carboxylic acid moiety.



Compd	Structure	[a]hDGAT-1 (% inhibition at 1 μM)	[b]hDGAT-1 (% inhibition at 0.1 μM)	[c]hDGAT-1 IC_{50} (nM)
16		79.6 ± 4.4	39.8 ± 2.1	NT
17		72.2 ± 7.8	61.8 ± 2.6	23.4 ± 3.4
18		48.9 ± 0.8	36.7 ± 2.4	NT
19		51.7 ± 3.0	42.0 ± 2.9	NT
20		88.1 ± 1.2	59.0 ± 7.0	39.9 ± 0.3
8		86.4 ± 3.2	85.7 ± 0.5	7.8 ± 1.3

The compounds were primarily tested for their DGAT-1 inhibitory activities at 1 μM[a] and 0.1 μM[b]. The compounds with inhibitory activities >50% at 0.1 μM were further investigated to determine their respective IC_{50} (half of the maximal inhibitory concentration) values[c]. All data are shown as the mean ± standard deviation (SD) from three independent experiments performed in duplicate. NT indicates not tested.

3-CF_3- (**22**) and 3-Cl-substituted ones (**28**). The fatty chain (**31**) and saturated carbon ring substitutions (**32**) caused a loss of the inhibitory activity. Changing the urea group to a thiocarbamide (**34**) and an amide (**36**, **40**) led to an obvious decline in or loss of the DGAT-1 inhibitory activity.

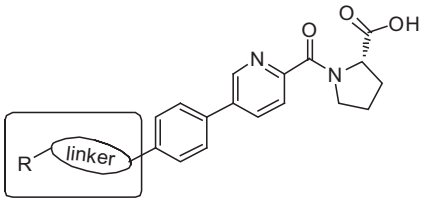
Compounds **22**, **27** and **28** showed the good solubility in the different Buffers (Table 3). Permeability tests of compounds **22**, **27** and **28** were performed using the bidirectional Caco-2 monolayer assay model (Table 3).[34] The data revealed that compound **22** exhibited a lower efflux ratio than compounds **27** and **28**.

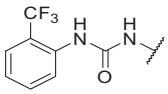
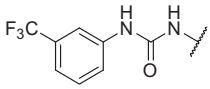
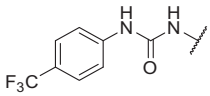
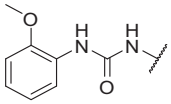
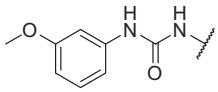
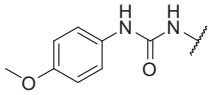
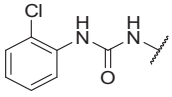
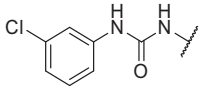
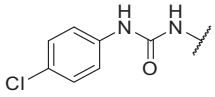
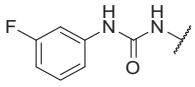
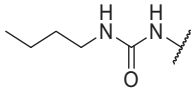
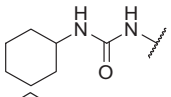
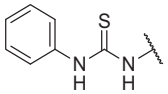
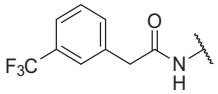
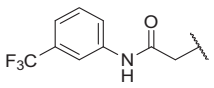
After differentiating for 4 days, the 3T3-L1 cells were maintained in the presence of the DGAT-1 inhibitors (compound **8**, LCQ908 or compound **22** at 20 μM) for 4 days; then, the cells in each group were subjected to Oil Red O staining (A) and cellular triglyceride content determination (B). Scale bar, 200 μm. Asterisks indicate significant differences relative to the control group ($^{***}p < 0.001$). Following treatment with the test compounds for 2 h, Caco-2 and HepG2 cells were incubated in the absence or presence of oleic acid (OA) for 48 h. Then, the cellular triglyceride contents of each group were determined (C-G). Asterisks indicate significant differences relative to the group treated with OA only ($^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$). The figures shown are representative of at least two independent experiments, each of which was performed in triplicate. A one-way analysis of variance (ANOVA) was performed to determine the *p* values.

The effects of compound **22** on the triglyceride contents in differentiated 3T3-L1 adipocytes, enterocyte-like Caco-2 cells and hepatoma-derived HepG2 cells, in which DGAT-1 is highly expressed, were investigated. Lipid accumulation was examined by Oil Red O staining and quantitative analysis of the cellular triglyceride content (Fig. 3 A and B). The treatment of differentiated 3T3-L1 adipocytes with compound **22** for 4 days significantly decreased the triglyceride contents. Additionally, after incubation with exogenous OA, Caco-2 cells and HepG2 cells exhibited significantly increased triglyceride generation, which was abolished by compound **8**, LCQ908 and compound **22** in a dose-dependent manner (Fig. 3 C–G).

The pharmacokinetic profile of compound **22** was evaluated in Sprague-Dawley rats (Table 4). The results showed that compound **22** exhibited lower plasma clearance (CL: 0.33 L/h/kg) and lower tissue distribution (Vss: 0.18 L/kg) after intravenous administration. After intragastric administration, the plasma peak time was 1.8 h, with low plasma exposure and only 0.28% of the absolute bioavailability. Based on these results, compound **22** might distribute specifically to some tissues. To validate this hypothesis, the tissue distribution of compound **22** was also determined (Table 5). After a 10-mg/kg oral dose of compound **22** in C57 mice, the drug levels in the plasma, liver and different segments of the intestine (duodenum, jejunum, and ileum) were determined at three time points (1, 2, and 5 h) and are provided in Table 5. As expected, compound **22** showed higher concentrations

Table 2
Structure-activity relationships of the urea groups.



Compd	Structure	[a]hDGAT-1 (% inhibition 1 μM)	[b]hDGAT-1 (% inhibition 0.1 μM)	[c]hDGAT-1 IC_{50} (nM)
21		65.3 ± 1.6	59.9 ± 1.6	59.2 ± 6.8
22		90.5 ± 2.6	80.5 ± 1.3	3.5 ± 0.9
23		68.3 ± 3.9	55.8 ± 1.1	30.3 ± 8.6
24		84.2 ± 1.7	73.2 ± 4.3	15.9 ± 3.2
25		74.8 ± 6.5	62.4 ± 2.0	32.1 ± 1.8
26		78.0 ± 0.2	68.3 ± 0.6	18.4 ± 3.0
27		98.4 ± 1.2	70.7 ± 0.7	8.5 ± 0.6
28		84.0 ± 1.6	73.6 ± 1.0	3.7 ± 0.6
29		78.9 ± 8.5	56.9 ± 5.8	8.8 ± 1.5
30		76.0 ± 1.9	68.0 ± 4.0	37.7 ± 6.9
31		29.0 ± 5.0	13.4 ± 2.6	NT
32		59.4 ± 4.8	40.5 ± 1.7	NT
34		70.3 ± 2.3	40.8 ± 1.3	NT
36		68.6 ± 1.3	36.7 ± 0.5	NT
40		23.1 ± 1.7	7.9 ± 4.0	NT
8		86.4 ± 3.2	85.7 ± 0.5	7.8 ± 1.3

The compounds were primarily tested for their DGAT-1 enzyme inhibitory activities at 1 μM[a] and 0.1 μM[b]. The compounds with inhibitory activities >50% at 0.1 μM were further investigated to determine their IC_{50} (half of the maximal inhibitory concentration) values[c]. All data are shown as the mean ± SD from three independent experiments performed in duplicate. NT indicates not tested.

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Table 3
Permeability profiles of compounds **22**, **27** and **28**.

Assay	Parameters	22	27	28
Solubility (μM)[a]	Buffer A	50	50	50
	Buffer B	50	50	50
Caco-2 Permeability	A → B P_{app} (10^{-6} cm·s^{-1})[b]	0.23	0.07	0.04
	B → A P_{app} (10^{-6} cm·s^{-1})[b]	3.82	1.75	2.01
	Efflux ratio	16.4	26.9	46.1

[a] Determined using nephelometry. Buffer A: Hank's balanced salt solution (HBSS)/10-mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)/0.1% bovine serum albumin (BSA), pH 6.8; Buffer B: HBSS/10-mM HEPES/0.1% BSA, pH 7.4; Buffer.
[b] P_{app}: apparent permeability rate coefficient.

(45- to 1380-fold) in the liver and different segments of the intestine than in the plasma at all time points. The preferential distribution in the liver and intestine over the plasma indicated that compound **22** is a novel, low-systemic-exposure DGAT-1 inhibitor that could act in certain locations to target DGAT-1.

The *in vivo* lipid-lowering effects of compound **22** were also evaluated using an acute lipid challenge test (Fig. 4). First, a model of an acute lipid challenge test was established (Fig. 4A). Male C57/KSJ mice were dosed with compound **8** or vehicle an hour prior to the administration of the olive oil bolus. After 2 h, the serum triglyceride levels of the vehicle-treated animals were maximized, and the increase in the serum triglyceride level was significantly suppressed by the administration of compound **8**. Thus, this model was appropriate for the evaluation of the *in vivo* triglyceride-lowering effects of these compounds. Second, the optimal time point for the administration of compound **22** was investigated (Fig. 4B). The data showed that the lipid-lowering effects of compound **22** were maximized when the compound and olive oil bolus were administered simultaneously (0 h). Finally, the dose-dependent activities of compound **22** were studied and compared with those of compounds **8** and LCQ908 (1, 3, and 10 mg/kg). Compound **22** was shown to significantly inhibit the acute high-serum triglyceride level induced by olive oil in a dose-dependent manner, and its effects were less potent than those of **8** but slightly more potent than those of LCQ908.

Six-week-old male mice (n = 6) were fasted for 16 h and orally dosed with either vehicle (0.1% w/v Tween 80) or the tested compound (10 mg/kg in A and B; 1, 3, and 10 mg/kg in C and D). One hour (A); 0, 1, and 3 h (B); or 0 h (C and D) after dosing, an olive oil bolus (15 mL/kg) was administered, and the mice in the control group were given an equal volume of water. Then, 1, 2, 3, and 4 h (A); or 2 h (B–D) later, blood samples were collected, and the serum triglyceride levels were measured. Asterisks indicate significant differences relative to the vehicle group ($^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$). A one-way ANOVA was performed to determine the *p* values.

3. Conclusions

Herein, we described the design and synthesis of a series of DGAT-1 inhibitors with a picolinoylpyrrolidine-2-carboxylic acid moiety based on compound **8** in which the phenyl linker was replaced by a six-membered heteroaryl group with a five-membered amino acid scaffold. Among them, compound **22** exhibited good inhibitory activity for hDGAT-1 and effectively reduced the intracellular triglyceride contents in the 3T3-L1, Caco-2 and HepG2 cell lines. Furthermore, an acute lipid challenge test of compound **22** revealed that it exerts dose-dependent lipid-lowering effects in mice. DGAT-1 inhibitor **22**, as an attractive lead candidate, warrants further investigation for the treatment of hyperlipidemia and other metabolic disorders because of its preferential distributions in the intestine and liver, which correlates perfectly with the location of DGAT-1.

4. Experimental section

4.1. General information

^{1}H NMR and ^{13}C NMR spectral data were recorded using CDCl3, DMSO-d_6 or MeOH-d_4 solutions with a Bruker Avance III 600, 500, 400 or 300 NMR spectrometer. Chemical shifts (δ) are reported in parts per million (ppm), and the signals are described as brs (broad singlet), s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), and m (multiplet). Coupling constants (J values) are given in Hz. The mass spectra were obtained using liquid chromatography mass spectrometry (LC-MS) on an Agilent 6120 instrument using electrospray ionization (ESI). Column chromatography was conducted using silica gel (200–300 mesh). All reactions were monitored using thin-layer chromatography (TLC) on silica gel plates. The purity (>95%) of final products prepared in this study was determined using chromatographic analysis with an Agilent 1200 series LC system (Agilent ChemStation Rev. B.03.01); column, ZORBAX Eclipse XDB-C18, 4.6 mm * 50 mm, 5 μm, or Nova Pak C18 3.9 mm * 150 mm, 4 μm; mobile phase, MeCN/H2O (0.2% triethylamine); flow rate, 1.0 mL/min; UV wavelength, maximal absorbance at 254 nm; temperature, ambient; and injection volume, 5 μL.

4.2. Procedure for the preparation of (S)-1-(4′-(3-phenylureido)-[1,1′-biphenyl]-4-carbonyl)pyrrolidine-2-carboxylic acid (**16**)

A mixture of 4-iodobenzoic acid (**11a**, 1.0 g, 4.0 mmol), (*S*)-methyl pyrrolidine-2-carboxylate (0.52 g, 4.0 mmol), DIC (0.56 g, 4.4 mmol), HOBt (0.60 g, 4.4 mmol) and DIPEA (1.32 mL, 8.0 mmol) in 30 mL of DCM was stirred at room temperature for 8 h, then 40 mL of water was added to reaction solution. The mixture was extracted with DCM (3 × 30 mL). The extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give a residue that was subjected to silica gel chromatography to afford **12a** as a white powder (1.19 g, 95% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.2 Hz, 2H), 7.32 (d, *J* = 8.2 Hz, 2H), 4.66 (brs, 1H), 3.78 (s, 3H), 3.68–3.46 (m, 2H), 2.40–2.25 (m, 1H), 2.13–1.84 (m, 3H).

A solution of **12a** (1.2 g, 3.3 mmol), 4-nitrophenylboronic acid pinacol ester (1.0 g, 4.0 mmol), KF (0.58 g, 9.9 mmol), and tetrakis (triphenylphosphine)palladium (92 mg, 0.08 mmol) in toluene/ethanol/H_2O (4/2/1 ratio, 20 mL) was heated to 80 °C for 8 h, cooled to room temperature, filtered through Celite, washed with ethyl acetate (3 × 20 mL), concentrated and purified on a silica gel chromatography to afford **13a** (0.99 g, 85% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (d, *J* = 8.6 Hz, 2H), 7.79–7.60 (m, 6H), 4.70 (dd, *J* = 8.3, 4.9 Hz, 1H), 3.79 (s, 3H), 3.76–3.65 (m, 1H), 3.60 (s, 1H), 2.40–2.25 (m, 1H), 2.11–1.90 (m, 3H).

A mixture of **13a** (0.90 g, 2.54 mmol), iron powder (0.43 g, 7.6 mmol), and NH_4Cl (136 mg, 2.54 mmol) in a mixture of solvents (75 mL ethanol and 25 mL water) was heated to 90 °C under N_2 for 8 h. The reaction mixture was filtered through Celite, treated with aqueous saturated sodium bicarbonate (30 mL) and extracted

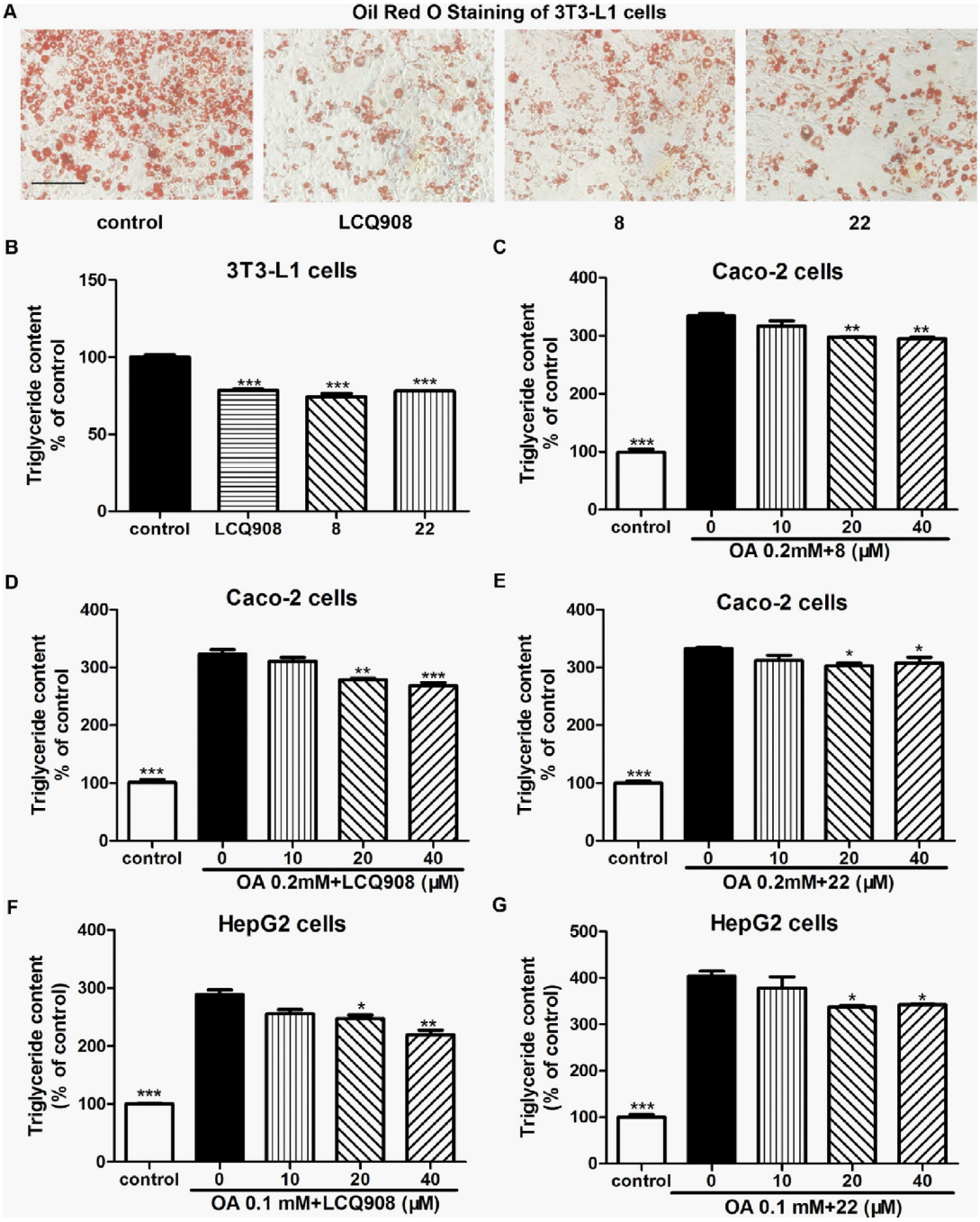

Fig. 3. Effects of the tested compounds on the intracellular triglyceride contents of 3T3-L1 cells, Caco-2 cells and HepG2 cells.

Table 4
Pharmacokinetic properties of compound **22**.[a]

	$AUC_{0\to\infty}$ (ng·h/mL)	MRT (h)	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/mL)	CL (L/h/kg)	Vss (L/kg)	F (%)
i.g.	215	3.4	2.0	1.8	51.6	/	/	0.28
i.v.	30075	0.53	0.91	/	/	0.33	0.18	/

[a] Determined using LC/MS/MS. $AUC_{0-\infty}$: the area under the concentration time curve; MRT: mean residence time; $t_{1/2}$: elimination half-life; T_{max}: the peak time; C_{max}: the peak concentration; CL: clearance; V_{ss}: steady-state distribution volume; F: absolute bioavailability.

with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, filtered, concentrated and purified on a silica gel chromatography to afford **14a** (0.58 g, 70% yield). To a solution of **14a** (250 mg, 0.77 mmol) and phenyl isocyanate (110 mg, 0.85 mmol) in dry THF (20 mL), triethylamine (0.28 mL, 1.54 mmol) was added. The mixture was stirred at 40 °C for 2 h, concentrated and purified by a flash column (20–50% EtOAc in petroleum ether) to provide **15a** (334 mg, 98% yield). Into a 100 mL round bottom flask, **15a**

Table 5
The mice plasma and tissue concentrations of compound **22**.[a]

Time (h)	Plasma (ng/ml)	Liver (ng/g)	Duodenum (ng/g)	Jejunum (ng/g)	Ileum (ng/g)
1	26.3 ± 5.8	7752 ± 3373	8043 ± 2052	36425 ± 5959	1245 ± 738
2	26.6 ± 9.3	4729 ± 1812	16535 ± 15208	9169 ± 1733	1262 ± 403
5	17.6 ± 15.0	3290 ± 1546	2623 ± 2800	5480 ± 3082	5516 ± 2681

[a] Determined using LC/MS/MS. All data are shown as the mean ± SD from three independent experiment.

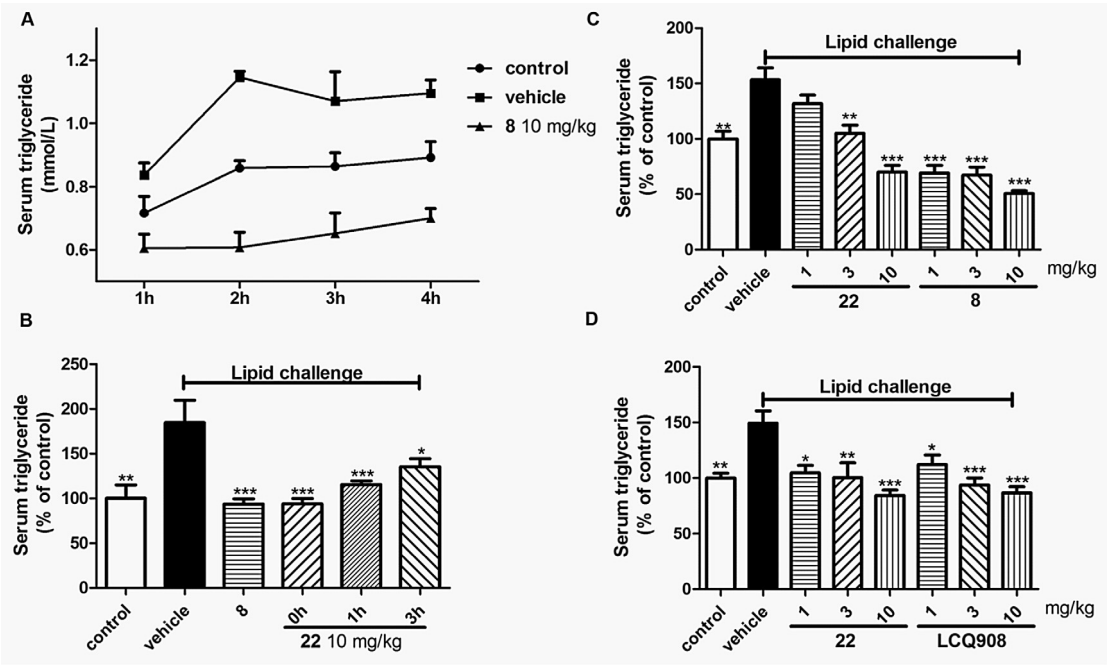

Fig. 4. The *in vivo* effects of compounds 22 in the acute lipid challenge test.

(195 mg, 0.44 mmol), LiOH (53 mg, 2.2 mmol), and 20 mL of 1:1 acetonitrile/water were added. The reaction mixtures were stirred at room temperature overnight. The pH-value of the solution was adjusted to between 4 and 7 by adding 1 M HCl. The precipitated solid was filtered and washed with a small amount of ethyl acetate to provide the title product **16** (334 mg, 96% yield). As a white solid: m.p. 173–174 °C. ^{1}H NMR (400 MHz, DMSO-D_6) δ 12.61 (s, 1H), 9.02 (s, 1H), 8.91 (s, 1H), 7.75–7.45 (m, 10H), 7.28 (t, J = 7.7 Hz, 2H), 6.97 (t, J = 7.2 Hz, 1H), 4.45–4.34 (m, 1H), 3.63–3.51 (m, 2H), 2.32–2.20 (m, 1H), 2.02–1.78 (m, 3H); ^{13}C NMR (101 MHz, DMSO-D_6) δ 173.5, 167.9, 152.5, 141.4, 140.7, 139.9, 139.7, 134.5, 132.4, 128.8, 127.9, 127.4, 127.2, 127.1, 125.8, 125.6, 121.9, 118.5, 118.2, 59.1, 49.6, 31.1, 29.0, 25.1, 22.3; LC-MS calcd. for $C_{25}H_{24}N_3O_4^+$ (*m/e*), 430.18, obsd. 430.2 (M+H). The purity of the compound **16** was 99.0% by High Performance Liquid Chromatography (HPLC).

*4.3. Procedure for Preparation of (S)-1-(5-(4-(3-phenylureido)phenyl) picolinoyl)pyrrolidine-2-carboxylic acid **(17)***

A mixture of 5-bromopyridine-2-carboxylic acid (**11b**, 2.02 g, 10.0 mmol), (*S*)-methyl pyrrolidine-2-carboxylate (1.42 g, 11.0 mmol), TBTU (3.53 g, 11.0 mmol) and DIPEA (3.31 mL, 20.0 mmol) in 20 mL of DMF was stirred at room temperature for 12 h, then DMF was removed by rotary evaporation. To the residue, 50 mL of water and 50 mL of ethyl acetate was added, extracted with ethyl acetate (50 mL × 3). The extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give a residue that was subjected to silica gel chromatography to afford **12b** as a white powder (3.31 g, 96% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (d, J = 2.3 Hz, 0.45H), 8.52 (d, J = 2.3 Hz, 0.55H), 7.99 (d, J = 8.4 Hz, 0.55H), 7.94–7.90 (m, 1H), 7.85 (d, J = 8.4 Hz, 0.45H), 5.11 (dd, J = 8.6, 3.1 Hz, 0.55H), 4.67 (dd, J = 8.5, 4.1 Hz, 0.45H), 4.07–3.94 (m, 1H), 3.93–3.73 (m, 2.35H), 3.66 (s, 1.65H), 2.35–2.22 (m, 1H), 2.21–1.90 (m, 3H).

A solution of **12b** (3.2 g, 10.2 mmol), 4-nitrophenylboronic acid pinacol ester (2.67 g, 10.7 mmol), KF (1.18 g, 20.4 mmol), and tetrakis(triphenylphosphine) palladium (230 mg, 0.2 mmol) in toluene/ethanol/H_2O (4/2/1 ratio, 70 mL) was heated to 80 °C for 12 h, cooled to room temperature, filtered through Celite, washed with ethyl acetate (3 × 50 mL), concentrated and purified on a silica gel chromatography to afford **13b** (3.15 g, 87% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.86 (d, J = 2.2 Hz, 0.44H), 8.72 (d, J = 2.3 Hz, 0.56H), 8.36 (d, J = 8.6, 0.88H), 8.35 (d, J = 8.6, 1.12H), 8.22 (d, J = 8.2 Hz, 0.56H), 8.07 (s, 0.44H), 8.03 (dd, J = 8.3, 2.3 Hz, 1H), 7.78 (d, J = 8.5, 0.88H), 7.76 (d, J = 8.5, 1.12H), 5.19 (dd, J = 8.7, 3.0 Hz, 0.56H), 4.71 (dd, J = 8.5, 4.1 Hz, 0.44H), 4.13–4.00 (m, 1H), 3.98–3.81 (m, 1H), 3.79 (s, 1.32H), 3.68 (s, 1.68H), 2.38–2.24 (m, 1H), 2.24–1.93 (m, 3H).

To a solution of **13b** (3.05 g, 8.6 mmol) in 50 mL ethanol at 0 °C under N_2, 10% Pd/C (0.30 g) and $NaBH_4$ (1.30 g, 34.4 mmol, portion-wise) were added. Then it was warmed to room temperature and stirred for another 6 h. To the reaction mixture, 1 M HCl was added until no bubbles come out. Ethanol was removed through rotary evaporation, 50 mL water and 50 mL ethyl acetate were added to residue, extracted with ethyl acetate (50 mL × 3). The extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give a residue that was subjected to silica gel chromatography to afford **14b** (2.10 g, 75% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.77 (s, 0.45H), 8.63 (s, 0.55H), 8.09 (d, J = 8.2 Hz, 0.55H), 7.96 (d, J = 8.1 Hz,

0.45H), 7.89 (d, J = 8.5 Hz, 1H), 7.46–7.37 (m, 2H), 6.83–6.72 (m, 2H), 5.24–5.16 (m, 1H), 4.70 (dd, J = 7.9, 3.2 Hz, 1H), 4.16–4.00 (m, 1H), 3.98–3.80 (m, 2H), 3.78 (s, 1.35H), 3.67 (s, 1.65H), 2.36–2.24 (m, 1H), 2.20–1.93 (m, 3H).

To a solution of **14b** (100 mg, 0.31 mmol) and phenyl isocyanate (40 mg, 0.34 mmol) in dry THF (10 mL), triethylamine (0.12 mL, 0.68 mmol) was added. The mixture was stirred at 40 °C for 2 h, concentrated and purified by a flash column (20–50% EtOAc in petroleum ether) to provide **15b** (131 mg, 95% yield). A 1:1 mixture of rotamers, ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.67 (d, J = 1.7 Hz, 0.5H), 8.59 (d, J = 1.9 Hz, 0.50H), 8.01 (d, J = 8.2 Hz, 0.50H), 7.93 (d, J = 4.2 Hz, 1H), 7.88 (d, J = 5.3 Hz, 1H), 7.82 (d, J = 8.2 Hz, 0.50H), 7.78–7.70 (m, 1H), 7.41 (t, J = 8.3 Hz, 2H), 7.37–7.23 (m, 6H), 7.03 (t, J = 7.3 Hz, 1H), 5.18 (dd, J = 8.6, 3.1 Hz, 0.50H), 4.75 (dd, J = 8.3, 4.3 Hz, 0.50H), 4.04–3.83 (m, 2H), 3.76 (s, 1.50H), 3.69 (s, 1.50H), 2.42–2.26 (m, 1H), 2.25–1.94 (m, 3H).

Into a 100 mL round bottom flask, **15b** (100 mg, 0.22 mmol), LiOH (27 mg, 1.1 mmol), and 30 mL of 1:1 acetonitrile/water were added. It was stirred at room temperature overnight. The pH-value of the solution was adjusted to between 4 and 7 by adding 1 M HCl. The precipitated solid was filtered and washed with a small amount of ethyl acetate to provide the title product **17** (95 mg, 98% yield). An orange solid as a 4:6 mixture of rotamers: m.p. 168–169 °C. ^{1}H NMR (600 MHz, CD_3OD) δ 9.04 (d, J = 1.5 Hz, 0.60H), 8.89 (d, J = 1.6 Hz, 0.40H), 8.62 (dd, J = 8.3, 2.0 Hz, 1H), 8.33 (dd, J = 8.3, 2.1 Hz, 1H), 8.21 (d, J = 8.3 Hz, 1H), 8.07 (d, J = 8.2 Hz, 1H), 7.77 (d, J = 8.6 Hz, 1H), 7.71–7.60 (m,, 3H), 7.46 (d, J = 8.2 Hz, 2H), 7.33–7.28(m, 2H), 7.05 (t, J = 7.3 Hz, 1H), 5.14 (dd, J = 8.5, 3.3 Hz, 0.4H), 4.68 (dd, J = 8.4, 4.9 Hz, 0.6H), 3.92 (t, J = 6.4 Hz, 1H), 3.88–3.72 (m, 1H), 2.47–2.37 (m, 1H), 2.26–2.00 (m, 3H); ^{13}C NMR (151 MHz, CD_3OD) δ 175.8, 174.9, 166.2, 163.8, 155.0, 154.9, 149.8, 146.5, 144.9, 143.6, 142.9, 142.1, 141.3, 140.9, 140.3, 140.3, 139.9, 138.1, 130.6, 129.9, 129.2, 129.1, 128.8, 126.6, 125.7, 124.1, 124.0, 120.6, 120.5, 120.4, 62.9, 61.7, 51.1, 32.7, 30.2, 26.5, 23.2; LC-MS calcd. for $C_{24}H_{23}N_4O_4^+$ (m/e), 431.17, obsd. 431.2 (M+H). The purity of the compound **17** was 99.6% by HPLC.

4.4. Procedure for the preparation of (S)-1-(6-(4-(3-phenylureido) phenyl)nicotinoyl)pyrrolidine-2-carboxylic acid (**18**)

A mixture of 6-bromonicotinic acid (**11c**, 1.01 g, 5.0 mmol), (*S*)-methyl pyrrolidine-2-carboxylate (0.71 g,5.5 mmol), TBTU (1.77 g, 5.5 mmol) and DIPEA (1.65 mL, 10.0 mmol) in 15 mL of DMF were stirred at room temperature for 12 h, then DMF was removed by rotary evaporation. To the residue, 50 mL of water and 50 mL of ethyl acetate were added, extracted with ethyl acetate (50 mL × 3). The extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give a residue that was subjected to silica gel chromatography to afford **12c** as a white powder (1.61 g, 93% yield). tetrakis(triphenylphosphine) palladium^{1}H NMR (300 MHz, $CDCl_3$) δ 8.97 (s, 1H), 8.35 (d, J = 8.5 Hz, 2H), 8.22 (d, J = 8.5 Hz, 2H), 8.07 (d, J = 8.2 Hz, 1H), 7.88 (d, J = 8.1 Hz, 1H), 4.72 (dd, J = 7.9, 4.4 Hz, 1H), 3.81 (s, 3H), 3.77–3.60 (m, 2H), 2.44–2.32 (m, 1H), 2.14–1.94 (m, 3H).

To a solution of **13c** (1.3 g, 3.7 mmol) in 30 mL ethanol at 0 °C under N_2, 10% Pd/C (130 mg) and $NaBH_4$ (560 mg, 14.8 mmol, portion-wise) were added. Then it was warmed to room temperature and stirred for another 6 h. To the reaction mixture, 1 M HCl was added until no bubbles come out. EtOH was removed through rotary evaporation, 50 mL of water and 50 mL of ethyl acetate was added to the residue, extracted with ethyl acetate (50 mL × 3). The extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give a residue that was subjected to silica gel chromatography to afford **14c** (926 mg, 77% yield). To a solution of **14c** (100 mg, 0.31 mmol) and phenyl isocyanate (40 mg, 0.34 mmol) in dry THF (10 mL), triethylamine (0.12 mL, 0.68 mmol) was added. The mixture was stirred at 40 °C for 2 h, concentrated and purified by a flash column (20–50% EtOAc in petroleum ether) to provide **15c** (131 mg, 95% yield). Into a 100 mL round bottom flask, **15c** (100 mg, 0.22 mmol), LiOH (27 mg, 1.1 mmol), and 30 mL of 1:1 acetonitrile/water were added. It was stirred at room temperature overnight. The pH-value of the solution was adjusted to between 4 and 7 by adding 1 M HCl. The precipitated solid was filtered and washed with a small amount of ethyl acetate to provide the title product **18** (90 mg, 93% yield). A light yellow solid as a 1:3 mixture of rotamers: m. p. 212–213 °C. ^{1}H NMR (400 MHz, DMSO-D_6) δ 9.54 (s, 1H), 9.29 (s, 1H), 8.80 (s, 0.72H), 8.68 (s, 0.26H), 8.19–7.97 (m, 4H), 7.65 (d, J = 8.2 Hz, 2H), 7.49 (d, J = 7.8 Hz, 2H), 7.29 (t, J = 7.6 Hz, 2H), 6.98 (t, J = 7.2 Hz, 1H), 4.55–4.51 (m, 0.26H), 4.48–4.41 (m, 0.72H), 3.62 (s, 2H), 2.36–2.24 (m, 1H), 2.06–1.79 (m, 3H); ^{13}C NMR (101 MHz, DMSO-D_6) δ 173.6, 173.1, 166.6, 165.4, 156.0, 155.5, 152.5, 146.5, 142.2, 139.7, 137.9, 137.2, 130.1, 129.2, 129.1, 128.9, 128.0, 127.9, 122.0, 120.0, 118.2, 117.9, 60.7, 59.3, 49.5, 46.8, 31.2, 29.0, 25.1, 22.4; LC-MS calcd. for $C_{24}H_{23}N_4O_4^+$ (m/e), 431.17, obsd. 431.2 (M+H). The purity of the compound **18** was 99.6% by HPLC.

4.5. Procedure for the preparation of (S)-1-(5-(4-(3-phenylureido) phenyl)pyrimidine-2-carbonyl)pyrrolidine-2-carboxylic acid (**19**)

A mixture of 5-bromopyrimidine-2-carboxylic acid (**11d**, 0.51 g, 2.5 mmol), (*S*)-methyl pyrrolidine-2-carboxylate (0.36 g, 2.75 mmol), TBTU (0.89 g, 2.75 mmol) and DIPEA (0.83 mL, 5.0 mmol) in 10mL of DMF was stirred at room temperature for 12 h, then DMF was removed by rotary evaporation. To the residue, 40 mL of water and 40 mL of ethyl acetate were added, extracted with ethyl acetate (40 mL × 3). The extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give a residue that was subjected to silica gel chromatography to afford **12d** (691 mg, 88% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (s, 1H), 8.87 (s, 1H), 4.87 (dd, J = 8.5, 2.8 Hz, 0.5H), 4.75 (dd, J = 8.5, 3.8 Hz, 0.5H), 3.98–3.72 (m, 3.5H), 3.68 (s, 1.5H), 3.36–2.26 (m, 1H), 2.24–1.95 (m, 3H).

A solution of **12d** (600 mg, 1.9 mmol), 4-nitrophenylboronic acid pinacol ester (523 mg, 2.1 mmol), KF (0.22 g, 3.8 mmol), and tetrakis(triphenylphosphine) palladium (46 mg, 0.04 mmol) in toluene/ethanol/H_2O (4/2/1 ratio, 30 mL) was heated to 80 °C for 12 h, cooled to room temperature, filtered through Celite, washed with ethyl acetate (3 × 40 mL), concentrated and purified on a silica gel chromatography to afford **13d** (534 mg, 79% yield). To a solution of **13d** (500 mg, 1.4 mmol)in 20 mL of ethanol at 0 °C under N_2, 10% Pd/C (50 mg) and $NaBH_4$ (212 mg, 5.6 mmol, portion-wise) were added. Then it was warmed to room temperature and stirred for another 6 h. To the reaction mixture, 1 M HCl was added until no bubbles come out. Ethanol was removed through rotary evaporation, 30 mL of water and 30 mL of ethyl acetate was added to residue, extracted with ethyl acetate (30 mL × 3). The extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give a residue that was subjected to silica gel chromatography to afford **14d** (324 mg, 71% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.96 (s, 1H), 8.92 (s, 1H), 7.43 (dd, J = 8.5, 1.9 Hz, 2H), 6.80 (dd, J = 8.4, 3.5 Hz, 2H), 4.93 (dd, J = 8.5, 2.9 Hz, 0.5H), 4.76 (dd, J = 8.5, 3.9 Hz, 0.5H), 4.02–3.76 (m, 5.5H), 3.68 (s, 1.5H), 2.36–2.23 (m, 1H), 2.21–1.97 (m, 3H).

To a solution of **14d** (100 mg, 0.31 mmol) and phenyl isocyanate (40 mg, 0.34 mmol) in dry THF (10 mL), triethylamine (0.12 mL, 0.68 mmol) was added. The mixture was stirred at 40 °C for 2 h, concentrated and purified by a flash column (20–50% EtOAc in petroleum ether) to provide **15d** (121 mg, 88% yield). Into a 100 mL round bottom flask, **15d** (100 mg, 0.22 mmol), LiOH (27 mg,

1.1 mmol), and 30 mL of 1:1 acetonitrile/water were added. It was stirred at room temperature overnight. The pH-value of the solution was adjusted to between 4 and 7 by adding 1 M HCl. The precipitated solid was filtered and washed with a small amount of ethyl acetate to provide the title product **19** (84 mg, 89% yield). A light yellow solid as a 1:1 mixture of rotamers: m.p. 196–197 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 9.17–9.07 (m, 2H), 7.77–7.61 (m, 4H), 7.45 (d, J = 7.8 Hz, 2H), 7.30 (t, J = 7.7 Hz, 2H), 7.03 (t, J = 7.6 Hz, 1H), 5.14–5.06 (m, 0.5H), 4.70–4.59 (m, 0.5H), 3.94–3.54 (m, 2H), 2.37 (s, 1H), 2.26–1.90 (m, 3H); ^{13}C NMR (126 MHz, DMSO-D_6) δ 173.5, 173.0, 163.8, 163.7, 159.3, 158.8, 154.2, 153.8, 152.4, 141.0, 139.5, 132.7, 132.5, 128.8, 127.6, 127.5, 126.2, 126.0, 122.0, 118.6, 118.3, 60.3, 58.7, 48.3, 47.1, 31.1, 28.9, 24.7, 22.1; LC-MS calcd. for $C_{23}H_{22}N_5O_4^+$ (*m/e*), 432.17, obsd. 430.2 (M+H). The purity of the compound **19** was 99.3% by HPLC.

4.6. Procedure for the preparation of (R)-1-(5-(4-(3-phenylureido) phenyl)picolinoyl)pyrrolidine-2-carboxylic acid (*20*)

A mixture of 5-bromopyridine-2-carboxylic acid (**11b**, 1.01 g, 5.0 mmol), (*R*)-methyl pyrrolidine-2-carboxylate (0.71 g,5.5 mmol), TBTU (1.77 g, 5.5 mmol) and DIPEA (1.65 mL, 10.0 mmol) in 15mL of DMF was stirred at room temperature for 12 h, then DMF was removed by rotary evaporation. To the residue, 50 mL water and 50 mL ethyl acetate was added, extracted with ethyl acetate (50 mL × 3). The extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give a residue that was subjected to silica gel chromatography to afford **12e** (1.66 g, 96% yield). A solution of **12e** (1.5 g, 4.2 mmol), 4-nitrophenylboronic acid pinacol ester (1.14 g, 4.6 mmol), KF (0.49 g, 8.4 mmol), and tetrakis(triphenylphosphine) palladium (92 mg, 0.08 mmol) in toluene/ethanol/H_2O (4/2/1 ratio, 40 mL) was heated to 80 °C for 12 h, cooled to room temperature, filtered through Celite, washed with ethyl acetate (3 × 40 mL), concentrated and purified on a silica gel chromatography to afford **13e** (1.42 g, 87% yield). To a solution of **13e** (1.3 g, 3.7 mmol) in 30 mL ethanol at 0 °C under N_2, 10% Pd/C (130 mg) and $NaBH_4$ (560 mg, 14.8 mmol, portion-wise) were added. Then it was warmed to room temperature and stirred for another 6 h. To the reaction mixture, 1 M HCl was added until no bubbles come out. EtOH was removed through rotary evaporation, 50 mL of water and 50 mL of ethyl acetate was added to residue, extracted with ethyl acetate (50 mL × 3). The extracts were dried over Na_2SO_4 and concentrated *in vacuo* to give a residue that was subjected to silica gel chromatography to afford **14e** (986 mg, 82% yield). To a solution of **14e** (100 mg, 0.31 mmol) and phenyl isocyanate (40 mg, 0.34 mmol) in dry THF (10 mL), trimethylamine (0.12 mL, 0.68 mmol) was added. The mixture was stirred at 40 °C for 2 h, concentrated and purified by a flash column (20–50% EtOAc in petroleum ether) to provide **15e** (128 mg, 93% yield). Into a 100 mL round bottom flask, **15e** (100 mg, 0.22 mmol), LiOH (27 mg, 1.1 mmol), and 30 mL of 1:1 acetonitrile/water were added. It was stirred at room temperature overnight. The pH-value of the solution was adjusted to between 4 and 7 by adding 1 M HCl. The precipitated solid was filtered and washed with a small amount of ethyl acetate to provide the title product **20** (95 mg, 98% yield). A light yellow solid as a 1:1 mixture of rotamers: m.p. 139–140 °C. ^{1}H NMR (400 MHz, DMSO-D_6) δ 12.51 (s, 1H), 8.98 (s, 1H), 8.94 (s, 0.5H), 8.83 (s, 1H), 8.80 (s, 0.5H), 8.19 (s, 1H), 7.92 (d, J = 8.1 Hz, 1H), 7.82 (d, J = 7.9 Hz, 1H), 7.74 (t, J = 8.3 Hz, 2H), 7.61 (d, J = 5.9 Hz, 2H), 7.47 (d, J = 7.6 Hz, 2H), 7.29 (t, J = 7.3 Hz, 2H), 6.98 (t, J = 6.7 Hz, 1H), 5.24–5.10 (m, 1H), 4.53–4.37 (m, 1H), 3.86 (d, J = 5.4 Hz, 1H), 3.73–3.59 (m, 1H), 2.36–2.20 (m, 1H), 2.08–1.76 (m, 3H); ^{13}C NMR (101 MHz, DMSO-D_6) δ 173.9, 173.3, 165.2, 164.9, 152.5, 151.1, 145.5, 144.6, 140.5, 139.6, 136.3, 136.3, 134.2, 134.1, 129.3, 129.1, 128.8, 127.5, 127.5, 124.3, 124.0, 122.0, 118. 6, 118.3, 60.7, 59.7, 49.4, 47.9, 31.3, 28.5, 25.1, 21.7; LC-MS calcd. for $C_{24}H_{23}N_4O_4^+$ (*m/e*), 431.17, obsd. 431.2 (M+H). The purity of the compound **20** was 99.9% by HPLC.

4.7. General procedure for the preparation of *21*–*30*

To a solution of corresponding aniline (0.46 mmol) and triphosgene (136 mg, 0.46 mmol) in dry DCM, triethylamine (0.64 mL, 4.6 mmol) was added, and the mixture was stirred for 10 min at room temperature. Without further purification, **14b** (100 mg, 0.31 mmol) was added, the reaction solution was stirred at 40 °C for 2 h, concentrated and purified by a flash column (20–50% EtOAc in petroleum ether) to provide **15f**–**q**. Into a 50 mL round bottom flask, **15f**–**q** (0.23 mmol), LiOH (27 mg, 1.1 mmol), and 20 mL of 1:1 acetonitrile/water were added. It was stirred at room temperature overnight. The pH-value of the solution was adjusted to between 4 and 7 by adding 1 M HCl. The precipitated solid was filtered and washed with a small amount of ethyl acetate to provide the title product **21**–**30**. (107 mg, 93% yield).

4.7.1. (S)-1-(5-(4-(3-(2-(Trifluoromethyl)phenyl)ureido)phenyl) picolinoyl)pyrrolidine-2-carboxylic acid (*21*)

A light yellow solid as a 1:1 mixture of rotamers: m.p. 141–142 °C. ^{1}H NMR (500 MHz, DMSO-D_6) δ 12.53 (s, 1H), 9.59 (s, 1H), 8.94 (d, J = 1.7 Hz, 0.5H), 8.80 (d, J = 1.9 Hz, 0.5H), 8.20 (ddd, J = 8.0, 5.6, 2.2 Hz, 1H), 8.16 (s, 1H), 7.96 (d, J = 7.1 Hz, 1H), 7.92 (d, J = 8.3 Hz, 0.5H), 7.83 (d, J = 8.2 Hz, 0.5H), 7.77 (dd, J = 10.9, 8.7 Hz, 2H), 7.69 (d, J = 7.9 Hz, 1H), 7.67–7.53 (m, 4H), 7.30 (t, J = 7.6 Hz, 1H), 5.17 (dd, J = 8.6, 3.4 Hz, 0.5H), 4.51–4.42 (m, 0.5H), 3.86 (t, J = 6.5 Hz, 1H), 3.71–3.63 (m, 1H), 2.34–2.20 (m, 1H), 2.07–1.77 (m, 3H); ^{13}C NMR (126 MHz, DMSO-D6) δ 173.9, 173.3, 165.2, 164.9, 152.4, 151.5, 151.2, 145.5, 144.6, 140.2, 136.3, 136.2, 134.3, 134.2, 133.0, 132.1, 132.1,131.5, 131.5, 129.7, 129.5, 128.8, 128.7, 127.6, 127.6,126.0 (q, J = 5.4 Hz), 125.9, 125.1, 124.3, 124.0, 124.0 (q, J = 272.9 Hz), 123.9, 1202, 120.1 (q, J = 31.6 Hz), 120.0, 118.6, 60.7, 59.7, 49.4, 47.9, 31.3, 28.5, 25.1, 21.7; LC-MS calcd. for $C_{25}H_{22}F_3N_4O_4$+ (m/e), 499.16, obsd. 499.2 (M+H). The purity of the compound **21** was 95.7% by HPLC.

4.7.2. (S)-1-(5-(4-(3-(3-(Trifluoromethyl)phenyl)ureido)phenyl) picolinoyl)pyrrolidine-2-carboxylic acid (*22*)

Yellow solid as a 1:1 mixture of rotamers: m.p. 146–147 °C. ^{1}H NMR (400 MHz, DMSO-D6) δ 12.51 (s, 1H), 9.18 (s, 1H), 9.07 (s, 1H), 8.94 (d, J = 2.1 Hz, 0.5H), 8.80 (d, J = 2.0 Hz, 0.5H), 8.19 (ddd, J = 8.4, 6.1, 2.3 Hz, 1H), 8.04 (s, 1H), 7.92 (d, J = 8.3 Hz, 0.5H), 7.83 (d, J = 8.2 Hz, 0.5H), 7.79–7.71 (m, 2H), 7.67–7.57 (m, 3H), 7.52 (t, J = 7.9 Hz, 1H), 7.32 (d, J = 7.6 Hz, 1H), 5.17 (dd, J = 8.6, 3.5 Hz, 0.5H), 4.50–4.42 (m, 0.5H), 3.87 (t, J = 6.5 Hz, 1H), 3.73–3.61 (m, 1H), 2.35–2.20 (m, 1H), 2.08–1.78 (m, 3H); ^{13}C NMR (101 MHz, DMSO-D_6) δ 174.0, 173.4, 165.2, 165.0, 152.5, 151.5, 151.3, 145.5, 144.7, 140.5, 140.1, 136.3, 136.2, 134.3, 134.2, 130.0,129.7, 129.6 (q, J = 31.4 Hz), 129.6, 127.5, 127.5, 124.3, 124.3 (q, J = 272.2 Hz), 124.0, 122.0, 118.9, 118.2 (q, J = 3.9 Hz), 114.2 (q, J = 3.9 Hz), 60.8, 59.8, 49.4, 47.9, 31.4, 28.5, 25.1, 21.7; LC-MS calcd. for $C_{25}H_{22}F_3N_4$-O_4+ (m/e), 499.16, obsd. 499.2 (M+H). The purity of the compound **22** was 95.4% by HPLC.

4.7.3. (S)-1-(5-(4-(3-(4-(Trifluoromethyl)phenyl)ureido)phenyl) picolinoyl)pyrrolidine-2-carboxylic acid (*23*)

Light yellow solid as a 1:1 mixture of rotamers: m.p. 139–140 °C. ^{1}H NMR (500 MHz, DMSO-D_6) δ 9.31 (s, 1H), 9.16 (s, 1H), 8.94 (s, 0.5H), 8.80 (d, J = 1.7 Hz, 0.5H), 8.25–8.16 (m, 1H), 7.92 (d, J = 8.2 Hz, 0.5H), 7.83 (d, J = 8.2 Hz, 0.5H), 7.80–7.73 (m, 2H), 7.71–7.60 (m, 6H), 5.17 (dd, J = 8.6, 3.4 Hz, 0.5H), 4.53–4.37 (m, 0.5H), 3.86 (t, J = 6.0 Hz, 1H), 3.71–3.62 (m, 1H), 2.37–2.21 (m, 1H), 2.08–1.77 (m, 3H); ^{13}C NMR (126 MHz, DMSO-D_6) δ 173.9,

173.3, 165.1, 164.9, 152.3, 151.4, 151.2, 145.5, 144.7, 143.4, 140.1, 136.3, 136.2, 134.3, 134.2, 129.7, 129.6, 127.6, 127.5, 126.1 (q, J = 3.7 Hz), 124.6 (q, J = 270.7 Hz), 124.3, 124.0, 121.9 (q, J = 31.9 Hz), 118.8, 117.9, 60.7, 59.7, 49.4, 47.9, 31.3, 28.5, 25.1, 21.7; LC-MS calcd. for $C_{25}H_{22}F_3N_4O_4$+ (m/e), 499.16, obsd. 499.2 (M+H). The purity of the compound **23** was 99.0% by HPLC.

*4.7.4. (S)-1-(5-(4-(3-(2-Methoxyphenyl)ureido)phenyl)picolinoyl) pyrrolidine-2-carboxylic acid (**24**)*

Light yellow solid as a 1:1 mixture of rotamers: m.p. 149–150 °C. ^{1}H NMR (400 MHz, DMSO-D$_6$) δ 12.62 (s, 1H), 9.72 (s, 1H), 8.93 (s, 1H), 8.79 (s, 1H), 8.38 (s, 1H), 8.22–8.10 (m, 2H), 7.90 (d, J = 8.1 Hz, 1H), 7.82 (d, J = 8.1 Hz, 1H), 7.78–7.70 (m, 2H), 7.66–7.52 (m, 3H), 7.05–6.87 (m, 3H), 5.15 (d, J = 6.1 Hz, 0.52H), 4.45 (s, 0.46H), 3.92–3.81 (m, 4H), 3.70–3.59 (m, 1H), 2.34–2.19 (m, 1H), 2.08–1.73 (m, 3H); ^{13}C NMR (126 MHz, DMSO-D$_6$) δ 174.5, 173.9, 165.7165.6, 152.9, 151.9, 148.3, 145.9, 145.1, 141.2, 136.8, 136.6, 134.5, 129.6, 129.5, 129.1, 128.0, 127.9, 124.6, 124.4, 122.4, 121.0, 119.0, 118.8, 111.3, 61.5, 60.4, 56.2, 49.8, 48.2, 31.8, 29.0, 25.5, 22.2; LC-MS calcd. for $C_{25}H_{25}N_4O_5^+$ (m/e), 461.18, obsd. 461.2 (M+H). The purity of the compound **24** was 95.2% by HPLC.

*4.7.5. (S)-1-(5-(4-(3-(3-Methoxyphenyl)ureido)phenyl)picolinoyl) pyrrolidine-2-carboxylic acid (**25**)*

Light yellow solid as a 7:13 mixture of rotamers: m.p. 147–148 °C. ^{1}H NMR (500 MHz, DMSO-D$_6$) δ 10.14–9.80 (m, 1.15H), 9.67 (s, 0.34H), 9.54 (s, 0.34H), 8.92 (s, 0.35H), 8.74 (s, 0.65H), 8.17 (dd, J = 8.2, 1.8 Hz, 1H), 8.04 (d, J = 6.7 Hz, 1H), 7.81 (d, J = 8.2 Hz, 0.35H), 7.78 (d, J = 8.2 Hz, 0.65H),, 7.72 (d, J = 8.5 Hz, 0.75H), 7.62 (d, J = 8.5 Hz, 0.75H), 7.55 (d, J = 8.3 Hz, 1.25H), 7.49 (d, J = 8.2 Hz, 1.25H), 7.24 (s, 1H), 7.19–7.11(m, 1H), 7.05–6.93 (m, 1H), 6.52 (t, J = 9.3 Hz, 1H), 5.09 (dd, J = 8.2, 3.4 Hz, 0.65H), 4.46 (dd, J = 8.1, 3.9 Hz, 0.35H), 3.86 (t, J = 6.1 Hz, 1H), 3.72 (s, 3H), 3.69–3.58 (m, 1H), 2.33–2.15 (m, 1H), 2.11–1.80 (m, 3H); ^{13}C NMR (126 MHz, DMSO-D$_6$) δ 175.5, 173.9, 165.9, 165.1, 159.7, 159.6, 152.8, 152.7, 152.1, 151.6, 145.4, 144.6, 141.6, 141.2, 140.9, 136.3, 135.8, 134.0, 133.6, 129.5, 129.4, 129.1, 128.7, 127.4, 127.0, 123.9, 123.9, 118.5, 118.3, 110.6, 107.1, 106.9, 104.0, 103.9, 61.9, 60.2, 54.9, 54.9, 49.4, 47.6, 31.5, 28.7, 25.1, 22.1; LC-MS calcd. for $C_{25}H_{25}N_4O_5^+$ (m/e), 461.18, obsd. 461.1 (M+H). The purity of the compound **25** was 98.6% by HPLC.

*4.7.6. (S)-1-(5-(4-(3-(4-Methoxyphenyl)ureido)phenyl)picolinoyl) pyrrolidine-2-carboxylic acid (**26**)*

Light yellow solid as a 1:1 mixture of rotamers: m.p. 156–157 °C. ^{1}H NMR (500 MHz, DMSO-D$_6$) δ 12.49 (s, 1H), 8.93 (s, 0.5H), 8.85–8.75 (m, 0.5H), 8.55 (s, 1H), 8.18 (s, 1H), 7.92 (d, J = 7.9 Hz, 0.5H), 7.82 (d, J = 8.1 Hz, 0.5H), 7.79–7.66 (m, 2H), 7.61 (s, 2H), 7.37 (d, J = 8.0 Hz, 2H), 6.88 (d, J = 8.1 Hz, 2H), 5.17 (d, J = 5.2 Hz, 0.5H), 4.46 (s, 0.5H), 3.87 (s, 1H), 3.72 (s, 3H), 3.70–3.68 (m, 1H), 2.38–2.16 (m, 1H), 2.09–1.75 (m, 3H); ^{13}C NMR (126 MHz, DMSO-D$_6$) δ 174.4, 173.8, 165.6, 165.4, 155.1, 153.1, 151.8, 151.6, 145.9, 145.0, 141.1, 136.8, 136.8, 134.6, 134.5, 133.0, 129.6, 129.4, 127.9, 127.9, 124.7, 124.4, 120.6, 119.0, 114.5, 61.2, 60.2, 55.6, 49.9, 48.3, 31.8, 29.0, 25.6, 22.2; LC-MS calcd. for $C_{25}H_{25}N_4O_5^+$ (m/e), 461.18, obsd. 461.2 (M+H). The purity of the compound **26** was 99.9% by HPLC.

*4.7.7. (S)-1-(5-(4-(3-(2-Chlorophenyl)ureido)phenyl)picolinoyl) pyrrolidine-2-carboxylic acid (**27**)*

Light yellow solid as a 1:1 mixture of rotamers: m.p. 148–149 °C. ^{1}H NMR (400 MHz, DMSO-D$_6$) δ 9.86 (s, 1H), 8.95 (s, 1H), 8.81 (s, 1H), 8.49 (s, 1H), 8.29–8.10 (m, 2H), 7.93 (d, J = 8.2 Hz, 1H), 7.84 (d, J = 7.7 Hz, 1H), 7.77 (t, J = 8.6 Hz, 2H), 7.65 (d, J = 7.3 Hz, 2H), 7.47 (d, J = 8.0 Hz, 1H), 7.31 (t, J = 7.6 Hz, 1H), 7.05 (t, J = 7.4 Hz, 1H), 5.17 (d, J = 7.3 Hz, 0.5H), 4.46 (s, 0.5H), 3.87 (s, 1H), 3.67–3.55 (m, 1H), 2.38–2.19 (m, 1H), 2.09–1.78 (m, 3H); ^{13}C NMR (126 MHz, DMSO-D$_6$) δ 173.9, 173.3, 165.0, 164.9, 152.1, 151.2, 151.1, 145.4, 144.6, 140.3, 136.4, 136.2, 135.9, 134.3, 129.5, 129.4, 129.3, 127.6, 127.6, 124.3, 124.0, 123.5, 122.2, 121.5, 118.6, 67.0, 60.7, 59.7, 49.4, 47.9, 31.3, 28.5, 25.1, 21.7; LC-MS calcd. For $C_{24}H_{22}ClN_4O_4^+$ (m/e), 465.13, obsd. 465.2 (M+H). The purity of the compound **27** was 95.6% by HPLC.

*4.7.8. (S)-1-(5-(4-(3-(3-Chlorophenyl)ureido)phenyl)picolinoyl) pyrrolidine-2-carboxylic acid (**28**)*

Light yellow solid as a 1:1 mixture of rotamers: m.p. 137–138 °C. ^{1}H NMR (500 MHz, DMSO-D$_6$) δ 12.51 (s, 1H), 9.03 (s, 1H), 9.02 (s, 1H), 8.94 (d, J = 1.8 Hz, 0.5H), 8.80 (d, J = 1.8 Hz, 0.5H), 8.20 (ddd, J = 8.2, 5.9, 2.3 Hz, 1H), 7.92 (d, J = 8.3 Hz, 1H), 7.83 (d, J = 8.2 Hz, 1H), 7.80–7.69 (m, 3H), 7.62 (dd, J = 8.6, 3.7 Hz, 2H), 7.36–7.26 (m, 2H), 7.07–6.99 (m, 1H), 5.17 (dd, J = 8.6, 3.4 Hz, 0.5H), 4.49–4.44 (m, 0.5H), 3.87 (t, J = 5.8 Hz, 1H), 3.71–3.62 (m, 1H), 2.34–2.22 (m, 1H), 2.07–1.79 (m, 3H); ^{13}C NMR (126 MHz, DMSO-D$_6$) δ 173.9, 173.3, 165.1, 164.9, 152.3, 151.4, 151.2, 145.5, 144.6, 141.2, 140.2, 136.3, 136.2, 134.2, 134.2, 133.2, 130.4, 129.6, 129.5, 127.5, 127.5, 124.3, 124.0, 121.6, 118.8, 117.6, 116.7, 60.7, 59.7, 49.4, 47.9, 31.3, 28.5, 25.1, 21.7; LC-MS calcd. for $C_{24}H_{22}ClN_4O_4^+$ (m/e), 465.13, obsd. 465.2 (M+H). The purity of the compound **28** was 98.7% by HPLC.

*4.7.9. (S)-1-(5-(4-(3-(4-Chlorophenyl)ureido)phenyl)picolinoyl) pyrrolidine-2-carboxylic acid (**29**)*

Light yellow solid as a 1:1 mixture of rotamers: m.p. 135–136 °C. ^{1}H NMR (400 MHz, DMSO-D$_6$) δ 12.53 (s, 1H), 9.00 (s, 1H), 8.96 (s, 1H), 8.94 (d, J = 1.8 Hz, 0.5H), 8.80 (d, J = 1.9 Hz, 0.5H), 8.19 (ddd, J = 8.0, 4.2, 2.4 Hz, 1H), 7.92 (d, J = 8.3 Hz, 1H), 7.83 (d, J = 8.2 Hz, 1H), 7.75 (t, J = 8.7 Hz, 2H), 7.61 (dd, J = 8.7, 2.7 Hz, 2H), 7.51 (d, J = 8.8 Hz, 2H), 7.34 (d, J = 8.8 Hz, 2H), 5.17 (dd, J = 8.6, 3.4 Hz, 0.5H), 4.51–4.41 (m, 0.5H), 3.86 (t, J = 6.5 Hz, 1H), 3.71–3.60 (m, 1H), 2.35–2.19 (m, 1H), 2.07–1.76 (m, 3H); ^{13}C NMR (101 MHz, DMSO-D$_6$) δ 173.9, 173.3, 165.1, 164.9, 152.4, 151.4, 151.4, 151.2, 145.5, 144.6, 140.3, 138.6, 136.3, 136.2, 134.2, 134.1, 129.5, 129.3, 128.7, 127.5, 127.5, 125.5, 124.3, 124.0, 119.8, 118.7, 60.7, 59.7, 49.4, 47.9, 31.3, 28.5, 25.1, 21.7; LC-MS calcd. For $C_{24}H_{22}ClN_4O_4^+$ (m/e), 465.13, obsd. 465.2 (M+H). The purity of the compound **29** was 99.9% by HPLC.

*4.7.10. (S)-1-(5-(4-(3-(3-Fluorophenyl)ureido)phenyl)picolinoyl) pyrrolidine-2-carboxylic acid (**30**)*

Light yellow solid as a 1:1 mixture of rotamers: m.p. 169–170 °C. ^{1}H NMR (400 MHz, DMSO-D$_6$) δ 10.13 (s, 1H), 10.05 (s, 1H), 8.93 (s, 0.5H), 8.79 (s, 0.5H), 8.20 (s, 1H), 7.90 (s, 0.5H), 7.86–7.67 (m, 2.5 H), 7.61 (s, 2H), 7.51 (d, J = 11.2 Hz, 1H), 7.29 (s, 1H), 7.11 (s, 1H), 6.75 (s, 1H), 5.16 (s, 0.5 H), 4.45 (s, 0.5H), 3.85 (s, 1H), 3.65 (s, 1H), 2.24 (s, 1H), 2.07–1.75 (m, 3H); 13CNMR (126 MHz, DMSO-D$_6$) δ 173.7, 173.1, 164.9, 164.8, 162.3 (d, J = 240.1 Hz), 152.5, 151.0, 150.9, 145.2, 144.4, 141.7, 141.6, 140.4, 136.3, 136.2, 134.2, 134.2, 130.3 (d, J = 9.4 Hz), 129.0, 128.9, 127.4 (d, J = 7.7 Hz), 124.1, 123.9, 118.1, 113.4, 107.9 (d, J = 21.0 Hz), 104.3 (d, J = 26.4 Hz), 60.6, 59.6, 49.3, 47.8, 31.3, 28.4, 25.0, 21.6; LC-MS calcd. For $C_{24}H_{22}FN_4O_4^+$ (m/e), 449.16, obsd. 449.2 (M+H). The purity of the compound **30** was 96.2% by HPLC.

*4.8. Procedure for preparation of **31** and **32***

Compounds **31** and **32** were prepared by using the same procedure as described for compound **17** substituting butyl isocyanate for and isocyanatocyclohexane for phenyl isocyanate.

*4.8.1. (S)-1-(5-(4-(3-Butylureido)phenyl)picolinoyl)pyrrolidine-2-carboxylic acid (**31**)*

Light yellow solid as a 1:1 mixture of rotamers: m.p. 183–184 °C. ^{1}H NMR (400 MHz, DMSO-D_6) δ 12.51 (s, 1H), 8.91 (s, 0.5H), 8.77 (s, 0.5H), 8.65 (s, 1H), 8.20–8.10 (m, 1H), 7.91 (d, J = 8.4 Hz, 0.5H), 7.81 (d, J = 8.1 Hz, 0.5H), 7.68 (t, J = 8.8 Hz, 2H), 7.55 (d, J = 6.3 Hz, 2H), 6.22 (t, J = 5.3 Hz, 1H), 5.17 (dd, J = 8.4, 3.1 Hz, 0.5H), 4.48–4.42 (m, 0.5H), 3.87 (t, J = 6.1 Hz, 1H), 3.65 (t, J = 7.2 Hz, 1H), 3.10 (q, J = 6.3 Hz, 2H), 2.37–2.21 (m, 1H), 2.07–1.76 (m, 3H), 1.47–1.38 (m, 2H), 1.37–1.27 (m, 2H); ^{13}C NMR (101 MHz, DMSO-D_6) δ 174.0, 173.3, 165.2, 165.0, 155.1, 151.2, 151.0, 145.4, 144.5, 141.4, 136.5, 136.4, 134.0, 133.9, 128.4, 128.2, 127.4, 127.3, 124.3, 124.0, 118.0, 60.8, 59.7, 49.4, 47.9, 38.8, 31.9, 31.3, 28.5, 25.2, 21.7, 19.6, 13.7; LC-MS calcd. For $C_{22}H_{27}N_4O_4^+$ (m/e), 411.20, obsd. 411.3 (M+H). The purity of the compound **31** was 99.9% by HPLC.

*4.8.2. (S)-1-(5-(4-(3-Cyclohexylureido)phenyl)picolinoyl)pyrrolidine-2-carboxylic acid (**32**)*

Light yellow solid as a 1:1 mixture of rotamers: m.p. 141–142 °C. ^{1}H NMR (400 MHz, DMSO-D_6) δ 8.91 (s, 0.5H), 8.77 (s, 0.5H), 8.60 (s, 1H), 8.16 (s, 1H), 7.91 (s, 0.5H), 7.82 (s, 0.5H), 7.68 (s, 2H), 7.53 (s, 2H), 6.20 (s, 1H), 5.17 (s, 1H), 4.45 (s, 1H), 3.86 (s, 1H), 3.65 (s, 1H), 3.48 (s, 1H), 2.24 (s, 1H), 2.06–1.52 (m, 9H), 1.38–1.08 (m, 5H); ^{13}C NMR (126 MHz, DMSO-D_6) δ 173.9, 173.3, 165.1, 164.9, 154.3, 151.1, 150.9, 145.3, 144.5, 141.4, 136.5, 136.4, 134.0, 134.0, 128.3, 128.2, 127.4, 127.3, 124.3, 124.0, 117.9, 60.7, 59.7, 49.4, 47.9, 47.6, 32.9, 31.3, 28.5, 25.3, 25.1, 24.4, 21.7; LC-MS calcd. for $C_{24}H_{29}N_4O_4^+$ (m/e), 437.22, obsd. 437.2 (M+H). The purity of the compound **32** was 99.9% by HPLC.

*4.9. Procedure for preparation of (S)-1-(5-(4-(3-phenylthioureido) phenyl)picolinoyl)pyrrolidine-2-carboxylic acid (**34**)*

Compound **34** was prepared by using the same procedure as described for compound **17** substituting phenyl isothiocyanate for phenyl isocyanate. Light yellow solid as a 1:1 mixture of rotamers: m.p. 114–115 °C. ^{1}H NMR (400 MHz, DMSO-D_6) δ 12.53 (s, 1H), 10.01 (s, 1H), 9.96 (s, 1H), 8.97 (d, J = 2.0 Hz, 1H), 8.83 (d, J = 2.1 Hz, 1H), 8.27–8.19 (m, 1H), 7.95 (d, J = 8.3 Hz, 0.5H), 7.85 (d, J = 8.2 Hz, 0.5H), 7.79 (t, J = 8.8 Hz, 2H), 7.69 (d, J = 7.5 Hz, 2H), 7.51 (d, J = 8.2 Hz, 2H), 7.36 (t, J = 7.8 Hz, 2H), 7.15 (t, J = 7.4 Hz, 1H), 5.18 (dd, J = 8.7, 3.4 Hz, 0.5H), 4.51–4.42 (m, 0.5H), 3.88 (t, J = 6.5 Hz, 1H), 3.72–3.61 (m, 1H), 2.36–2.21 (m, 1H), 2.08–1.79 (m, 3H); ^{13}C NMR (126 MHz, DMSO-D_6) δ 179.5, 173.9, 173.3, 165.1, 164.9, 151.7, 151.5, 145.7, 144.9, 140.2, 140.2,139.4, 136.2, 136.1, 134.6, 134.5, 131.8, 131.6, 128.5, 127.1, 127.1, 124.5, 124.3, 124.0, 123.6, 60.7, 59.7, 54.9, 49.4, 47.9, 31.3, 28.5, 25.1, 21.7; LC-MS calcd. for $C_{24}H_{23}N_4O_3S^+$ (m/e), 447.15, obsd. 447.2 (M+H). The purity of the compound **34** was 97.4% by HPLC.

*4.10. (S)-1-(5-(4-(2-(3-(Trifluoromethyl)phenyl)acetamido)phenyl) picolinoyl)pyrrolidine-2-carboxylic acid (**36**)*

A solution of **14b** (100 mg, 3.1 mmol), m-(trifluoromethyl) phenylacetic acid (70 mg, 0.34 mmol), TBTU (109 mg, 0.34 mmol) and DIPEA (1.02 mL, 6.2 mmol) in 10mL of DMF were stirred at room temperature for 12 h, then DMF was removed by rotary evaporation. To the reaction mixture, 50 mL of water and 50 mL of ethyl acetate were added to residue, extracted with ethyl acetate (50 mL × 3). The extracts were dried over Na2SO4 and concentrated in vacuo to give a residue that was subjected to silica gel chromatography to afford **35** (140 mg, 88% yield). Into a 50 mL round bottom flask, **35** (113 mg, 0.22 mmol), LiOH (27 mg, 1.1 mmol), and 20 mL of 1:1 acetonitrile/water were added. It was stirred at room temperature overnight. The pH-value of the solution was adjusted to between 4 and 7 by adding 1 M HCl. The precipitated solid was filtered and washed with a small amount of diethyl ether to provide the title product 36 (102 mg, 93% yield). Light yellow solid as a 1:1 mixture of rotamers: m.p. 154–155 °C. ^{1}H NMR (400 MHz, DMSO-D_6) δ 10.55 (s, 1H), 8.94 (s, 0.5H), 8.80 (s, 0.5H), 8.19 (d, J = 6.4 Hz, 1H), 7.92 (d, J = 8.4 Hz, 0.5H), 7.83 (d, J = 8.2 Hz, 0.5H), 7.81–7.70 (m, 5H), 7.69–7.51 (m, 4H), 5.16 (d, J = 5.6 Hz, 0.5H), 4.46 (d, J = 4.9 Hz, 0.5H), 3.84 (s, 3H), 3.71–3.59 (m, 1H), 2.37–2.16 (m, 1H), 2.11–1.73 (m, 3H); ^{13}C NMR (126 MHz, DMSO-D_6) δ 173.9, 173.3, 168.8, 165.1, 164.9, 151.5, 151.4, 145.6, 144.7, 139.7, 137.2, 136.2, 136.1, 134.4, 134.4, 133.5, 132.1, 131.5, 131.5, 130.8, 130.7, 129.3, 129.0 (q, J = 31.4 Hz), 128.8, 128.7, 127.5, 127.4, 125.9 (q, J = 2.7 Hz), 124.3 (q, J = 271.6 Hz), 124.3, 124.0, 123.4 (q, J = 3.7 Hz), 123.2, 119.6, 60.7, 59.7, 49.4, 47.9, 42.6, 31.3, 28.5, 25.1, 21.7; LC-MS calcd. for $C_{26}H_{23}F_3N_3O_4^+$ (m/e), 498.16, obsd. 498.2 (M+H). The purity of the compound **36** was 95.7% by HPLC.

*4.11. (S)-1-(5-(4-(2-Oxo-2-((3-(trifluoromethyl)phenyl)amino)ethyl) phenyl)picolinoyl)pyrrolidine-2-carboxylic acid (**40**)*

A solution of 4-bromophenylacetic acid (1.08 g, 5.0 mmol), 3-(trifluoromethyl)aniline (0.85 g, 5.3 mmol), TBTU (1.70 g, 5.3 mmol) and DIPEA (1.65 mL, 10.0 mmol) in 10 mL of DMF was stirred at room temperature for 12 h, then DMF was removed by rotary evaporation. To the residue, 50 mL of water and 50 mL of ethyl acetate were added, extracted with ethyl acetate (3 × 50 mL), concentrated and purified on a silica gel chromatography to afford **37**. A solution of **37** (1.50 g, 4.2 mmol), bis(pinacolato)diboron (1.12 g, 4.4 mmol), Pd(dppf)Cl2·CH2Cl2 (69 mg, 0.084 mg), and KOAc (0.82 g, 8.4 mmol) in DMF was stirred at 80 °C for 8 h, then DMF was removed by rotary evaporation. To the residue, 50 mL of water and 50 mL of ethyl acetate was added, extracted with ethyl acetate (2 × 50 mL), concentrated and purified on a silica gel chromatography to afford 38 (1.21 g, 71% yield). ^{1}H NMR (400 MHz, CDCl3) δ 7.73 (s, 1H), 7.65 (d, J = 7.7 Hz, 1H), 7.53 (d, J = 8.3 Hz, 2H), 7.41 (t, J = 8.0 Hz, 1H), 7.35 (d, J = 8.3 Hz, 1H), 7.27 (s, 1H), 7.21 (d, J = 8.3 Hz, 2H), 3.70 (s, 2H), 1.26 (s, 12H).

A solution of **38** (1.1 g, 2.7 mmol), **12b** (0.84 g, 2.7 mmol), KF (0.31 g, 5.4 mmol), and $Pd(PPh_3)_4$ (69 mg, 0.06 mmol) in toluene/ethanol/H_2O (4/2/1 ratio, 50 mL) was heated to 80 °C for 12 h, cooled to room temperature, filtered through Celite, washed with ethyl acetate (3 × 40 mL), concentrated and purified on a silica gel chromatography to afford **39** (0.86 g, 62% yield). Into a 50 mL round bottom flask, **39** (113 mg, 0.22 mmol), LiOH (27 mg, 1.1 mmol), and 30 mL of 1:1 acetonitrile/water was added. It was stirred at room temperature overnight. The pH-value of the solution was adjusted to between 4 and 7 by adding 1 M HCl. The precipitated solid was filtered and washed with a small amount of ethyl acetate to provide the product **40** (102 mg, 93% yield). Light yellow solid as a 1:1 mixture of rotamers: m.p. 173–174 °C. ^{1}H NMR (400 MHz, DMSO-D_6) δ 12.57 (s, 1H), 10.60 (s, 1H), 8.93 (s, 1H), 8.79 (s, 1H), 8.19 (t, J = 7.0 Hz, 1H), 8.10 (s, 1H), 7.91 (d, J = 8.1 Hz, 0.5H), 7.86–7.70 (m, 3.5H), 7.64–7.45 (m, 5H), 7.38 (d, J = 7.6 Hz, 1H), 5.13 (d, J = 5.5 Hz, 0.5H), 4.44 (d, J = 5.0 Hz, 0.5H), 3.83 (t, J = 7.6 Hz,1H), 3.74 (s, 2H), 3.67–3.58 (m, 1H), 2.31–2.18 (m, 1H), 2.04–1.77 (m, 3H); ^{13}C NMR (126 MHz, DMSO-D_6) δ 173.9, 173.3, 169.5, 165.1, 165.0, 152.0, 151.9, 146.0, 145.1, 140.0, 136.4, 136.3, 134.9 (q, J = 5.8 Hz), 134.7, 134.6, 133.12, 133.1, 132.3, 132.1, 131.5, 131.4, 130.1, 130.0, 129.5 (q, J = 31.4 Hz), 128.8, 128.7, 127.1, 127.0, 124.3, 124.1 (q, J = 272.5 Hz), 124.0, 122.6, 120.86, 119.6, 115.1, 60.8, 59.8, 49.4, 47.8, 42.9, 31.3, 28.5, 25.1, 21.7; LC-MS calcd. for $C_{26}H_{23}F_3N_3O_4^+$ (*m/e*), 498.16, obsd. 498.2 (M+H). The purity of the compound **40** was 95.1% by HPLC.

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4.12. In vitro DGAT enzyme assay

The recombinant full length human DGAT-1 protein were expressed in Sf9 insect cells for 48 h, and the microsome containing DGAT-1 enzyme was prepared by differential centrifugation[1,20] and stored at −80 °C for DGAT-1 enzyme assay.

The DGAT-1 inhibitory activities of compounds were determined by Phospholipid FlashPlate assay as described before.[26] In brief, the assay mixture containing 20 mM HEPES (pH 7.5), 2 mM $MgCl_2$, 0.04% BSA, 50 μM 1, 2-didecanoyl-*sn*-glycerol (DAG, Chyman), 5 μM palmitoyl-1-^{14}C coenzyme A (PerkinElmer) and test compounds (1 μL) dissolved in DMSO was added to each well of a phospholipid FlashPlate (PerkinElmer) in a total volume of 100 μL. The reaction was initiated by the addition of DGAT-1 enzyme (1.5 μg/well), followed by incubation for 60 min at 37 °C and was stopped by the addition of 100 μL isopropanol. Plates were sealed, incubated for 12 h and read on 1450 microbeta trilux microplate Scintillation and luminescence Counter (PerkinElmer).

4.13. Cell-based assays

Mouse 3T3-L1 preadipocytes, human colon carcinoma Caco-2 cells and hepatoma HepG2 cells were maintained in Dulbecco's modified Eagle medium (DMEM, Gibco) with 10% fetal bovine serum (FBS, Hyclone). The 3T3-L1 cells were treated as follows: two days after confluence, the differentiation of 3T3-L1 preadipocytes were induced by 1 μg/mL insulin (Sigma), 1 μM dexamethasone (Sigma), and 0.5 mM 3-isobutyl-1-methyl-xanthine (IBMX; Sigma) in DMEM with 10% FBS for 2 days. Then, the medium was changed to DMEM with 10% FBS containing 1 μg/mL insulin. Two days later, the medium was changed back to DMEM with 10% FBS and the cells were treated with test compounds for 4 days. The medium was renewed every 2 days. Then the cells in each group were subjected to Oil Red O staining and cellular triglyceride content determination. Following the treatment with test compounds for 2 h, Caco-2 cells and HepG2 cells were incubated in the absence or presence of oleic acid (OA, Sigma) for 48 h in DMEM with 10% FBS, respectively. Then oil red O staining and cellular triglyceride content determination experiments were performed as previously described.[35]

4.14. Permeability assay

The Caco-2 method was used to study permeability. Caco-2 cells (American Type Culture Collection) were cultured on Millicell-24 cell culture insert plates (Merck Millipore). For basolateral and apical compartment, pH value of transport buffer (HBSS/HEPES 10 mM/0.1% BSA) was adjusted to 7.4 and 6.8 respectively. Permeation time lasted 95 min. The kinetics were performed at 35 and 95 min. The test compounds were analysed by LC-MS/MS (Xevo TQ-S coupled to UPLC H—Class, Waters). The apparent permeability was calculated as follows: Papp (cm/s) = $\Delta Q/\Delta t * 1/(A * C0)$, where, $\Delta Q/\Delta t$ is the product quantity which appears in the receiver by time unit; A is the membrane area (cm^2); C0 is the initial concentration in the donor compartment (μM). The efflux ratio was the quotient of the Papp from apical side to the basolateral side divided by the Papp from basolateral side to the apical side.

4.15. In vivo assay

All animal care and experiments were permitted by Institutional Animal Care and Use Committees of Shanghai Institute of Materia Medica. For the acute lipid challenge study, 6-week-old male C57/KSJ mice (SLAC, 20 ± 2 g, n = 6) were fasted 16 h and then orally dosed with either vehicle (0.1% w/v Tween 80) or test compounds, then an olive oil bolus (15 mL/kg) was orally administered 2 h later, and the mice of control group were given equal volume water. The blood samples were collected and the serum triglyceride levels were subsequently measured by a commercially triglyceride assay kit (Shanghai Mind).

4.16. Pharmacokinetic study of **22** in SD rats

7 Sprague dawley male rats (200–220 g) were randomly divided into 2 groups, each group 4/3, intragastric or intravenous administration of the compounds respectively. The compound **22** were prepared with 5%DMSO, 5% Tween, and 80/90% physiological saline. Fasting 12 h before the test, free drinking water, after 2 h feeding. Pharmacokinetic parameters were calculated using a non-compartmental model of Phoenix 1.3 software (US Pharsight Corporation). The peak concentration C_{max} and the peak time T_{max} are measured; the area under the concentration time curve of AUC_{0-t} value was calculated by the trapezoidal method; $AUC_{0-\infty} = AUC_{0-t} + C_t/k_e$, C_t was the last blood concentration that can be measured, k_e was elimination rate constant; elimination half-life $t_{1/2} = 0.693/k_e$; mean residence time MRT = AUMC/AUC; clearance $CL = D/AUC_{0-\infty}$; steady-state distribution volume V_{ss}=CL × MRT; absolute bioavailability$F = (AUC_{i.g.} \times D_{i.v.})/(AUC_{i.v.} \times D_{i.g.}) \times 100\%$.

4.17. Tissue distribution of compound **22** in C57 mice

9 C57 male mice (18–24 g, 8 weeks old) were randomly divided into 3 groups. Oral administration, after anesthesia with isoflurane, 0.1 mL blood (from orbit) and tissue (liver, duodenum, jejunum and ileum) was taken, EDTAK2 anti-freezing, and p.o. group was collected at 1 h, 2 h, 5 h after giving **22**. Blood samples were collected on ice, centrifugal separation of plasma in 1 h. Collected plasma were stored at −80 °C; after weighing, adding water according to 1:10 (m/v), homogenating, tissues were kept at low temperature. 10 μL to 1.5 mL centrifuge tube, adding 100 μL internal standard solution (5 ng/mL vera pammy and 50 ng/mL glibenclamide acetonitrile solution), vortex 60 s and centrifugating for 3 min; the supernatant (75 μL for plasma, 50 μL tissue samples) were added to 96-well plates. The concentration of 22 in plasma was determined by LC/MS/MS (Agilent 1200, API 4000 QTRAP). The data acquisition and control system software is Analyst1.5.1 (Applied Biosystem). The peak integral mode of the sample is automatic integration; the ratio of the sample peak area and internal standard peak area is used as the index, and the concentration of the sample was regressed. Linear regression, the weight coefficient is 1/X2.

4.18. Statistical analysis

Values in figures were expressed as mean ± SEM. The comparison of different groups was assessed by one-way ANOVA followed by Tukey's multiple comparison test. It was considered to be statistically significant when a p value < 0.05.

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