Regular Article

Discovery and Biological Evaluation of Potent and Orally Active Human 11β-Hydroxysteroid Dehydrogenase Type 1 Inhibitors for the Treatment of Type 2 Diabetes Mellitus

Takanori Koike,* Ryota Shiraki, Daisuke Sasuga, Mitsuru Hosaka, Tomoaki Kawano, Hiroki Fukudome, Kazuo Kurosawa, Ayako Moritomo, Shinya Mimasu, Hirofumi Ishii, and Seiji Yoshimura

Drug Discovery Research, Astellas Pharma Inc.; 21 Miyukigaoka, Tsukuba, Ibaraki 305–8585, Japan.

Received March 4, 2019; accepted May 21, 2019

We synthesized and evaluated novel 5-[2-(thiophen-2-yl)propan-2-yl]-4H-1,2,4-triazole derivatives as 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) inhibitors. Optimization of the thiophene ring and the substituents on the 1,2,4-triazole ring produced 3,4-dicyclopropyl-5-{2-[3-fluoro-5-(trifluoromethyl)thiophen-2-yl]propan-2-yl}-4H-1,2,4-triazole monohydrochloride (9a), which showed potent and selective inhibitory activity against human 11β-HSD1. Compound 9a was also metabolically stable against human and mouse liver microsomes. Oral administration of 9a to diabetic ob/ob mice lowered corticosterone levels in adipose tissue, and thereby reduced plasma glucose and insulin levels in a dose-dependent manner.

Key words type II diabetes mellitus; 11β-hydroxysteroid dehydrogenase type 1; liver microsomal stability

Introduction

Diabetes mellitus is a metabolic disease in which hyperglycemia persists chronically as a result of deficient insulin action due to failed insulin secretion in the pancreas or insulin resistance in peripheral tissues. Diabetes mellitus is divided into two types according to the cause: type 1 diabetes is caused by insulin deficiency due to destruction of pancreatic β cells; and type 2 diabetes is caused by environmental factors such as genetic factors, obesity, binge eating, lack of exercise and a high fat diet. Numerous therapeutic agents for type 2 diabetes have been studied, and insulin secretagogues and insulin sensitizers have been developed. However, owing to limited pharmacological effects and harmful side effects, glycemic control in many type 2 diabetic patients remains insufficient. Therefore, development of drugs with new mechanisms of action is warranted.

11β-Hydroxysteroid dehydrogenase is an enzyme that catalyzes the conversion of glucocorticoids. Two isozymes have been identified (Fig. 1): 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) converts inactive glucocorticoids into active glucocorticoids in the liver and adipose tissue, while 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) converts active glucocorticoids into inactive glucocorticoids in the kidney. Reports have identified a relationship between abnormal activation of glucocorticoids in adipose tissue and diabetes. Reports have also demonstrated increased 11β-HSD1 activity in the adipose tissue of obese patients, and a correlation between 11β-HSD1 activity and body mass index (BMI), homeostasis model assessment of insulin resistance (HOMA-IR) and fasting blood glucose level. In addition, a transgenic mouse selectively overexpressing 11β-HSD1 in adipose tissue had raised glucocorticoids levels in adipose tissue and insulin resistance and hyperlipidemia. Therefore, an 11β-HSD1 selective inhibitor is expected to suppress glucocorticoid action by inhibiting the conversion of inactive glucocorticoids to active glucocorticoids, and improve metabolic abnormalities caused by glucocorticoids such as hyperglycemia, insulin resistance and hyperlipidemia. However, inhibition of 11β-HSD2 in the kidney is also known to induce hypertension, making selectivity for 11β-HSD1 over 11β-HSD2 of paramount importance. Various 11β-HSD1 inhibitors have been reported to date, and clinical trials of several compounds have been conducted (Fig. 2), but none of the compounds have been launched yet. Therefore, we conceptualized that the discovery of a new type of 11β-HSD1 inhibitor is necessary to overcome the current stagnation in clinical trials and initiated research from ground up.
We have been focusing on developing novel 11β-HSD1 inhibitors as a new class of drugs for type 2 diabetes. High throughput screening (HTS) of the Astellas compound library identified compound 1 as a novel 11β-HSD1 inhibitor. In our initial study, to find patentable compounds, we converted the linker moiety, chlorobenzene moiety and the substituents at the 3 and 4 positions on the triazole ring of compound 1 to produce lead compound 9d (unpublished data; Fig. 3). Compound 9d had no inhibitory activity against human 11β-HSD2, but showed moderate inhibitory activity against human 11β-HSD1 (IC₅₀ = 23 nM) and insufficient metabolic stability in mice (CLₐᵥ = 1122 mL/min/kg).

Therefore, we aimed to further improve the inhibitory activity of compound 9d against human 11β-HSD1 and its metabolic stability in mice for testing in a diabetes mouse model. Here, we describe further optimization of 9d as a novel 11β-HSD1 inhibitor, including structure–activity relationship (SAR) studies and evaluation of its in vivo efficacy.

Results and Discussion

Chemistry

Synthesis of compounds 9a–p is shown in Chart 1. Esterification of carboxylic acid 2 followed by heating of 3a and 3b with hydrazine monohydrate in ethanol (EtOH) yielded acylhydrazine 4a and 4b. Compound 5 was converted to 6, which was hydrolyzed to 7. Condensation of 7 and hydrazine monohydrate, followed by cyclization of acylhydrazine and N-cyclopropylcyclopropanecarboxamide (8a) using methyl trifluoromethanesulfonate yielded triazole 9c. Compounds 9a, 9b, and 9d–p were synthesized in a similar manner to 9c.

Chart 2 shows the synthesis of 10c, 10d and 10o. Compound 9d was also brominated with N-bromosuccinimide to yield 11, which was treated with n-butyl lithium and N,N-dimethylformamide (DMF) to give aldehyde 12. Compound 12 was converted to 13 using Wolff–Kishner reduction. Iodization of 9d and subsequent cyanation with copper(I) cyanide gave nitrile 15.

Synthesis of 19 is depicted in Chart 3. Cyclization of 16 and 8a using methyl trifluoromethanesulfonate followed by removal of the tert-butoxycarbonyl (Boc) group from 17 with hydrochloric acid yielded amine 18. Treatment of 18 with 2,5-dimethoxytetrahydrofuran gave pyrrole 19.

Preparation of 21 is shown in Chart 4. Treatment of 20 with thionyl chloride and subsequent cyclization with formyl hydrazine yielded 21.

Compounds 25a–e were synthesized as shown in Chart 5. Compound 4d was condensed with cyclopropanecarbonyl chloride to give 22, which was cyclized with trifluoromethanesulfonic anhydride to give oxadiazole 23. Compound 24 was prepared by bromination of 23, and 24 was reacted with several amines by microwave irradiation to give 25a–e.

Chart 6 shows the synthesis of 29a and 29b. Condensation of 26a and 26b with cyclopropanecarbonyl chloride followed by cyclization of 27a and 27b by treatment with phosphorus oxychloride gave 28a and 28b. Reaction of 28a and 28b with cyclopropylamine by microwave irradiation yielded 29a and 29b.

Biological Evaluation

The compounds’ enzymatic inhibitory activity against human 11β-HSD1, 11β-HSD2 and mouse 11β-HSD1 was determined by using a homogeneous time-resolved fluorescence method (HTRF®). Truncated recombinant human and mouse 11β-HSD1 proteins (residues 24–292 with His-tag at N-terminal) were produced from Escherichia coli (E. coli), and full length recombinant human 11β-HSD2 protein was produced from HEK-293. Selected compounds were measured for metabolic stability against mouse and human hepatic CYPs. CLogP values were calculated using ACD LogP prediction software (ACD/Percepta).17

The effect of the conversion of the thienyl moiety is shown in Table 1, along with the corresponding data for 9d for comparison. Pyridine derivatives 29a and 29b showed lower inhibitory activity against human 11β-HSD1 than 9d, and pyrazine derivative 9p showed a dramatic loss in activity. Replacement with pyrrole (19) resulted in a moderate decrease...
inhibitory activity against human 11β-HSD1. These results suggest that nitrogen-containing heteroaromatics may be unfavorable at the terminal thiophene ring position. Meanwhile, compound 19 showed improved mouse liver microsomal (MLM) stability. The difference in CLogP values between 9d and 19 indicated that the improved MLM stability was due to the decrease in lipophilicity. On the basis of these results, we concluded that the thiophene ring was the most suitable substituent at the R position.

Table 2 summarizes the effects of substituents at the 3-position of the triazole ring. Removal of the 3-position substituent (21) resulted in loss of inhibitory activity against human 11β-HSD1, suggesting that substituents are necessary at the 3-position of the triazole ring. Substitution of the cyclopropyl group with an n-propyl (9e) or cyclopropylmethyl (9f) group decreased inhibitory activity against human 11β-HSD1 by about 2-fold. tert-Butyl (9g), cyclobutyl (9h), cyclopentyl (9i) and cyclohexyl (9j) derivatives showed a slight improvement in inhibitory activity, indicating that bulky substituents are preferable at the 3-position of the triazole ring. Compounds 9e–j showed decreased MLM stability, possibly due to the high lipophilicity compared to 9d. In contrast, neither group of compounds showed inhibitory activity against human 11β-HSD2. These results indicate that the cyclopropyl group was the most suitable substituent at the 3-position of the triazole ring.

To suppress oxidative metabolism of the thiophene ring, we studied the effects of substituent groups such as electron withdrawing groups on the thiophene ring of compound 9d (Table 3). Substitution with a halogen atom, such as fluorine (9m), chlorine (10d) and bromine (11), at the 5-position of the thiophene ring increased inhibitory activity against human 11β-HSD1, but was ineffective in improving MLM stability. A 5-methyl derivative (13) showed comparable activity to 9d, but had 2-fold lower MLM stability. Meanwhile, substitution with a 5-cyano group (15) resulted in about a 4-fold reduction in inhibitory activity, but 3-fold increase in MLM stability compared to 9d. This improvement in MLM stability was probably due to a decrease in lipophilicity. Substitution with a 5-trifluoromethyl group (9b) resulted in activity equivalent...
to that of 9d. Interestingly, compound 9b had about 3-fold higher MLM stability, despite an increased CLogP value. This suggests that the improved MLM stability may be due to suppression of oxidative metabolism of the thiophene ring. 3-Fluoro (9c), 4-bromo (9n) and 3-chloro (9o) analogues had increased inhibitory activity against human 11β-HSD1 but no improvement MLM stability. In particular, the 3-chloro derivative showed a significant decrease in MLM stability, indicating that substitution with a chloro group is unfavorable at the 3-position of the thiophene ring.

Next, we investigated 3,5-disubstituted derivatives. A 3-fluoro-5-chloro derivative (10c) and 3,5-dichloro derivative (10o) showed improved inhibitory activity compared to 9d. However, they had deteriorated MLM stability, especially 10o, which showed a dramatic decrease similar to 9o. Interestingly, a 3-fluoro-5-trifluoromethyl derivative (9a) was the most potent human 11β-HSD1 inhibitor generated in this series (IC50 = 4.8 nM), and had enhanced MLM stability (mouse CClint = 578 mL/min/kg).

Results from compounds 9c, 10c and 9a suggested the fluoro group was the most suitable substituent at the 3-position of the thiophene ring for increasing inhibitory activity against human 11β-HSD1. In addition, results from compounds 9a and 9b indicated that substitution of a trifluoromethyl group at the 5-position of the thiophene ring was most effective for improving MLM stability. Neither group of compounds had inhibitory activity against human 11β-HSD2. The above results suggest that the 3-fluoro-5-trifluoromethyl group was the most suitable substituent for the thiophene ring.

The effect of substituents at the 4-position of the triazole ring is shown in Table 4. To improve efficiency for SAR studies, we converted 5-bromothiophene derivatives, which were easier to synthesize than 3-fluoro-5-trifluoromethyli thiophene derivatives. The corresponding data for 11 are also shown.

Chart 2. Synthesis of 10c, 10d, 10o, 11, 13 and 15

Chart 3. Synthesis of 19

Chart 4. Synthesis of 21
in Table 4 for comparison. Replacement of the cyclopropyl group with a methyl group (9k) resulted in a slight attenuation of inhibitory activity, indicating that a certain bulkiness was required at the 4-position of the thiophen ring. The alkyl derivatives 9l and 25a–c showed inhibitory activity equivalent to 11, but 25a–c had decreased MLM stability. Substitution with benzyl (25d) and phenethyl (25e) groups resulted in a decrease in inhibitory activity against human 11β-HSD1, indicating that an alkyl group at the R position may be more suitable than a benzene ring. Neither group of compounds showed inhibitory activity against human 11β-HSD2. On the basis of these results, we concluded that a cyclopropyl group was the most suitable substituent at the 4-position of the triazole ring.

Table 1. Conversion of the Thiophen Moiety

| Compound | R          | Human 11β-HSD1 IC₅₀ (nM) | Human 11β-HSD2 IC₅₀ (nM) | Mouse CLᵥ (mL/min/kg) | CLogP |
|----------|------------|--------------------------|--------------------------|----------------------|-------|
| 9d       |            | 23                       | >10000                   | 1122                 | 2.95  |
| 29a      |            | 591                      | NT                       | NT                   | 2.08  |
| 29b      |            | 343                      | NT                       | NT                   | 2.08  |
| 9p       |            | >3000                    | NT                       | NT                   | 1.52  |
| 19a      |            | 134                      | >30000                   | 368                  | 2.39  |

a) Hydrochloride salt. b) Not tested.
ated and showed the inhibitory activity of compound 9d against mouse 11β-HSD1 for comparison. Although compound 9a showed only moderate inhibitory activity against mouse 11β-HSD1, it had potent inhibitory activity against human 11β-HSD1 and was metabolically stable against human liver microsomes (Table 5). As shown in Fig. 4, we further tested compound 9a to determine its activity after oral administration. Seven-week-old male diabetic ob/ob mice were treated twice daily with 0.3, 1, 3 and 10 mg/kg of 9a for four weeks. At 12 h after the final administration, plasma and retroperitoneal adipose tissue samples were collected, and plasma glucose, plasma insulin and corticosterone levels in retroperitoneal adipose tissue were measured. Compound 9a dose-dependently reduced plasma glucose and insulin levels, and decreased corticosterone levels in adipose tissue. Although compound 9a showed only moderate inhibitory activity against mouse 11β-HSD1 in vitro, its activity was sufficient to lower corticosterone levels in adipose tissue, resulting in a decrease in plasma glucose and insulin levels in vivo.

Table 3. Conversion of Substituent on the Thiophene Ring

| Compound | R    | Human 11β-HSD1 IC50 (nM) | Human 11β-HSD2 IC50 (nM) | Mouse CLint (mL/min/kg) | CLogP |
|----------|------|--------------------------|--------------------------|-------------------------|-------|
| 9d       | H    | 23                       | >10000                   | 1122                    | 2.95  |
| 9m       | 5-F  | 11                       | >3000                    | 1461                    | 3.00  |
| 10d      | 5-Cl | 15                       | >10000                   | 1494                    | 3.34  |
| 11       | 5-Br | 7.9                      | >3000                    | 1445                    | 3.54  |
| 13       | 5-Me | 19                       | >10000                   | 1983                    | 3.06  |
| 15       | 5-CN | 95                       | >3000                    | 385                     | 2.49  |
| 9b       | 5-CF3 | 24                       | >10000                   | 423                     | 3.59  |
| 9n       | 4-Br | 12                       | >10000                   | 1674                    | 3.50  |
| 9c       | 3-F  | 12                       | >10000                   | 1666                    | 2.90  |
| 9o       | 3-Cl | 14                       | >10000                   | 6791                    | 3.34  |
| 10c      | 3-F, 5-Cl | 8.7               | >3000                    | 2116                    | 3.06  |
| 10o      | 3,5-diCl | 13              | >10000                   | >9066                   | 3.71  |
| 9a       | 3-F, 5-CF3 | 4.8            | >3000                    | 578a                    | 3.53  |

Table 4. Conversion of the 4-Position Substituent of 1,2,4-Triazole

| Compound | R        | Human 11β-HSD1 IC50 (nM) | Human 11β-HSD2 IC50 (nM) | Mouse CLint (mL/min/kg) | CLogP |
|----------|----------|--------------------------|--------------------------|-------------------------|-------|
| 11c      | -Pr      | 7.9                      | >3000                    | 1445                    | 3.54  |
| 9k       | Me       | 26                       | NTb                     | NTb                     | 3.30  |
| 9l       | Et       | 9.6                      | >3000                    | 1298                    | 3.62  |
| 25a      | n-Pr     | 8.6                      | >3000                    | 2771                    | 4.12  |
| 25b      | i-Pr     | 14                      | >10000                   | 5746                    | 4.07  |
| 25c      | Cyclopropyl methyl | 12              | >10000                   | 4190                    | 4.03  |
| 25d      | Bn       | 22                       | NTb                     | NTb                     | 5.16  |
| 25e      | Phenethyl | 43              | >10000                   | NTb                     | 5.39  |

Table 5. Profile of Compound 9a

| Compound | Human 11β-HSD1 IC50 (nM) | Human 11β-HSD2 IC50 (nM) | Mouse CLint (mL/min/kg) | CLogP |
|----------|--------------------------|--------------------------|-------------------------|-------|
| 9d       | 23                       | >10000                   | NTb                     | 1122  |
| 9a       | 4.8                      | 20                       | >10000                   | NTb   | 578b |

Fig. 4. Effects of Repeated Administration of 9a in Male ob/ob Mice (0.3–10 mg/kg, Twice Daily for 4 Weeks, n = 7–8)

Values are presented as the mean ± standard error (S.E.). *p < 0.05, **p < 0.01 versus vehicle-treated group, using the Dunnett’s multiple comparisons test.
Finally, to explore the reason behind the very potent inhibitory activity of 9a against human 11β-HSD1, we performed a docking analysis of the interaction between 9a and human 11β-HSD1. The human 11β-HSD1 model was created based on the crystal structure of human 11β-HSD1 in a complex with a triazole inhibitor (PDB code 3D5Q). 18) Compound 9a was docked using GOLD version 5.5.1,19–21) as shown in Fig. 5. The docking study suggested that 9a was positioned in the steroid substrate binding site and that the nitrogen atom at the 1-position of the triazole ring interacted with Tyr183, while the nitrogen atom at the 2-position of the triazole ring interacted with Ser170. In addition, this model indicated that the thiophene moiety of 9a fit into the hydrophobic pocket, which contained Val180. The corresponding residue to Val180 in mice was isoleucine (Ile), which is more sterically bulky than Val as shown in Fig. 6b. Therefore the trifluoromethyl moiety of 9a cannot exist at this position in mouse 11β-HSD1 due to steric hindrance, and this may be an explanation for the attenuated inhibitory activity of 9a against mouse 11β-HSD1 compared to human 11β-HSD1.

Conclusion

In our investigation of novel 11β-HSD1 inhibitors, optimization of 9d resulted in the discovery of compound 9a, 3,4-dicyclopentanyl-5-[2-{3-fluoro-5-(trifluoromethyl)-thiophen-2-yl]propan-2-yl]-4H-1,2,4-triazole monohydrochloride. These findings may form the basis for the potent inhibitory activity of 9a against human 11β-HSD1.
ride, which showed potent and selective inhibitory activity against human 11β-HSD1, and good human and mouse liver microsomal stability. Oral administration of compound 9a reduced corticosterone levels in adipose tissue, resulting in reduced plasma glucose and insulin levels in diabetic ob/ob mice. Therefore, 9a may be a new type of insulin sensitizer with the potential to be an effective treatment for type II diabetes.

Experimental

Chemistry Starting materials and reagents are commercially available. 1H-NMR spectra were recorded on Varian 300-MR, Varian 400-MR, Varian VNS-400 or JEOL LAMBD A and chemical shifts were expressed in δ (ppm) values with trimethylsilane as an internal reference (s = singlet, d = doublet, dd = double doublet, sdd = double double doublet, t = triplet, q = quartet, m = multiplet, br = broad and brs = broad singlet). MS were recorded on JEOL JMS-LX2000, Waters ZQ2000, Thermo Electron TRACE DSQ, Thermo Electron LQc Advantage or Thermo Scientific Exact spectrometry. Elemental analyses were performed with Elementar Vario EL III (C, H, N) and Dionex ICS-5000 (S, halogen) instruments, and results were within ±0.4% of theoretical values. All reactions were carried out using commercially available reagents and solvents without further purification. The following abbreviations are used: AcOH, acetic acid; DMF, dimethylformamide; DMSO, dimethyl sulfox ide; Et2O, diethyl ether; EtOAc, ethyl acetate; EtOH, ethanol; Et3N, triethylamine; HOBt, 1-hydroxybenzotriazole; MeOH, methanol; THF, tetrahydrofuran; WSCD, water-soluble carbodiimide; CAS No., Chemical Abstracts Service Registry Number.

Methyl 2-[3-Fluoro-5-(trifluoromethyl)thiophen-2-yl]-2-methylpropanoate (3a) Dipotassium carbonate (6.20 g, 44.9 mmol) and iodomethane (2.27 mL, 36.5 mmol) were added to an ice-cooled solution of 2-[3-fluoro-5-(trifluoromethyl)thiophen-2-yl]-2-methyl propanoic acid (2, 7.67 g, 29.9 mmol, CAS No.; 950604-93-0) in DMF (30 mL), and the mixture was stirred at r.t. for 15 h. The reaction mixture was diluted with water (60 mL) and extracted with Et2O. The organic layer was washed with water, dried over Na2SO4, filtered and concentrated in vacuo. The resulting residue was purified by column chromatography on silica gel (hexane-EtOAc) to give the title compound 3a (6.81 g, 25.2 mmol, 84%) as a pale yellow syrup. 1H-NMR (400 MHz, CDCl3) δ: 1.65 (6H, s), 3.73 (3H, s), 7.12–7.13 (1H, m).

2-[3-Fluoro-5-(trifluoromethyl)thiophen-2-yl]-2-methylpropanehydrazide (4a) Hydrazine monohydrate (1.84 g, 12.0 mmol) in EtOH (2 mL) and the mixture was stirred at 60°C for 24 h. The reaction mixture was concentrated in vacuo. The resulting residue was diluted with saturated aqueous sodium hydrogen carbonate solution and extracted with CHCl3. The organic layer was dried over Na2SO4, filtered and concentrated in vacuo. The resulting residue was purified by column chromatography on silica gel (CHCl3–MeOH) to give the title compound 4a (286 mg, 1.06 mmol, 89%) as a pale yellow syrup. 1H-NMR (400 MHz, CDCl3) δ: 1.66 (6H, s), 2.10–3.90 (2H, br), 6.80–7.04 (1H, m), 7.17 (1H, s). Chemical ionization mass spectrometry (CI-MS) m/z: 271 [M + H]+.

2-Methyl-2-[5-(trifluoromethyl)thiophen-2-yl]propane-2,4-diol (5a) The title compound was prepared in a similar manner to that described for 4a from ethyl 2-methyl-2-[5-(trifluoromethyl)thiophen-2-yl]propanoate (3b, 1.51 g, 5.67 mmol, CAS No.; 950604-90-7) at 89% yield (1.27 g, 5.03 mmol). 1H-NMR (400 MHz, DMSO-d6) δ: 1.56 (6H, s), 4.27 (2H, brs), 7.04–7.07 (1H, m), 7.51–7.55 (1H, m), 9.09 (1H, brs). Electrospray ionization (ESI)-MS m/z: 253 [M + H]+.

2-(3-Fluorothiophen-2-yl)-2-methylpropanenitrile (6) A mixture of iodomethane (2.14 mL, 34.4 mmol) and (3-fluorothiophen-2-yl)acetonitrile (5, 1.62 g, 11.5 mmol, CAS No.; 950604-64-5) in DMF (15 mL) was slowly added to an ice-cooled mixture of sodium hydride (NaH) (60% in mineral oil; 1.19 g, 29.8 mmol) in DMF (25 mL), and the mixture was stirred at r.t. for 20 min. The reaction mixture was poured into ice water and extracted with EtOAc. The organic layer was washed with brine, dried over MgSO4, filtered and concentrated in vacuo. The resulting residue was purified by column chromatography on silica gel (EtOAc–hexane) to give the title compound 6 (1.75 g, 10.3 mmol, 90%) as a colorless oil. 1H-NMR (300 MHz, CDCl3) δ: 1.81 (6H, s), 6.81 (1H, d, J = 5.6 Hz), 7.08–7.13 (1H, m).

2-(3-Fluorothiophen-2-yl)-2-methylpropanoic Acid (7) Potassium hydroxide (1.74 g, 31.0 mmol) was added to a solution of 6 (1.75 g, 10.3 mmol) in ethylene glycol (17.5 mL) and the mixture was stirred at 190°C for 2.5 h. The reaction mixture was cooled to r.t. and water was added to the mixture. The mixture was washed with Et2O and the aqueous layer was cooled in an ice bath, and conc. hydrochloric acid (ca. 30 mL) was added. The mixture was extracted with Et2O and washed with 1 M hydrochloric acid and brine. The organic layer was dried over MgSO4, filtered and concentrated in vacuo, then dried to give the title compound 7 (1.91 g, 10.1 mmol, 98%) as an ochre solid. 1H-NMR (300 MHz, CDCl3) δ: 1.67 (6H, s), 6.76 (1H, d, J = 5.6 Hz), 7.04 (1H, dd, J = 3.7, 5.6 Hz).

3,4-Dicyclopropyl-5-[2-(3-fluorothiophen-2-yl)propan-2-yl]-1H-1,2,4-triazole (9c) WSCD·HCl (2.41 g, 12.6 mmol) was added to an ice-cooled solution of 7 (2.15 g, 11.4 mmol), hydrazine monohydrate (1.11 mL, 22.9 mmol) and HOBT·H2O (1.84 g, 12.0 mmol) in CH2Cl2 (21.5 mL), and the mixture was stirred at r.t. overnight. WSCD·HCl (240 mg, 1.25 mmol) was added and the reaction mixture was stirred for r.t. for 2 h. Saturated aqueous sodium hydrogen carbonate solution was added and the reaction mixture was extracted with CHCl3, and washed with H2O and brine. The organic layer was dried over MgSO4, filtered and concentrated in vacuo, then dried to give a pale yellow solid (2.10 g, 10.4 mmol). A mixture of N-cyclopropylcyclopropenecarboxamide (8a, 1.56 g, 12.5 mmol) and methyl trifluoromethanesulfonate (1.59 mL, 13.5 mmol) was stirred at 60°C for 30 min. Toluene (26 mL), Et3N (1.88 mL, 13.5 mmol) and the obtained solid described above (2.10 g, 10.4 mmol) were added and the mixture was stirred at 60°C for 2 d and 100°C for 2 h. CHCl3 and saturated aqueous sodium hydroxide solution were added to the mixture, and the organic layer was separated. The organic layer was washed with brine, dried and concentrated in vacuo. The resulting residue was purified by column chromatography on silica gel (EtOAc–MeOH) to give a pale yellow solid. CHCl3 was added to the resulting solid and the precipitated solid was filtered off. Disopropyl ether was added to the resulting residue and solidified. The obtained solid was washed with a solvent comprising hexane, EtOAc and Et2O, followed
by washing with diisopropyl ether to give the title compound 9e (878 mg, 3.01 mmol, 26%) as a white solid. \(^{1}H\)-NMR (300 MHz, CDCl\(_3\)) \(\delta\): 0.79–1.05 (6H, m), 1.15–1.22 (2H, m), 1.88–2.00 (1H, m), 1.93 (6H, s), 2.70–2.79 (1H, m), 6.71 (1H, d, \(J = 5.6\) Hz), 7.01–7.05 (1H, m). ESI-MS \(m/z\): 276 [M + H].

High resolution (HR)-MS (ESI) \(m/z\): 276.1524 [M + H]\(^+\)

(Calcd for \(C_{12}H_{22}N_{5}S\): 276.1529)

4-Cyclopropyl-3-(cyclopropylmethyl)-5-[2-(thiophen-2-yl)propan-2-yl]-4H-1,2,4-triazole (9f) The title compound was prepared in a similar manner to that described for 9a, except for the salt-forming procedure, from 4d (883 mg, 4.79 mmol) and \(N\)-2-dicyclopropylacetamide (1.00 g, 7.18 mmol) at 51% yield (709 mg, 2.47 mmol) as a white solid. \(^{1}H\)-NMR (400 MHz, CDCl\(_3\)) \(\delta\): 0.23–0.29 (2H, m), 0.48–0.60 (4H, m), 0.80–0.87 (2H, m), 1.15–1.24 (1H, m), 1.96 (6H, s), 2.71–2.78 (3H, m), 6.73 (1H, dd, \(J = 1.2, 3.6\) Hz), 6.90 (1H, dd, \(J = 3.5, 5.1\) Hz), 7.15 (1H, dd, \(J = 1.1, 5.1\) Hz). FAB-MS \(m/z\): 288 [M + H]\(^+\). HR-MS (ESI) \(m/z\): 288.1527 [M + H]\(^+\)

(Calcd for \(C_{13}H_{24}N_{5}S\): 288.1529)

3,4-Dicyclopropyl-5-[2-[3-fluoro-5-(trifluoromethyl)thiophen-2-yl]-4-yl]propan-2-yl)-4H-1,2,4-triazole (9g) The title compound was prepared in a similar manner to that described for 9a, except for the salt-forming procedure, from 4d (814 mg, 4.42 mmol) and \(N\)-cyclopropyl-2,2-dimethylpropanamide (2.50 g, 17.7 mmol) at 6.5% yield (83.0 mg, 0.287 mmol) as a white solid. \(^{1}H\)-NMR (300 MHz, DMSO-\(d_6\)) \(\delta\): 0.44–0.51 (2H, m), 0.86–0.94 (2H, m), 1.44 (9H, s), 1.85 (6H, s), 3.09–3.19 (1H, m), 6.76 (1H, dd, \(J = 1.1, 3.5\) Hz), 6.95 (1H, dd, \(J = 3.5, 5.1\) Hz), 7.37 (1H, dd, \(J = 1.1, 5.1\) Hz). ESI-MS \(m/z\): 290 [M + H]\(^+\). HR-MS (ESI) \(m/z\): 290.1690 [M + H]\(^+\)

(Calcd for \(C_{16}H_{22}N_{5}S\): 290.1685)

3-Cyclobutyl-4-cyclopropyl-5-[2-(thiophen-2-yl)propan-2-yl]-4H-1,2,4-triazole (9h) The title compound was prepared in a similar manner to that described for 9a, except for the salt-forming procedure, from 4d (1.32 g, 7.18 mmol) and \(N\)-cyclopropylcyclohexanecarboxamide (1.00 g, 7.18 mmol) at 29% yield (590 mg, 2.05 mmol) as a colorless solid. \(^{1}H\)-NMR (400 MHz, DMSO-\(d_6\)) \(\delta\): 0.27–0.33 (2H, m), 0.74–0.81 (2H, m), 1.80–1.92 (1H, m), 1.85 (6H, s), 1.95–2.08 (1H, m), 2.25–2.41 (4H, m), 2.80–2.87 (1H, m) 3.63–3.73 (1H, m), 6.77 (1H, dd, \(J = 1.1, 3.6\) Hz), 6.94 (1H, dd, \(J = 3.5, 5.1\) Hz), 7.37 (1H, dd, \(J = 1.1, 5.1\) Hz). FAB-MS \(m/z\): 288 [M + H]\(^+\). HR-MS (ESI) \(m/z\): 288.1534 [M + H]\(^+\)

(Calcd for \(C_{16}H_{22}N_{5}S\): 288.1529)

3-Cyclopentyl-4-cyclopropyl-5-[2-(thiophen-2-yl)propan-2-yl]-4H-1,2,4-triazole (9i) The title compound was prepared in a similar manner to that described for 9a, except for the salt-forming procedure, from 4d (1.00 g, 5.43 mmol) and \(N\)-cyclopropylcyclopentanecarboxamide (1.00 g, 7.18 mmol) at 37% yield (611 mg, 2.03 mmol) as a white solid. \(^{1}H\)-NMR (400 MHz, CDCl\(_3\)) \(\delta\): 0.51–0.57 (2H, m), 0.84–0.91 (2H, m), 1.59–1.72 (2H, m), 1.84–2.15 (6H, m), 1.96 (6H, s), 2.71–2.78 (1H, m), 3.21–3.32 (1H, m), 6.72–6.75 (1H, m), 6.91 (1H, dd, \(J = 3.8, 4.8\) Hz), 7.15–7.19 (1H, m). ESI-MS \(m/z\): 302 [M + H]\(^+\). HR-MS (ESI) \(m/z\): 302.1682 [M + H]\(^+\)

(Calcd for \(C_{17}H_{24}N_{5}S\): 302.1682)

3-Cyclohexyl-4-cyclopropyl-5-[2-(thiophen-2-yl)propan-2-yl]-4H-1,2,4-triazole (9j) The title compound was prepared in a similar manner to that described for 9a, except for the salt-forming procedure, from 4d (1.00 g, 5.43 mmol) and \(N\)-cyclopropylcyclohexanecarboxamide (1.18 g, 7.06 mmol) at 40% yield (687 mg, 2.18 mmol) as a white solid. \(^{1}H\)-NMR (400 MHz, CDCl\(_3\)) \(\delta\): 0.50–0.55 (2H, m), 0.84–0.90 (2H, m), 1.27–1.43 (3H, m), 1.71–2.01 (7H, m), 1.95 (6H, s), 2.68–2.76 (1H, m), 2.82–2.92 (1H, m), 6.73 (1H, dd, \(J = 1.2, 3.6\) Hz), 6.90

(2H, m), 1.01–1.11 (5H, m), 1.87–2.03 (2H, m), 2.00 (6H, s), 2.95–3.02 (1H, m), 3.10–3.18 (2H, m), 6.80 (1H, dd, \(J = 1.2, 3.6\) Hz), 6.97 (1H, dd, \(J = 3.5, 5.3\) Hz), 7.23–7.28 (1H, m).
(1H, dd, J = 3.6, 5.1 Hz), 7.16 (1H, dd, J = 1.1, 5.1 Hz). ESI-MS m/z: 316 [M + H]+. HR-MS (ESI) m/z: 316.1843 [M + H]+ (Caled for C16H23N3S: 316.1842).

3-[2-(5-Bromothiophen-2-yl)propan-2-yl]-5-cyclopropyl-4-methyl-1H-1,2,4-triazole Monohydrochloride (9k) The title compound was prepared in a similar manner to that described for 9a from 2-(5-bromothiophen-2-yl)-2-methylpropenehydrazide (690 mg, 2.62 mmol, CAS No.: 950604-87-2) and N-methyleclocypropanecarboxamide (390 mg, 3.93 mmol) at 13% yield (127 mg, 0.355 mmol) as a solid. 1H-NMR (400 MHz, DMSO-d6) δ: 1.16–1.21 (4H, m), 1.78 (6H, s), 2.13–2.21 (1H, m), 3.45 (3H, s), 6.88 (1H, d, J = 3.9 Hz), 7.16 (1H, d, J = 3.9 Hz). ESI-MS m/z: 326 [M + H]+. HR-MS (ESI) m/z: 326.0324 [M + H]+ (Caled for C17H19N3SBr: 326.0321).

3-[2-(5-Bromothiophen-2-yl)propan-2-yl]-5-cyclopropyl-4-ethyl-1H-1,2,4-triazole Monohydrochloride (9l) The title compound was prepared in a similar manner to that described for 9a from 2-(5-bromothiophen-2-yl)-2-methylpropenehydrazide (500 mg, 1.90 mmol) and N-ethylclocypropanecarboxamide (323 mg, 2.85 mmol) at 16% yield (111 mg, 0.295 mmol) as a solid. 1H-NMR (400 MHz, CDCl3) δ: 1.20 (3H, t, J = 7.2 Hz), 1.39–1.46 (2H, m), 1.87–1.94 (3H, m), 1.89 (6H, s), 4.02 (2H, q, J = 7.3 Hz), 6.67 (1H, d, J = 3.8 Hz), 6.97 (1H, d, J = 3.8 Hz). ESI-MS m/z: 340 [M + H]+. HR-MS (ESI) m/z: 340.0479 [M + H]+ (Caled for C17H19N3BrS: 340.0478).

3,4-Dicyclopropyl-5-[(2-5-fluorothiophen-2-yl)propan-2-yl]-1H-1,2,4-triazole Monohydrochloride (9m) The title compound was prepared in a similar manner to that described for 9a from 2-(5-fluorothiophen-2-yl)-2-methylpropenehydrazide (515 mg, 2.55 mmol CAS No.: 950604-98-8) and 8a (637 mg, 5.09 mmol) at 19% yield (161 mg, 0.491 mmol) as a colorless solid. 1H-NMR (400 MHz, DMSO-d6) δ: 0.74–0.80 (2H, m), 1.00–1.07 (2H, m), 1.19–1.31 (4H, m), 1.85 (6H, s), 2.23–2.32 (1H, m), 3.21–3.28 (1H, m), 6.60–6.63 (2H, m). FAB-MS m/z: 292 [M + H]+. HR-MS (ESI) m/z: 292.1276 [M + H]+. (Caled for C16H19N3SBr: 292.1278).

3-[2-(4-Bromothiophen-2-yl)propan-2-yl]-4,5-dicyclopropyl-1H-1,2,4-triazole (9n) The title compound was prepared in a similar manner to that described for 9a, except for the salt-forming procedure, from 2-(4-bromothiophen-2-yl)-2-methylpropenehydrazide (7.4 g, 38.0 mmol, CAS No.: 950604-78-1) and 8a (4.58 g, 36.66 mmol) at 41% yield (4.07 g, 11.6 mmol) as a white solid. 1H-NMR (300 MHz, DMSO-d6) δ: 0.47–0.55 (2H, m), 0.87–1.02 (6H, m), 1.84 (6H, s), 1.98–2.08 (1H, m), 2.98–3.08 (1H, m), 6.88 (1H, d, J = 1.5 Hz), 7.54 (1H, d, J = 1.4 Hz). ESI-MS m/z: 352 [M + H]+. HR-MS (ESI) m/z: 352.0478 [M + H]+ (Caled for C17H19N3SBr: 352.0478).

3-[2-(3-Chlorothiophen-2-yl)propan-2-yl]-4,5-dicyclopropyl-1H-1,2,4-triazole (10a) The title compound was prepared in a similar manner to that described for 10d from 9e (100 mg, 0.325 mmol) at 63% yield (106 mg, 0.325 mmol) as a white solid. 1H-NMR (300 MHz, CDCl3) δ: 0.87–0.96 (2H, m), 0.97–1.07 (4H, m), 1.14–1.22 (2H, m), 1.85–1.99 (1H, m), 1.91 (6H, s), 2.77–2.88 (1H, m), 6.61 (1H, s). ESI-MS m/z: 326 [M + H]+. HR-MS (ESI) m/z: 326.0888 [M + H]+. (Caled for C16H19N3ClS: 326.0889).

3,4-Dicyclopropyl-5-[2-(3,5-dichlorothiophen-2-yl)propan-2-yl]-1H-1,2,4-triazole (10b) The title compound was prepared in a similar manner to that described for 10d from 9o (100 mg, 0.325 mmol) at 51% yield (56.8 mg, 0.166 mmol) as a white solid. 1H-NMR (300 MHz, CDCl3) δ: 0.89–1.05 (6H, m), 1.13–1.20 (2H, m), 1.87–1.98 (1H, m), 1.90 (6H, s), 2.57–2.66 (1H, m), 6.71 (1H, s). ESI-MS m/z: 342 [M + H]+. HR-MS (ESI) m/z: 342.0596 [M + H]+. (Caled for C17H19N3Cl2S: 342.0593).

3-[2-(5-Bromothiophen-2-yl)propan-2-yl]-4,5-dicyclopropyl-1H-1,2,4-triazole (11) N-Bromosuccinimide (68 mg, 0.38 mmol) was added to a solution of 9d (100 mg, 0.366 mmol) in AcOH (3 mL) and the mixture was stirred at 80°C for 2h. The reaction mixture was concentrated in vacuo and diluted with CHCl3. One molar aqueous sodium hydroxide solution was added and the reaction mixture was extracted with CHCl3. The organic layer was washed with brine, dried over MgSO4 filtered and concentrated in vacuo. The resulting residue was purified by column chromatography on silica gel (CHCl3–MeOH). The obtained product was washed with Et2O and dried to give the title compound 11 (72.8 mg, 0.207 mmol, 57%) as a colorless solid. 1H-NMR (400 MHz, DMSO-d6) δ: 0.54–0.60 (2H, m), 0.89–1.01 (6H, m), 1.83 (6H, s), 1.99–2.07 (1H, m), 3.00–3.06 (1H, m), 6.67 (1H, d, J = 3.6 Hz), 7.06 (1H, d, J = 3.9 Hz). FAB-MS m/z: 354 [M + H]+. HR-MS (ESI) m/z: 354.0983 [M + H]+. HR-MS (ESI) m/z: 354.0983.
Potassium hydroxide (140 mg, 2.49 mmol) was added and the mixture was stirred at −78°C for 45 min. A solution of DMF (1.27 mL, 16.4 mmol) in THF (10 mL) was added and the mixture was stirred at −78°C for 30 min. The reaction mixture was poured into water and extracted with CHCl₃. The organic layer was washed with brine, dried over MgSO₄, filtered and concentrated. The residue was purified by column chromatography on silica gel (CHCl₃–MeOH) to give the title compound (400 MHz, DMSO–d₆) as a colorless solid. 1H-NMR (400 MHz, DMSO–d₆–MeOH) to give the title compound

The resulting residue was purified by column chromatography on silica gel (CHCl₃–MeOH). The obtained product was washed with diisopropyl ether and dried to give the title compound

The resulting residue was purified by column chromatography on silica gel (CHCl₃–MeOH). The obtained product was washed with diisopropyl ether and dried to give the title compound

The resulting residue was purified by column chromatography on silica gel (CHCl₃–MeOH). The obtained product was washed with diisopropyl ether and dried to give the title compound

The resulting residue was purified by column chromatography on silica gel (CHCl₃–MeOH). The obtained product was washed with diisopropyl ether and dried to give the title compound

The resulting residue was purified by column chromatography on silica gel (CHCl₃–MeOH). The obtained product was washed with diisopropyl ether and dried to give the title compound

The resulting residue was purified by column chromatography on silica gel (CHCl₃–MeOH). The obtained product was washed with diisopropyl ether and dried to give the title compound

The resulting residue was purified by column chromatography on silica gel (CHCl₃–MeOH). The obtained product was washed with diisopropyl ether and dried to give the title compound

The resulting residue was purified by column chromatography on silica gel (CHCl₃–MeOH). The obtained product was washed with diisopropyl ether and dried to give the title compound

The resulting residue was purified by column chromatography on silica gel (CHCl₃–MeOH). The obtained product was washed with diisopropyl ether and dried to give the title compound

The resulting residue was purified by column chromatography on silica gel (CHCl₃–MeOH). The obtained product was washed with diisopropyl ether and dried to give the title compound

The reaction mixture was stirred at 60°C for 2h and 100°C for 1h. CHCl₃ and saturated aqueous sodium hydrogen carbonate solution were added to the mixture, and the organic layer was separated and washed with brine, dried over MgSO₄ filtered and concentrated in vacuo. The mixture was filtered and concentrated in vacuo.

The reaction mixture was stirred at 60°C for 2h and 100°C for 1h. CHCl₃ and saturated aqueous sodium hydrogen carbonate solution were added to the mixture, and the organic layer was separated and washed with brine, dried over MgSO₄ filtered and concentrated in vacuo. The mixture was filtered and concentrated in vacuo.

The reaction mixture was stirred at 60°C for 2h and 100°C for 1h. CHCl₃ and saturated aqueous sodium hydrogen carbonate solution were added to the mixture, and the organic layer was separated and washed with brine, dried over MgSO₄ filtered and concentrated in vacuo. The mixture was filtered and concentrated in vacuo.

The reaction mixture was stirred at 60°C for 2h and 100°C for 1h. CHCl₃ and saturated aqueous sodium hydrogen carbonate solution were added to the mixture, and the organic layer was separated and washed with brine, dried over MgSO₄ filtered and concentrated in vacuo. The mixture was filtered and concentrated in vacuo.
mixture. The mixture was stirred at 70°C for 20 h, and CHCl₃ and 1 M aqueous sodium hydroxide solution were added. The organic layer was separated and washed with brine, dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (CHCl₃–MeOH). The obtained product was washed with hexane and dried to give the title compound 21 (565 mg, 2.42 mmol, 25%) as a colorless solid. ³H-NMR (400 MHz, DMSO-d₆) δ: 0.74–0.80 (4H, m), 1.86 (6H, s), 2.75–2.81 (1H, m), 6.89 (1H, dd, J = 1.1, 3.5 Hz), 6.97 (1H, dd, J = 3.5, 5.3 Hz), 7.42 (1H, dd, J = 1.2, 5.1 Hz). EI-MS m/z: 233 [M⁺]. HR-MS (ESI) m/z: 234.1053 [M + H⁺] (Calcd for C₂₅H₂₆N₄S: 234.1059).

N′-2-Methyl-2-(thiophen-2-yl)propanoyl)cyclopropanecarboxydrazide (22) Et₃N (15 mL, 108 mmol) was added to a solution of 4d (368 mg, 2.42 mmol, 92%) as a white solid. ¹H-NMR (400 MHz, CDCl₃) δ: 2.85 (1H, d, J = 3.9 Hz), 3.82 (1H, d, J = 3.9 Hz), 6.98 (1H, d, J = 3.8 Hz). FAB-MS m/z: 356 [M + H⁺]. HR-MS (ESI) m/z: 354.0634 [M + H⁺] (Calcd for C₂₅H₂₆N₄SBrS: 354.0634).

3-[[2-(5-Bromothiophen-2-yl)propan-2-yl]-5-cyclopropyl-4-propyl-4H-1,2,4-triazole Monohydrochloride (25a) 5.28 g, 14.14 g (8H, s), 1.96–2.08 (3H, m), 4.52–4.63 (1H, m), 6.62 (1H, d, J = 3.9 Hz), 6.97 (1H, d, J = 3.9 Hz). FAB-MS m/z: 356 [M + H⁺]. HR-MS (ESI) m/z: 354.0635 [M + H⁺] (Calcd for C₂₅H₂₆N₄SBrS: 354.0634).

3-[[2-(5-Bromothiophen-2-yl)propan-2-yl]-5-cyclopropyl-4-(cyclopropylmethyl)-4H-1,2,4-triazole Monohydrochloride (25b) The title compound was prepared in a similar manner to that described for 25a from 24 (500 mg, 1.60 mmol) and isopropylamine (1.37 mL, 16.7 mmol) as a white solid. ¹H-NMR (400 MHz, CDCl₃) δ: 1.41–1.48 (8H, m), 1.87 (6H, s), 1.96–2.08 (3H, m), 4.52–4.63 (1H, m), 6.62 (1H, d, J = 3.9 Hz), 6.97 (1H, d, J = 3.9 Hz). FAB-MS m/z: 356 [M + H⁺]. HR-MS (ESI) m/z: 354.0635 [M + H⁺] (Calcd for C₂₅H₂₆N₄SBrS: 354.0634).

1-[2-([5-Bromothiophen-2-yl]propan-2-yl)-5-cyclopropyl-4-propyl-4H-1,2,4-triazole Monohydrochloride (25c) Cyclopropylaminoethylamine (568 mg, 7.99 mmol) was added to a solution of 24 (500 mg, 1.60 mmol) in AcOH (5 mL) and reacted at 170°C for 40 min using a microwave reaction device. The reaction mixture was cooled to r.t. and concentrated in vacuo. The residue was washed with EtOAc and dried to give the title compound 25c (231 mg, 0.591 mmol, 37%) as a white solid. ¹H-NMR (400 MHz, CDCl₃) δ: 0.87 (3H, t, J = 7.4 Hz), 1.37–1.53 (4H, m), 1.84–2.00 (3H, m), 1.90 (6H, s), 3.86–3.93 (2H, m), 6.70 (1H, d, J = 3.9 Hz), 6.98 (1H, d, J = 3.8 Hz). FAB-MS m/z: 356 [M + H⁺]. HR-MS (ESI) m/z: 354.0634 [M + H⁺] (Calcd for C₂₅H₂₆N₄SBrS: 354.0634).

4-Benzyl-3-[[2-(5-bromothiophen-2-yl)propan-2-yl]-5-cyclopropyl-4H-1,2,4-triazole Monohydrochloride (25d) The title compound was prepared in a similar manner to that described for 25a from 24 (500 mg, 1.60 mmol) and benzylamine (1.74 mL, 15.9 mmol) at 33% yield (231 mg, 0.526 mmol) as a white solid. ¹H-NMR (400 MHz, CDCl₃) δ: 1.14–1.21 (2H, m), 1.62–1.70 (1H, m), 1.77–1.85 (2H, m), 1.83 (6H, s), 5.20 (2H, s), 6.64 (1H, d, J = 3.9 Hz), 6.80–6.87 (2H, m), 6.86 (1H, d, J = 3.9 Hz), 7.34–7.38 (3H, m). FAB-MS m/z: 404 [M + H⁺]. HR-MS (ESI) m/z: 402.0634 [M + H⁺] (Calcd for C₂₅H₂₆N₄SBrS: 402.0634).
4-(2-Phenethyl)-4H-1,2,4-triazole (25e) The title compound was prepared in a similar manner to that described for 25a, except for the salt-forming procedure, from 24 (500 mg, 1.60 mmol) and 2-phenylethylamine (2.00 mL, 15.8 mmol) at 20% yield (131 mg, 0.314 mmol) as a white solid. $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$: 1.03–1.09 (2H, m), 1.19–1.24 (2H, m), 1.64–1.73 (1H, m), 1.85 (6H, s), 2.63–2.70 (2H, m), 3.88–3.94 (2H, m), 6.64 (1H, d, $J = 3.7$ Hz), 6.95 (1H, d, $J = 3.8$ Hz), 6.96–7.00 (2H, m), 7.22–7.33 (3H, m). FAB-MS $m/z$: 416 [M + H]$^+$. 

$N^\prime$-[2-Methyl-2-(pyridin-2-yl)propanoyl]cyclopropanecarboxyhydrazide (27a) Et$_2$N (3.42 mL, 24.6 mmol) was added to a solution of 2-methyl-2-(pyridin-2-yl)propanohydrazide (26a, 4.00 g, 22.3 mmol, CAS No.; 880166-60-9) in dichloromethane (20 mL), and then a solution of cyclopropycarbonyl chloride (2.11 mL, 23.4 mmol) in dichloromethane (20 mL) was added dropwise to the mixture at 0°C. The reaction mixture was stirred at 3h and saturated aqueous sodium hydrogen carbonate solution was added. The mixture was extracted with CHCl$_3$, and the organic layer was washed with brine, dried over MgSO$_4$, filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel (CHCl$_3$–MeOH) to give the title compound 27a (5.25 g, 21.2 mmol, 76%) as a white solid. $^1$H-NMR (300 MHz, DMSO–D$_6$) at 45% yield (103 mg, 0.418 mmol) as (pyridin-4-yl) propanehydrazide ($27a$). The title compound was prepared in a similar manner to that described for 27b (5.81 g, 25.3 mmol, 62%) as a colorless oil. $^1$H-NMR (300 MHz, DMSO–D$_6$) at 45% yield (103 mg, 0.418 mmol) as (pyridin-4-yl) propanehydrazide ($27a$). The title compound was prepared in a similar manner to that described for 27a from 2methyl-2-(pyridin-4-yl)propanohydrazide (26b, 166 mg, 0.926 mmol, CAS No.; 950604-79-2) at 45% yield (103 mg, 0.418 mmol) as a white solid. $^1$H-NMR (300 MHz, DMSO–D$_6$) $\delta$: 0.68–0.76 (4H, m), 1.45–1.63 (1H, m), 1.47 (6H, s), 7.38–7.42 (2H, m), 8.47–8.51 (2H, m), 9.47 (1H, brs), 9.87 (1H, brs). ESI-MS $m/z$: 248 [M + H]$^+$. 

2-[2-(5-Cyclopropyl-1,3,4-oxadiazol-2-yl)propan-2-yl]pyridine (28a) A mixture of 27a (10.1 g, 40.8 mmol) and phosphorus oxychloride (20 mL), and then a solution of cyclopropanecarbonyl chloride (2.00 mL, 15.8 mmol) at 0°C, was added to a solution of 27a from 2methyl-2-(pyridin-4-yl)propanohydrazide (26b, 166 mg, 0.926 mmol, CAS No.; 950604-79-2) at 45% yield (103 mg, 0.418 mmol) as a white solid. $^1$H-NMR (300 MHz, DMSO–D$_6$) $\delta$: 1.03–1.11 (4H, m), 1.83 (6H, s), 2.03–2.16 (1H, m), 7.12–7.21 (2H, m), 7.60–7.68 (1H, m), 8.53–8.59 (1H, m). ESI-MS $m/z$: 230 [M + H]$^+$. 

2-[2-(4,5-Dicyclopropyl-4H-1,2,4-triazol-3-yl)propan-2-yl]pyridine Monohydrochloride (29a) Cyclopropyl amine (900 µL, 13.1 mmol) was slowly added to a solution of 28a (300 mg, 1.31 mmol) in AcOH (3 mL) at 0°C, and the mixture was reacted at 175°C for 40 min using a microwave reaction device. The reaction mixture was neutralized with 1 M aqueous sodium hydroxide solution and extracted with CHCl$_3$. The organic layer was washed with brine, dried over MgSO$_4$, filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc–MeOH then 90:10) to give a colorless oil. The title compound was prepared in a similar manner to that described for 29a from 28b (36.4 mg, 0.159 mmol) at 45% yield (10.5 mg, 0.0344 mmol) as a white solid. $^1$H-NMR (300 MHz, DMSO–D$_6$) $\delta$: 0.40–0.47 (2H, m), 0.74–0.82 (2H, m), 1.23–1.30 (4H, m), 1.85 (6H, s), 2.26–2.36 (1H, m), 3.05–3.14 (1H, m), 7.35–7.40 (1H, m), 7.53 (1H, d, $J = 8.0$ Hz), 7.90 (1H, ddd, $J = 1.8$, 7.8, 7.8 Hz), 8.47–8.51 (1H, m). ESI-MS $m/z$: 269 [M + H]$^+$. HR-MS (ESI) $m/z$: 269.1759 [M + H]$^+$. (Caled for C$_{16}$H$_{21}$N$_4$O$_2$: 269.1761). 

4-[2-(4,5-Dicyclopropyl-4H-1,2,4-triazol-3-yl)propan-2-yl]pyridine Monohydrochloride (29b) The title compound was prepared in a similar manner to that described for 29a from 28b (36.4 mg, 0.159 mmol) at 45% yield (10.5 mg, 0.0344 mmol) as a white solid. $^1$H-NMR (300 MHz, DMSO–D$_6$) $\delta$: 0.53–0.60 (2H, m), 0.81–0.89 (2H, m), 1.18–1.26 (4H, m), 1.86 (6H, s), 2.17–2.28 (1H, m), 3.03–3.12 (1H, m), 7.74 (2H, d, $J = 6.5$ Hz), 8.82 (2H, d, $J = 6.4$ Hz). ESI-MS $m/z$: 269 [M + H]$^+$. HR-MS (ESI) $m/z$: 269.1759 [M + H]$^+$. (Caled for C$_{16}$H$_{21}$N$_4$O$_2$: 269.1761).

11$\beta$-HSD1 and 11$\beta$-HSD2 Assay Reombinant human and mouse 11$\beta$-HSD1 enzymes were prepared in accordance with a previous study. Test compounds were dissolved in DMSO and diluted to the desired concentrations. The reaction was initiated by adding the compound to a reaction mixture containing 10 mM phosphate buffer (pH 6.6), 200 nM cortisone, 40 µM reduced nicotinamide adenine dinucleotide phosphate (NADPH) and the 11$\beta$-HSD1 enzyme, and incubating at r.t. for 1 h.

The 11$\beta$-HSD2 assay was conducted using the same method as that used for the 11$\beta$-HSD1 assay, except for the enzyme reaction conditions. The human 11$\beta$-HSD2 enzyme was produced in HEK293 cells transfected with an expression vector (pcDNA3.1, Invitrogen) encoding human 11$\beta$-HSD2. The crude extract from HEK293 cell homogenates was used as the enzyme source for the human 11$\beta$-HSD2 assay. The enzyme reaction was initiated by adding the compound to a reaction mixture containing 40 nM Tris–HCl buffer (pH 8.0), 200 nM cortisone, 200 µM nicotinamide adenine dinucleotide (NAD) and the 11$\beta$-HSD2 enzyme, and incubating at 37°C for 2 h. After the enzymatic reaction, the enzymatic inhibitory activity was measured by detecting cortisol using a HTRF®. Each of the XL-665-labeled cortisol containing 400 µM carbamoxolone and cryptate-labeled cortisol antibody (Cibisio Biosyssays, Codolet, France) was added and incubated at r.t. for 2 h, and the fluorescence intensity was measured using a fluorophotometer (ARVO HTS, PerkinElmer, Inc., Waltham, MA, U.S.A.). The enzymatic inhibitory activity was calculated from the fluorescence intensity ratio at two wavelengths.
(665nm/620nm). The ratio when DMSO was added instead of the compound was regarded as 0% and the ratio when neither 11β-HSD1 nor 11β-HSD2 was added was regarded as 100%.

Effect of Repeated Administration in ob/ob Mice All experiments were performed in accordance with the regulation of the Animal Ethics Committee of Astellas Pharma Inc.

Male diabetic ob/ob mice were purchased from Charles River Laboratories Japan (Kanagawa, Japan). Compound 9a was dissolved in 6% 2-hydroxypropyl-β-cyclodextrin (Sigma-Aldrich Co. LLC., St. Louis, MO, U.S.A.). Seven-week-old male ob/ob mice (n = 7–8) were orally administered vehicle or 0.3–10mg/kg of 9a twice daily for four weeks. At 12h after the final administration, plasma and retroperitoneal adipose tissue samples were collected. Plasma glucose levels were determined using the Glucose CII test (Wako, Osaka, Japan). Plasma insulin levels were determined using the Insulin enzyme-linked immunosorbent assay (ELISA) kit (Shibayagi, Gunma, Japan). Corticosterone levels in retroperitoneal adipose tissue were determined using the Corticosterone ELISA kit (Enzo Life Sciences, Farmingdale, NY, U.S.A.).

In Vitro Intrinsic Clearance with Mouse and Human Liver Microsomes To estimate the metabolic stability of compounds against mouse or human hepatic CYPs, the test compound (0.2μM) was incubated with pooled male CD1 mouse or human liver microsomes (0.2 mg protein/mL), NADPH (1mM) and ethylenediaminetetraacetic acid (EDTA) (0.1 mM) in pH 7.4 Na+-K+ phosphate buffer (100mM) at 37°C. Incubations were conducted for 0, 15, 30, and 45min. The peak area of the compound and internal standard was measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and analyzed to calculate CLint (mL/min/kg).

Molecular Modeling Human 11β-HSD1 Model

The crystal structure of human 11β-HSD1 was obtained from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB code: 3D5Q). After deleting C and D chains, hydrogen atoms were added to the protein using the Protonate3D module with the Amber10EHT force field in MOE. Docking Study

The ligand molecule was prepared using LigPrep and MacroModel and energy-minimized conformation was used to input molecules. Compounds were docked to the human 11β-HSD1 model using the docking program GOLD version 5.5.1. The ligand molecule was docked 10 times. The top scoring position, as assessed by GoldScore, was employed for discussion.

Sequence Homology between Human and Mouse

The crystal structure of mouse 11β-HSD1 was down-loaded from RCSB (PDB code: 4K26). The homology between human 11β-HSD1 (PDB code: 3D5Q) and mouse 11β-HSD1 (PDB code: 4K26) sequences was evaluated using MOE.

Acknowledgments The authors thank Koji Aoyama and Taisuke Nakazawa for their contribution to the biological evaluations, Susumu Watanuki for his helpful support in preparing this manuscript, and Yuichiro Sato and Hiroshi Kayakiri for their support. The authors also thank the staff of Astellas Research Technologies Co., Ltd., for conducting the metabolic clearance assay, elemental analysis, and spectral measurements.

Conflict of Interest The authors declare no conflict of interest.

References

1) Taylor S. L., Accili D., Imai Y., Diabetes, 43, 735–740 (1994).
2) American Diabetes Association, Diabetes Care, 35 (Suppl. 1), S64–S71 (2012).
3) Ross S. A., Guive E. A., Wang M., Chem. Rev., 104, 1255–1282 (2004).
4) Montague C. T., O’Rahilly S., Diabetes, 49, 883–888 (2000).
5) Kask E., Olsson T., Söderberg S., Andrew R., Livingstone D. E., Johnson D., Walker B. R., J. Clin. Endocrinol. Metab., 86, 1418–1421 (2001).
6) Lindsay R. S., Wake D. J., Nair S., Bunt J., Livingstone D. E., Permana P. A., Tatarannii P. A., Walker B. R., J. Clin. Endocrinol. Metab., 88, 2738–2744 (2003).
7) Masuzaki H., Paterson J., Shinuya H., Morton N. M., Mullins J. J., Seckl J. R., Flier J. S., Science, 294, 2166–2170 (2001).
8) Masuzaki H., Yamamoto H., Kenyon C. J., Elmqist J. K., Morton N. M., Paterson J. M., Shinuya H., Sharp M. G., Fleming S., Mullins J. J., Seckl J. R., Flier J. S., J. Clin. Invest., 112, 83–90 (2003).
9) Mune T., Rogerson F. M., Nikkili H., Agarwal A. K., White P. C., Nat. Genet., 10, 394–399 (1995).
10) Goldberg F. W., Dossetger A. G., Scott J. S., Robb G. R., Boyd S., Groombridge S. D., Kemmitt P. D., Sjogren T., Gutierrez P. M., deSchoolmeester J., Swales J. G., Turnbull A. V., Wild M. J., J. Med. Chem., 57, 970–986 (2014).
11) Abrahamsen L., Nilsson J., Opperman U., Svensson U., WO2005068646A1, 2005.
12) Siau M., Johnson T. O., Wang Y., Nair S. K., Taylor W. D., Cripps S. J., Matthews J. J., Edwards M. P., Pauly T. A., Ermoliev J., Castro A., Hosca N. A., LaPaglia A., Fanjul A. N., Vogel J. E., Bioorg. Med. Chem. Lett., 19, 3493–3497 (2009).
13) Feig P. U., Shah S., Hermanowski-Vosatkja A., Plotkin D., Springer M. S., Donahue S., Thich C., Klein F. J., Lai E., Kaufman K. D., Diabetes Obes. Metab., 13, 498–504 (2011).
14) Veniant M. M., Hale C., Hangate R. W., Gahm K., Emery M. G., Jons A., Joseph S., Adams J., Hague A., Moniz G., Zhang J., Bart- beger M. D., Li N., Syed R., Jordan S., Komorowski R., Chen M. M., Cupples R., Kim K. W., Steen D. J. Jr., Johansson L., Hen- riksson M. A., Williams M., Vallgårda J., Fotsch C., Wang M., J. Med. Chem., 53, 4481–4487 (2010).
15) Zhaung L., Tse C. M., Xu Z., Zhao W., Cacatian S., Ye Y. J., Singh S. B., Lindblom P., McKeever B. M., Krosby P. M., Zhao Y., Lala D., Kruk B. A., Meng S., Howard L., Johnson J. A., Bukhtiyarov Y., Panemangalore R., Guo J., Guo R., Himmelsbach F., Hamilton B., Scheler-Metz A., Schauerte H., Gregg R., McGeehan G. M., LeFtheris K., Clareon D. A., Bioorg. Med. Chem., 25, 3649–3657 (2017).
16) Wan Z.-K., Chenell E., Li H.-Q., Kendall C., Wang Y., Gingras S., Xiang J., Massafi S. W., Mansour T. S., Saiha E., J. Org. Chem., 56, 7048–7055 (2011).
17) ACD/Percepta, version 14.0.0, Advanced Chemistry Development, Inc., Toronto, ON, Canada, www.acdlabs.com, 2015.
18) Tu H., Powers J. P., Liu J., Ursu S., Sudom A., Yan X., Xu H., Meininger D., DeGravenreid M., He X., Jaen J. C., Sun D., Labelle M., Yamamoto H., Shan B., Walker N. P., Wang Z., Bioorg. Med. Chem., 16, 8922–8931 (2008).
19) GOLD5.5.1, The Cambridge Crystallographic Data Centre, Cambridge, U.K.
20) Jones G., Willett P., Glen R. C., J. Comput. Aided Mol. Des., 9, 525–540 (1995).
21) Jones G., Willett P., Glen R. C., Leach A. R., Taylor R., J. Mol.
22) Molecular Operating Environment (MOE), version MOE2016.0802, Chemical Computing Group Inc., 1010 Sherbrooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2016.

23) Walker E. A., Clark A. M., Hewison M., Ride J. P., Stewart P. M., J. Biol. Chem., 276, 21343–21350 (2001).

24) Naritomi Y., Terashita S., Kimura S., Suzuki A., Kagayama A., Sugiyama Y., Drug Metab. Dispos., 29, 1316–1324 (2001).

25) Tu H., Powers J. P., Liu J., Ursu S., Sudom A., Yan X., Xu H., Meininger D., Degrassenreid M., He X., Jaen J. C., Sun D., Labelle M., Yamamoto H., Shan B., Walker N. P., Wang Z., Bioorg. Med. Chem., 16, 8922–8931 (2008).

26) LigPrep (version 44011), Schrodinger, LLC, New York, NY.

27) MacroModel, Schrodinger, LLC, New York, NY.

28) Böhme T., Engel C. K., Farjot G., Güssregen S., Haack T., Tschank G., Ritter K., Bioorg. Med. Chem. Lett., 23, 4685–4691 (2013).