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Discovery of *N*-(1-(3-(4-phenoxyphenyl)-1,2,4-oxadiazol-5-yl)ethyl) acetamides as novel acetyl-CoA carboxylase 2 (ACC2) inhibitors with peroxisome proliferator-activated receptor α/δ (PPARα/δ) dual agonistic activity

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ABSTRACT

Acetyl-CoA carboxylases (ACCs) catalyze a critical step in *de novo* lipogenesis, and are considered as promising targets for treatment of obesity, dyslipidemia and type 2 diabetes mellitus. On the other hand, peroxisome proliferator-activated receptors (PPARs) are well-established therapeutic targets for these metabolic syndrome-related diseases. Therefore, we considered that dual modulators of ACC and PPARs would be promising candidates as therapeutic agents. Here, we designed a series of acetamides based on the molecular similarity between ACC inhibitors and PPAR agonists. Screening of the synthesized compounds identified *N*-(1-(3-(4-phenoxyphenyl)-1,2,4-oxadiazol-5-yl)ethyl)acetamides as novel ACC2 inhibitors with PPARα/PPARδ dual agonistic activity. Structure–activity relationship studies and further structural elaboration afforded compounds with distinct activity profiles. Our findings should be helpful for the discovery of candidate agents with an appropriate balance of ACC-inhibitory and PPAR-activating activities for therapeutic lipid control.

1. Introduction

The term metabolic syndrome refers to a cluster of risk factors, including central obesity, hypertension (high blood pressure), elevated fasting plasma glucose, dyslipidemia (e.g., elevated triglycerides and low high-density lipoprotein cholesterol levels), and insulin resistance, that lead to type 2 diabetes mellitus (T2DM) as well as cardiovascular diseases (CVD). It is a serious public health problem worldwide.[1] Although the molecular mechanisms of the pathogenesis of metabolic syndrome are not fully understood, it is established that disordered lipid metabolism is closely related to the development of obesity and insulin resistance.[2–5] For example, ectopic lipid accumulation in non-adipose tissues such as liver and skeletal muscle is associated with insulin resistance.[2] Therefore, rebalancing of lipid metabolism is expected to be beneficial for a whole range of complications associated with metabolic syndrome.[5] Inhibition of acetyl-CoA carboxylases (ACCs) has recently received particular attention in this area.[6] ACCs catalyze the ATP-dependent carboxylation of acetyl-CoA to form malonyl-CoA. Malonyl-CoA is a substrate of fatty acid synthetase (FAS) in *de novo* lipogenesis and a negative regulator of carnitine palmitoyltransferase 1 (CPT1), a mitochondrial outer membrane protein that transports free fatty acyl-CoAs into mitochondria for β-oxidation (fatty acid catabolism step). In humans, there are two ACC isoforms; cytosolic ACC1 is primarily expressed in lipid-rich tissues (liver and adipose) and is involved in lipogenesis, whereas mitochondrial ACC2 is primarily found in oxidative tissues (muscle) and mainly controls fatty acid oxidation. Knockdown of ACC1, ACC2 or both significantly increases fatty acid oxidation in rat hepatocytes[7], and an ACC inhibitor, soraphen A, improves insulin sensitivity in mice fed a high-fat diet.[8] These data indicate that ACC inhibition could be useful in the treatment of T2DM and obesity.

The peroxisome proliferator-activated receptors (PPARs) have also been recognized as molecular targets for such metabolic syndrome-related diseases.[9] PPARs are ligand-dependent transcription factors belonging to the nuclear hormone receptor superfamily. They regulate transcription of a large number of different genes involved in lipid metabolism, glucose homeostasis and adipogenesis, in response to the binding of endogenous lipophilic ligands (e.g. prostaglandins, fatty acids and their metabolites). There are three

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subtypes of PPAR, commonly designated as PPARα, PPARγ and PPARδ (also known as PPARβ). PPARα is found in tissues with high rates of fatty acid catabolism, such as brown adipose tissue and liver. PPARγ is predominantly expressed in adipose tissue, whereas the third subtype, PPARδ, is expressed in many tissues. PPARα agonists, represented by fibrates (e.g. bezafibrate, fenofibrate), have been clinically used for the treatment of dyslipidemia and insulin resistance. PPARγ agonists, for example thiazolidine-2,4-diones such as pioglitazone, which improve insulin sensitivity and glycemic control, are currently being used to treat patients with T2DM. The therapeutic potential of PPARδ agonists is still under investigation, but a potent subtype-selective PPARδ agonist GW501516 was shown to improve insulin sensitivity in animal models of T2DM.[10]

Because both ACCs and PPARs are key targets for regulation of lipid metabolism, compounds that simultaneously inhibit ACCs and activate PPARs may offer synergistic benefits. Reviews of multi-target drugs,[11] the magic shotgun approach[12] and polypharmacology[13] have suggested that drugs engaging two or more targets can be therapeutically superior to single-target drugs. For example, dual modulators that target PPAR and soluble epoxide hydrolase (sEH)[14,15] or angiotensin II type 1 receptor (AT1)[16] have recently been developed. However, to our knowledge, there is no report on ACC/PPAR dual modulators. Currently available fibrates have significant therapeutic benefits, but their disadvantages include weak agonistic activities and dose-dependent side effects;[17] it is possible that these issues might be overcome by multi-targeting. Driven largely by these concepts, we designed a library of candidate dual modulators of ACC and PPAR on the basis of the molecular similarity between known ACC inhibitors and PPAR agonists. Screening of the synthesized compounds resulted in identification of *N*-(1-(3-(4-phenoxyphenyl)-1,2,4-oxadiazol-5-yl)ethyl)acetamides as novel ACC2 inhibitors with PPARα/δ dual agonistic activity. Further structural elaboration based on structure–activity relationship (SAR) studies led to (***S***)-**32**, which inhibited ACC2 and activated PPARα and PPARδ at submicromolar concentrations [(***S***)-**32**: IC_{50} (ACC2) = 0.22 μM, EC_{50} (PPARα) = 0.15 μM, EC_{50} (PPARδ) = 0.29 μM].

2. Results and discussion

2.1. Molecular design

Compounds **1**–**3** have been reported as ACC1/2 dual inhibitors with low to moderate ACC2 selectivity (Fig. 1).[18,19] These compounds have linear lipophilic structures with a pendant polar acetamide group. Similar molecular features are seen in known PPAR agonists (Fig. 2), i.e., bezafibrate, pioglitazone and GW501516 have linear lipophilic cores with an acidic head group (carboxylic acid or thiazolidine-2,4-dione). This observation suggests that there may be an overlapping pharmacophore for ACC inhibitors and PPAR agonists. We thus speculated that derivatives of these compounds might be dual modulators of ACC and PPAR. Although the binding mode of **1**–**3** is unknown, the acetamide group and adjacent methyl group, and terminal alkoxy/phenoxy group seem to be important for the ACC-inhibitory activity according to previously reported SAR studies.[18] Taking into account the fact that PPARs have large ligand-binding cavities,[9,20] we designed and prepared a series of acetamide-based compounds as candidate dual modulators (Fig. 3, compounds **4**–**11**). 1,2,4-Oxadiazoles were designed because of the ease of preparation of acetamides from nitriles and *N*-acetylamino acids (e.g., *N*-acetylalanine).

2.2. Synthesis and evaluation of the designed compounds

The designed compounds **4**–**7** were synthesized as outlined in Scheme 1. 4-Isopropoxyphenol, prepared from hydroquinone,[21] was treated with 4-fluorobenzonitrile in the presence of K_2CO_3 to afford **12**. Compound **13** was similarly obtained from commercially available 4-benzyloxyphenol. Compounds **12** and **13** were converted into amidoximes **14** and **15**, which in turn were condensed with *N*-acetylamino acids to afford the desired compounds **4** and **5**, as well as *O*-benzyl-protected **16**. Removal of the benzyl group of **16** gave **6**, which was alkylated with 1-iodopropane to afford **7**. Benzimidazoles **8**–**10** were prepared as shown in Scheme 2. First, 2-chloro-5-cyanobenzimidazole, synthesized from 3,4-

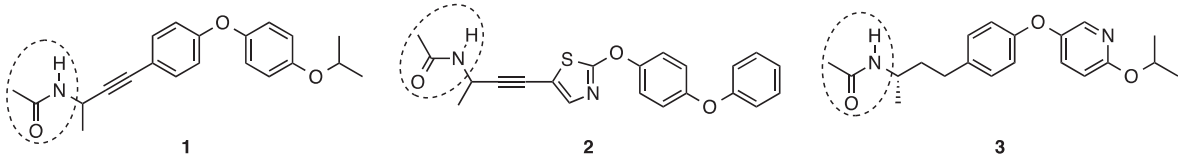

Figure 1. Chemical structures of known ACC1/2 dual inhibitors **1**–**3**.

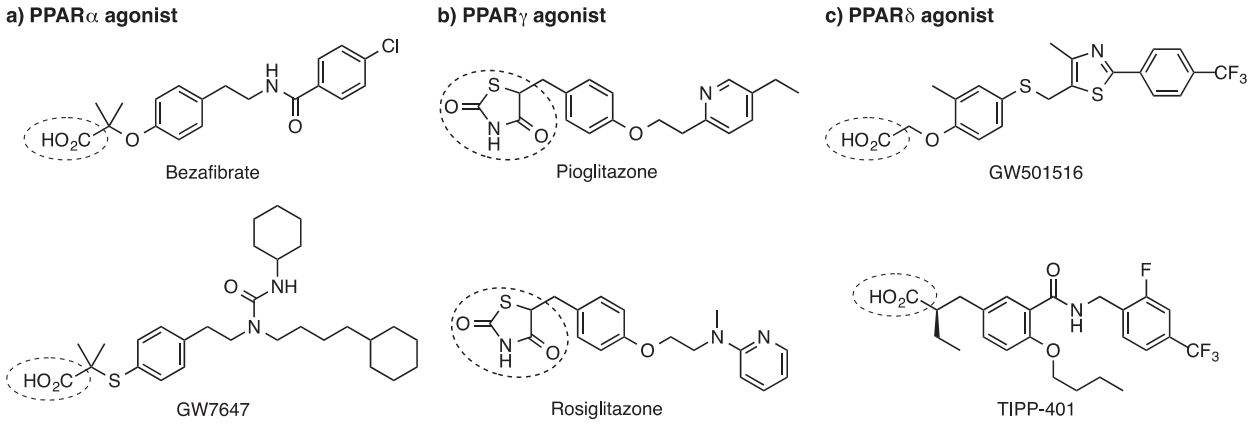

Figure 2. Chemical structures of PPARα agonists (bezafibrate and GW7647), PPARγ agonists (pioglitazone and rosiglitazone) and PPARδ agonists (GW501516 and TIPP-401).

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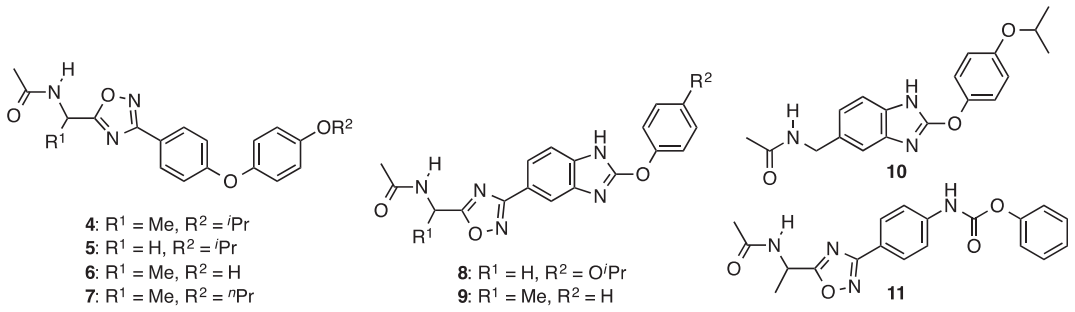
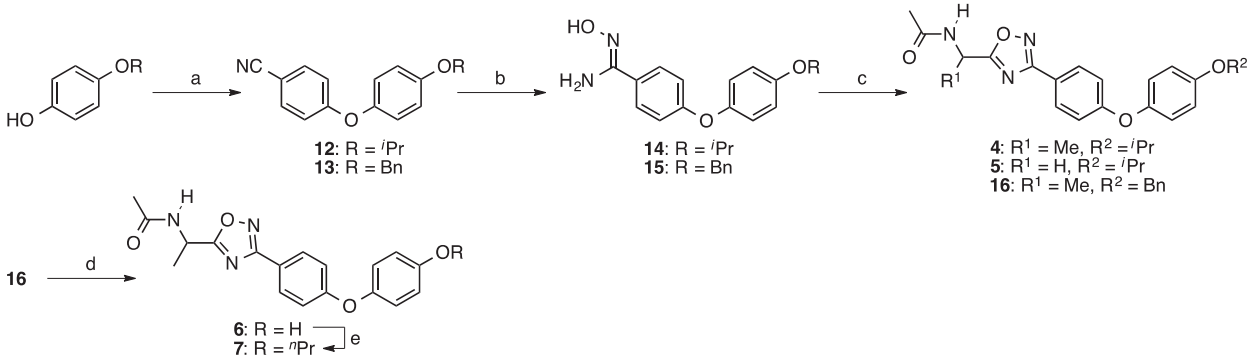
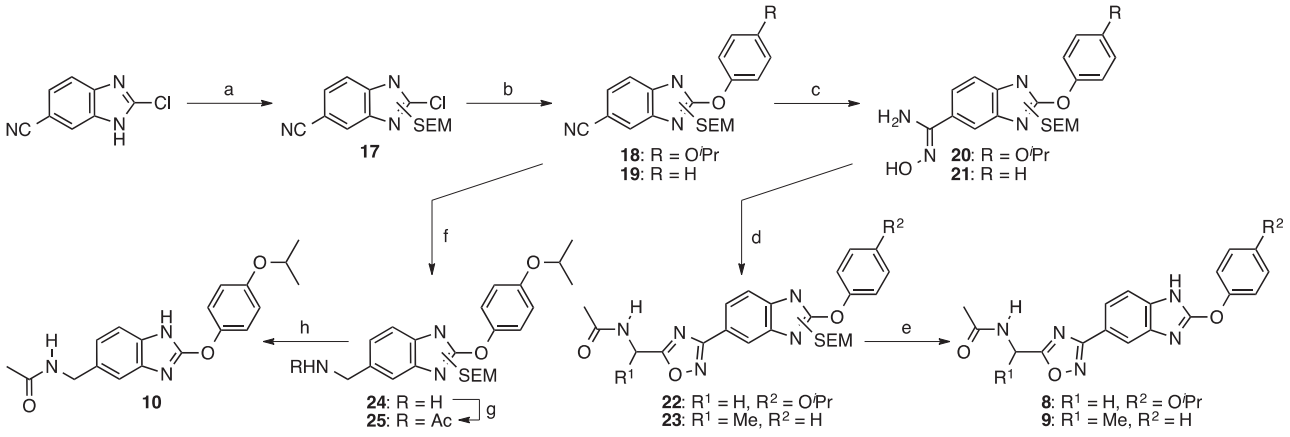


Figure 3. The designed acetamide-based compounds.



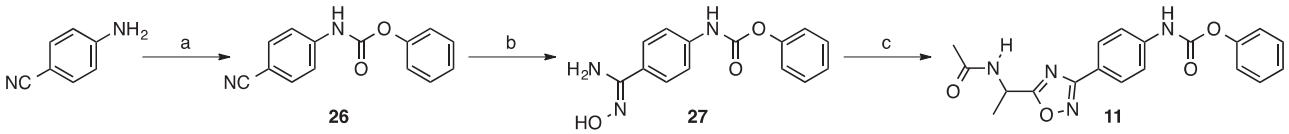
Scheme 1. Reagents and conditions: (a) 4-fluorobenzonitrile, K_2CO_3, DMF, 150 °C, 97% (for **12**), 97% (for **13**); (b) aq NH_2OH, EtOH, reflux, 58% (for **14**), 52% (for **15**); (c) *N*-acetyl-DL-alanine (for **4** and **16**), *N*-acetylglycine (for **5**), HATU, DIPEA, DMF, rt to 80–100 °C, 46% (for **4**), 65% (for **5**), 70% (for **16**); (d) BBr_3, CH_2Cl_2, −50 to −20 °C, 91% ; (e) 1-iodopropane, Cs_2CO_3, DMF, 80 °C, 94%.



Scheme 2. Reagents and conditions: (a) SEMCl, Et_3N, THF, rt, 87%; (b) 4-isopropoxyphenol (for **18**), phenol (for **19**), Cs_2CO_3, DMF, rt, 99% (for **18**), 99% (for **19**); (c) aq NH_2OH, EtOH, reflux, 87% (for **20**), 93% (for **21**); (d) *N*-acetylglycine (for **22**), *N*-acetyl-DL-alanine (for **23**), HATU, DIPEA, DMF, rt to 90 °C; (e) TBAF, THF, reflux, 13% in 2 steps (for **8**), 22% in 2 steps (for **9**); (f) H_2, Raney Ni, aq NH_3, EtOH, rt, 97%; (g) Ac_2O, Et_3N, CH_2Cl_2, rt, 85%; (h) TBAF, THF, reflux, 55%.

diaminobenzonitrile,[22] was protected using 2-(trimethylsilyl) ethoxymethyl chloride (SEMCl) to give **17** as a 1:1 regioisomeric mixture (1-*N*-SEM and 3-*N*-SEM). Coupling of **17** with phenols provided the corresponding 2-phenoxybenzimidazoles **18** and **19**. Compounds **8** and **9** were synthesized from **18** and **19** similarly to the above 1,2,4-oxadiazole synthesis. Compound **10** was prepared by reduction of the cyano group of **18** followed by acetylation and SEM deprotection. Carbamate **11** was simply obtained from 4-aminobenzonitrile (Scheme 3).

The prepared compounds were evaluated for ACC-inhibitory and PPAR-agonistic activities (Table 1). We used recombinant human ACC2, and performed ATP consumption assay[19] (see Section 4). Some of compounds exhibited partial inhibition. Thus, the IC_{50} values were determined from the dose–response curves, and the values of percent inhibition at 30 μM were calculated relative to the maximum ACC2 inhibition of **1**. For evaluation of PPAR-agonistic activities, we adopted a cell-based reporter gene assay using CMX-GAL4N-hPPAR LBD as a recombinant receptor gene, TK-MH100x4-LUC as a reporter gene, and CMX β-galactosidase gene for normalization, as previously reported.[23] The EC_{50} values were determined from the dose–response curves, and the maximum efficacies (E_{max}) were calculated relative to those of known PPAR agonists (GW7647 for PPARα, rosiglitazone for PPARγ, and GW501516 for PPARδ).



Scheme 3. Reagents and conditions: (a) phenyl chloroformate, pyridine, THF, 0 °C to rt, 98%; (b) aq NH_2OH, EtOH, 50 °C to reflux, 29%; (c) *N*-acetyl-DL-alanine, HATU, DIPEA, DMF, rt to 90 °C, 78%.

Table 1
ACC2-inhibitory and PPAR-agonistic activities of acetamide-based compounds

Compound	ACC2[a] IC_{50} [μM] (inhibition [%])[c,d]	PPARα[b] EC_{50} [μM] (efficacy [%])[c,e]	PPARγ[b] EC_{50} [μM] (efficacy [%])[c,e]	PPARδ[b] EC_{50} [μM] (efficacy [%])[c,e]
4	0.10 (74)	ia.[f]	ia.[f]	ia.[f]
5	1.1 (83)	ia.[f]	ia.[f]	ia.[f]
6	(24)	1.0 (13–33)	ia.[f]	1.0 (2–9)
7	0.61 (88)	ia.[f]	ia.[f]	ia.[f]
8	ia.[f]	ia.[f]	ia.[f]	ia.[f]
9	ia.[f]	ia.[f]	ia.[f]	ia.[f]
10	ia.[f]	ia.[f]	ia.[f]	ia.[f]
11	ia.[f]	ia.[f]	ia.[f]	ia.[f]
1	0.011 (100)	ia.[f]	ia.[f]	ia.[f]
GW7647	—	0.021 (100)	—	—
Rosiglitazone	—	—	0.26 (100)	—
GW501516	—	—	—	0.0059 (100)

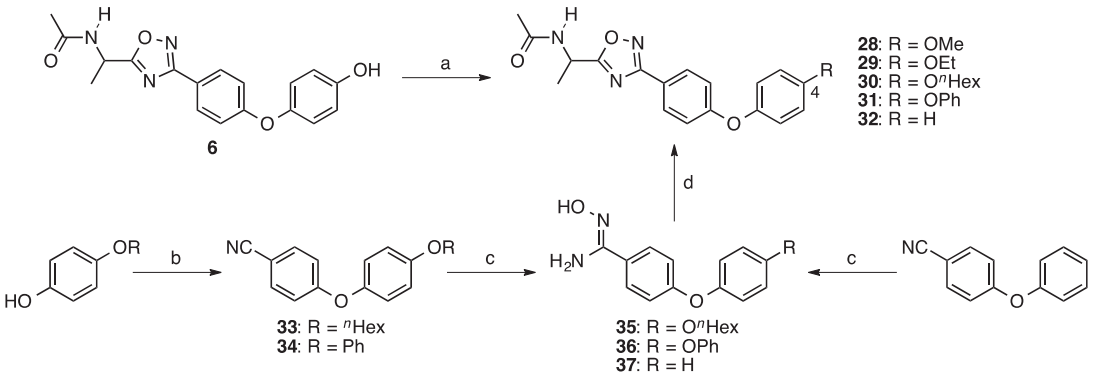
[a] Inhibitory activity towards recombinant human ACC2.
[b] Agonistic activities towards PPAR(LBD)-GAL4 chimeric receptors in transiently transfected HEK-293 cells.
[c] Evaluations were performed in triplicate, and the EC_{50} and IC_{50} values were determined from the dose–response curves.
[d] Percent inhibition at 30 μM is shown in parenthesis. The maximum ACC2 inhibition by **1** was defined as 100%.
[e] Maximum PPAR-agonistic activities from several independent evaluations are shown in parenthesis. The maximal efficacies of GW7647, rosiglitazone and GW501516 were defined as 100% for PPARα, PPARγ and PPARδ, respectively.
[f] ia. = inactive at <20 μM.

Among the designed compounds, **4**, **5**, **6** and **7** exhibited ACC2-inhibitory activity, and **4**, which has a similar structure to **1**, was the most potent. Neither **4** nor **1** showed activity against PPARs. However, **6** showed both ACC2-inhibitory activity and partial PPARα/δ dual agonistic activity, although the former activity was weak (24% inhibition at 30 μM). PPARγ agonistic activity was not seen at concentrations up to 20 μM.

2.3. Structure–activity investigations of the dual modulator 6

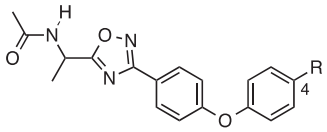
To improve the ACC2-inhibitory activity and the PPARα/δ dual agonistic activity of **6**, we initially focused on exploring substituents at the 4-position of the phenoxyphenyl group because it is known that terminal alkoxy/phenoxy group affects greatly to the ACC-inhibitory activity.[19,24] For this purpose, compounds **28**–**32** were prepared using a similar route to that in Scheme 1 (Scheme 4), and the obtained compounds were evaluated for ACC2-inhibitory activity (Table 2). The order of ACC2-inhibitory activity was OH (**6**) ≈ OMe (**28**) < OEt (**29**) < H (**32**) ≈ O^{n}Hex (**30**) < O^{i}Pr (**4**) < OPh (**31**), which is consistent with the reported SAR of acetamide-based ACC inhibitors (OEt ≪ O^{i}Pr ⩽ OPh).[18,24] Agonistic activities towards PPARs were also evaluated, and we found that **28**, **29** and **32** were active at submicromolar concentrations. Compounds **29** and **32** were identified as more potent dual modulators of ACC2 and PPARα/δ. Further, their maximum efficacies towards PPARα and PPARδ were similar to those of compound **6**. It is interesting that structurally similar compounds exhibited dramatically different activities (Table 2). For example, compounds **6** and **28** are PPAR-selective, whereas the phenoxy analogue (**31**) exhibits selective ACC inhibition. These compounds might be useful tools to clarify the roles of ACC and PPAR in lipid metabolism.

We next focused on the methylene bridge between the acetamide and the oxadiazole. Compounds **38** and **39** were prepared by coupling with *N*-acetylglycine (Scheme 5), and their activities



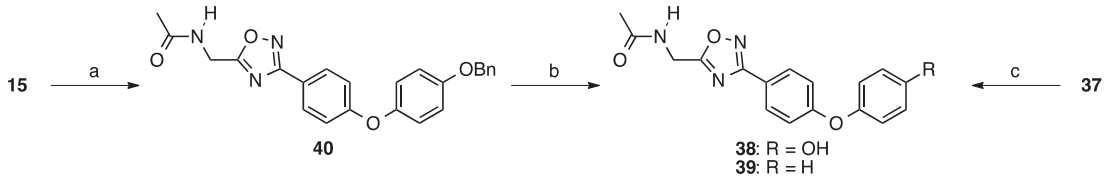
Scheme 4. Reagents and conditions: (a) Me_2SO_4 (for **28**), K_2CO_3 (for **28**), EtI (for **29**), Cs_2CO_3 (for **29**), DMF, 90 °C, 23% (for **28**), 83% (for **29**); (b) 4-fluorobenzonitrile, K_2CO_3, DMF, 150 °C, 99% (for **33**), 81% (for **34**); (c) aq NH_2OH, EtOH, reflux, 73% (for **35**), 79% (for **36**), 96% (for **37**); (d) *N*-acetyl-DL-alanine, HATU, DIPEA, DMF, rt to 90 °C, 47% (for **30**), 92% (for **31**), 87% (for **32**).

Table 2
SAR investigations of substituents at the 4-position of the phenoxyphenyl group



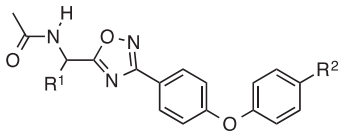
Compound	R	ACC2[a] IC_{50} [μM] (inhibition [%])[c,d]	PPARα[b] EC_{50} [μM][c]	PPARγ[b] EC_{50} [μM][c]	PPARδ[b] EC_{50} [μM][c]
28	OMe	(17)	0.21	ia.[e]	0.39
29	OEt	0.42 (35)	0.33	ia.[e]	0.37
30	O^{n}Hex	0.26 (93)	ia.[e]	ia.[e]	ia.[e]
31	OPh	0.048 (76)	ia.[e]	n.t.[e]	ia.[e]
32	H	0.29 (45)	0.32	ia.[e]	0.32
6	OH	(24)	1.0	ia.[e]	1.0

[a] Inhibitory activity towards recombinant human ACC2.
[b] Agonistic activities towards PPAR(LBD)-GAL4 chimeric receptors in transiently transfected HEK-293 cells. All active compounds showed partial agonistic properties, as did compound **6** (see Table 1).
[c] Evaluations were performed in triplicate, and the EC_{50} and IC_{50} values were determined from the dose–response curves.
[d] Percent inhibition at 30 μM is shown in parenthesis. The maximum ACC2 inhibition by **1** was defined as 100%.
[e] ia. = inactive at <20 μM. n.t. = not tested.



Scheme 5. Reagents and conditions: (a) *N*-acetylglycine, HATU, DIPEA, DMF, rt to 90 °C, 76%; (b) BBr_3, CH_2Cl_2, −50 to −20 °C, 92% (for **38**); (c) *N*-acetylglycine, HATU, DIPEA, DMF, rt to 90 °C, 62% (for **39**).

Table 3
SAR investigations on the methylene bridge between acetamide and oxadiazole



Compound	R^1	R^2	ACC2[a] IC_{50} [μM] (inhibition [%])[c,d]	PPARα[b] EC_{50} [μM][c]	PPARγ[b] EC_{50} [μM][c]	PPARδ[b] EC_{50} [μM][c]
38	H	OH	(3)	ia.[e]	n.t.[e]	n.t.[e]
6	Me	OH	(24)	1.0	ia.[e]	1.0
39	H	H	ia.[e]	ia.[e]	n.t.[e]	n.t.[e]
32	Me	H	0.29 (45)	0.32	ia.[e]	0.32

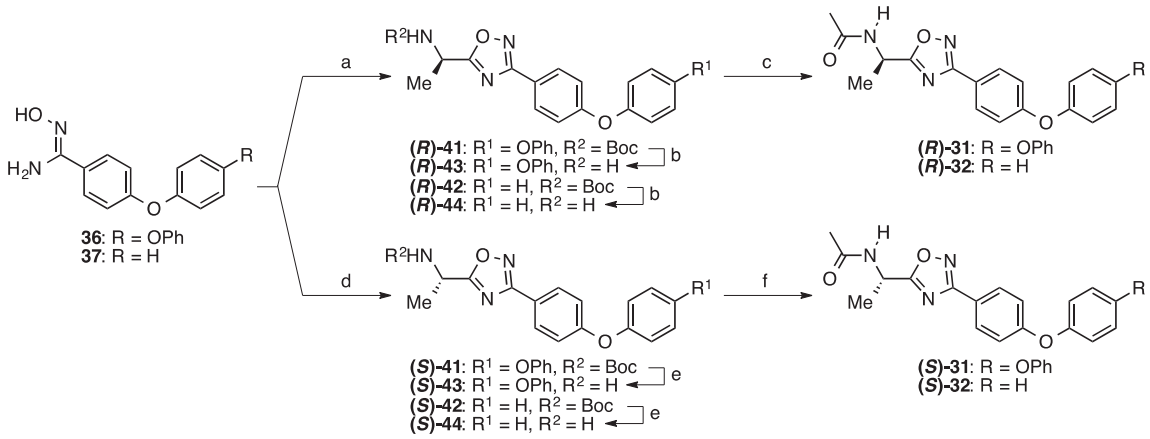
[a] Inhibitory activity towards recombinant human ACC2.
[b] Agonistic activities towards PPAR(LBD)-GAL4 chimeric receptors in transiently transfected HEK-293 cells. All active compounds showed partial agonistic properties, as did compound **6** (see Table 1).
[c] Evaluations were performed in triplicate, and the EC_{50} and IC_{50} values were determined from the dose–response curves.
[d] Percent inhibition at 30 μM is shown in parenthesis. The maximum ACC2 inhibition by **1** was defined as 100%.
[e] ia. = inactive at <20 μM (for PPAR) or <30 μM (for ACC). n.t. = not tested.

were evaluated (Table 3). Conversion of the monomethyl methylene bridge (–CHMe–) to methylene ($-CH_2-$) considerably decreased the PPARα-agonistic activity as well as the ACC2-inhibitory activity. The results indicated that the methyl group at the methylene bridge is critical for both ACC-inhibitory activity and PPAR-agonistic activity.

Next, we synthesized both enantiomers of the most potent ACC2 inhibitor **31** and dual modulator **32** by using commercially available chiral *N*-Boc-alanines (Scheme 6). The (*S*)-isomers of **31** and **32** were found to show higher ACC2-inhibitory activities than the corresponding (*R*)-isomers (Table 4). This result is consistent with previous reports on other acetamide-based ACC inhibitors.[18,24] However, it is noteworthy that (***S***)-**32** exhibited PPARα/δ dual agonistic activities, whereas (***R***)-**32** did not. Thus, among the synthesized compounds, (***S***)-**32** was the most potent dual modulator of ACC2 and PPARα/δ.

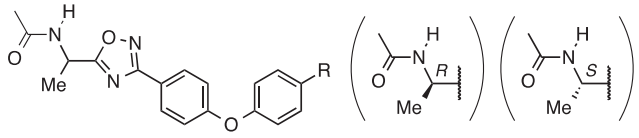
3. Conclusions

We designed a series of candidate dual modulators of ACC and PPAR based on the molecular similarity between known ACC inhibitors and PPAR agonists. Among the synthesized compounds, **6** showed weak ACC2-inhibitory activity together with PPARα/δ dual agonistic activity. Structure-activity investigations and structural



Scheme 6. Reagents and conditions: (a) *N*-(*tert*-butoxycarbonyl)-D-alanine, HATU, DIPEA, DMF, rt to 90 °C, 65% (for (***R***)-**41**), 69% (for (***R***)-**42**); (b) TFA, CH_2Cl_2, 0 °C to rt, quant. (for (***R***)-**43**), 90% (for (***R***)-**44**); (c) Ac_2O, pyridine, CH_2Cl_2, 0 °C to rt, 73% (for (***R***)-**31**), 90% (for (***R***)-**32**); (d) *N*-(*tert*-butoxycarbonyl)-L-alanine, HATU, DIPEA, DMF, rt to 90 °C, 69% (for (***S***)-**41**), 61% (for (***S***)-**42**); (e) TFA, CH_2Cl_2, 0 °C to rt, quant. (for (***S***)-**43**), 98% (for (***S***)-**44**); (f) Ac_2O, pyridine, CH_2Cl_2, 0 °C to rt, 74% (for (***S***)-**31**), 79% (for (***S***)-**32**).

Table 4
SAR investigations on the stereochemistry of the methyl group near acetamide



Compound	Me	R	ACC2[a] IC_{50} [μM] (inhibition [%])[c,d]	PPARα[b] EC_{50} [μM][c]	PPARγ[b] EC_{50} [μM][c]	PPARδ[b] EC_{50} [μM][c]
31	*rac*	OPh	0.048 (76)	ia.[e]	ia.[e]	ia.[e]
(***R***)-**31**	*R*	OPh	7.5 (67)	ia.[e]	n.t.[e]	ia.[e]
(***S***)-**31**	*S*	OPh	0.016 (86)	ia.[e]	n.t.[e]	ia.[e]
32	*rac*	H	0.29 (45)	0.32	ia.[e]	0.32
(***R***)-**32**	*R*	H	(37)	ia.[e]	n.t.[e]	ia.[e]
(***S***)-**32**	*S*	H	0.22 (65)	0.15	n.t.[e]	0.29

[a] Inhibitory activity towards recombinant human ACC2.
[b] Agonistic activities towards PPAR(LBD)-GAL4 chimeric receptors in transiently transfected HEK-293 cells. All active compounds showed partial agonistic properties, as did compound **6** (see Table 1).
[c] Evaluations were performed in triplicate, and the EC_{50} and IC_{50} values were determined from the dose–response curves.
[d] Percent inhibition at 30 μM is shown in parenthesis. The maximum ACC2 inhibition of **1** was defined as 100%.
[e] ia. = inactive at <20 μM. n.t. = not tested.

elaboration of **6** led us to the more potent dual modulator **32** and the ACC2-selective inhibitor **31**. The (*S*)-isomers of **32** and **31** were identified as the eutomers for ACC2 inhibition and PPARα/δ activation [(***S***)-**32**: IC_{50} (ACC2) = 0.22 μM, EC_{50} (PPARα) = 0.15 μM, EC_{50} (PPARδ) = 0.29 μM; (***S***)-**31**: IC_{50} (ACC2) = 0.016 μM]. Importantly, some of our compounds show distinct activity profiles; for example, compound **31** selectively inhibits ACC2, whereas compound **28** mainly exhibits PPARα/δ agonistic activity. These compounds are expected to be useful tools for investigating the appropriate balance of ACC inhibition and PPAR activation to achieve therapeutic lipid control.[25]

4. Experimental

4.1. Chemistry

4.1.1. General

All chemical reagents and solvents were purchased from Sigma–Aldrich, Kanto Chemical, Tokyo Chemical Industry and Wako Pure Chemical Industries, and used without further purification. Moisture-sensitive reactions were performed under an atmosphere of argon, unless otherwise noted, and monitored by thin-layer chromatography (TLC, Merck silica gel 60 F254 plate). Bands were visualized using UV light or by application of appropriate reagents followed by heating. Flash chromatography was carried out with silica gel (Silica gel 60N, 40–50 μm particle size) purchased from Kanto Chemical. NMR spectra were recorded on a JEOL JNM-ECA500 (500 MHz) spectrometer, operating at 500 MHz for ^{1}H NMR and at 125 MHz for ^{13}C NMR. Proton and carbon chemical shifts are expressed in δ values (ppm) relative to internal tetramethylsilane (0.00 ppm), residual $CHCl_3$ (7.26 ppm), CHD_2OD (3.31 ppm) or C_2HD_5SO (2.49 ppm) for ^{1}H NMR, and internal tetramethylsilane (0.00 ppm) or $CDCl_3$ (77.16 ppm), methanol-d_4 (49.00 ppm) or dimethyl sulfoxide-d_6 (39.50 ppm) for ^{13}C NMR. Data are reported as follows: chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad), coupling constants (Hz), integration. High-resolution mass spectra were recorded using a Bruker micrOTOF II mass spectrometer. Fast atom bombardment (FAB) mass spectra were recorded on a JEOL JMA-HX110 mass spectrometer with *m*-nitrobenzyl alcohol as a matrix.

4.1.2. General procedure A: coupling reaction of phenol and 4-fluorobenzonitrile

To a solution of phenol (1.2 equiv) and 4-fluorobenzonitrile (1.0 equiv) in anhydrous DMF was added K_2CO_3 (1.2 equiv). The

mixture was stirred at 150 °C for 2–9 h, then poured into ice-cold water, and extracted with CH_2Cl_2 or AcOEt. The combined organic layer was washed with water, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford the product.

4.1.3. General procedure B: reaction of benzonitrile and hydroxylamine

To a solution of benzonitrile (1.0 equiv) in EtOH was added 50% aq hydroxylamine (4.0–5.0 equiv). The mixture was refluxed for 1–3 h, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford the product.

4.1.4. General procedure C: condensation of amidoxime and amino acid

A solution of amino acid (1.5 equiv), HATU (1.5 equiv) and DIEA (1.5 equiv) in anhydrous DMF was stirred at room temperature for 15–60 min. Amidoxime (1.0 equiv) was then added, and the resulting mixture was further stirred at room temperature for 25–120 min and then at 80–100 °C for 0.5–9 h. After the reaction, the mixture was diluted with AcOEt and washed with aq $NaHCO_3$. The organic layer was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography to afford the product.

4.1.5. General procedure D: removal of Bn group

BBr_3 (0.2–0.4 equiv, 1 M in CH_2Cl_2) was slowly added to a solution of benzyloxy compound (1.0 equiv) in anhydrous CH_2Cl_2 at −50 °C, and the mixture was stirred at −20 °C for 1.5 h. The reaction was then quenched by addition of water. The resulting mixture was warmed to ambient temperature, diluted with CH_2Cl_2 and aq HCl, and extracted with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford the product.

4.1.6. General procedure E: removal of SEM group

To a solution of SEM-protected compound (1.0 equiv) in THF was added TBAF (3.0 equiv, 1 M in THF). The mixture was refluxed for 11–20 h, then cooled to room temperature, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography or PTLC to afford the product.

4.1.7. General procedure F: removal of Boc group

To a solution of Boc-protected compound (1.0 equiv) in CH_2Cl_2 was added TFA (10 equiv) at 0 °C. The mixture was stirred at room temperature for 1–5 h, and then concentrated under reduced pressure to afford the product.

4.1.8. General procedure G: acetylation of amine

To a solution of amine (1.0 equiv) in CH_2Cl_2 was added pyridine (5.0 equiv) and acetic anhydride (1.2–2.0 equiv) at 0 °C. The mixture was stirred at room temperature for 2–14 h, and then diluted with CH_2Cl_2 and 0.5 N aq HCl. The organic layer was washed with 0.5 N aq HCl and saturated aq $NaHCO_3$, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by recrystallization (*n*-hexane/AcOEt) to afford the product.

4.1.9. 4-(4-Isopropoxyphenoxy)benzonitrile (12)

General procedure A: A white solid (97%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57 (ddd, J = 9.2, 2.6, 2.6 Hz, 2H), 7.00–6.97 (m, 2H), 6.96 (ddd, J = 9.2, 2.3, 2.3 Hz, 2H), 6.92 (ddd, J = 9.2, 3.1, 3.1 Hz, 2H), 4.52 (sep, J = 6.3 Hz, 1H), 1.35 (d, J = 6.3 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.7, 155.4, 147.8, 134.2, 121.9, 119.1, 117.4, 117.3, 105.4, 70.7, 22.2; MS (FAB) m/z 254 $(M+H)^+$.

4.1.10. 4-(4-Benzyloxyphenoxy)benzonitrile (13)

General procedure A: A white solid (97%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57 (ddd, J = 9.2, 2.3, 2.3 Hz, 2H), 7.46–7.40 (m, 4H), 7.33–7.37 (m, 1H), 7.01 (s, 4H), 6.96 (ddd, J = 9.2, 2.3, 2.3 Hz, 2H), 5.08 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.5, 156.3, 148.2, 136.8, 134.2, 128.8, 128.3, 127.6, 121.9, 119.1, 117.3, 116.4, 105.4, 70.6; MS (FAB) m/z 302 $(M+H)^+$.

4.1.11. 4-(4-Isopropoxyphenoxy)benzamidoxime (14)

General procedure B: An yellow solid (58%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56 (ddd, J = 9.7, 2.6, 2.6 Hz, 2H), 6.98–6.93 (m, 4H), 6.88 (ddd, J = 9.0, 2.3, 2.3 Hz, 2H), 4.84 (s, 2H), 4.50 (sep, J = 5.7 Hz, 1H), 1.34 (d, J = 5.7 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.3, 154.7, 152.5, 149.4, 127.5, 126.6, 121.3, 117.4, 117.3, 70.7, 22.2; MS (FAB) m/z 287 $(M+H)^+$.

4.1.12. 4-(4-Benzyloxyphenoxy)benzamidoxime (15)

General procedure B: A white solid (52%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.54 (s, 1H), 7.63 (ddd, J = 9.4, 2.3, 2.3 Hz, 2H), 7.47–7.44 (m, 2H), 7.41–7.38 (m, 2H), 7.35–7.31 (m, 1H), 7.06–7.00 (m, 4H), 6.88 (ddd, J = 9.4, 2.3, 2.3 Hz, 2H) 5.78 (s, 2H), 5.08 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.6, 154.9, 150.4, 149.2, 137.0, 128.5, 127.9, 127.7, 127.1, 120.9, 116.6, 116.1, 69.6; MS (FAB) m/z 335 $(M+H)^+$.

4.1.13. *N*-(1-(3-(4-(4-Isopropoxyphenoxy)phenyl)-1,2,4-oxadiazol-5-yl)ethyl)acetamide (4)

General procedure C: A white solid (46%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (ddd, J = 9.2, 2.5, 2.5 Hz, 2H), 7.00 (ddd, J = 9.4, 2.0, 2.0 Hz, 4H), 6.90 (ddd, J = 9.2, 2.9, 2.9 Hz, 2H), 6.23 (d, J = 6.9 Hz, 1H), 5.47 (dq, J = 7.4, 6.9 Hz, 1H), 4.51 (sep, J = 6.3 Hz, 1H), 2.09 (s, 3H), 1.63 (d, J = 7.4 Hz, 3H), 1.35 (d, J = 6.3 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.5, 169.6, 168.0, 161.5, 154.9, 148.9, 129.3, 121.6, 120.4, 117.4, 117.3, 70.7, 43.0, 23.3, 22.2, 20.1; ESI-TOF-HRMS calcd for $C_{21}H_{24}N_3O_4$ (m/z) $[M+Na]^+$ 404.1581, found 404.1583.

4.1.14. *N*-((3-(4-(4-Isopropoxyphenoxy)phenyl)-1,2,4-oxadiazol-5-yl)methyl)acetamide (5)

General procedure C: A white solid (65%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (dd, J = 8.9, 1.4 Hz, 2H), 7.00 (d, J = 9.2 Hz, 4H), 6.90 (ddd, J = 9.2, 2.9, 2.9 Hz, 2H), 6.25 (s, 1H), 4.74 (dd, J = 5.7, 1.1 Hz, 2H), 4.51 (sep, J = 6.3 Hz, 1H), 2.12 (d, J = 1.1 Hz, 3H), 1.35 (d, J = 6.3 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.9, 170.3, 168.1, 161.6, 154.9, 148.9, 129.3, 121.6, 120.3, 117.4, 117.3, 70.7, 36.0, 32.1, 22.2; ESI-TOF-HRMS calcd for $C_{20}H_{21}N_3O_4$ (m/z) $[M+Na]^+$ 390.1424, found 390.1423.

4.1.15. *N*-(1-(3-(4-(4-Benzyloxyphenoxy)phenyl)-1,2,4-oxadiazol-5-yl)ethyl)acetamide (16)

General procedure C: A yellow solid (70%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (ddd, J = 8.6, 2.9, 2.9 Hz, 2H), 7.47–7.43 (m, 2H), 7.42–7.39 (m, 2H), 7.36–7.33 (m, 1H), 7.03–6.98 (m, 6H), 6.15 (d, J = 8.0 Hz, 1H), 5.46 (dq, J = 7.4, 7.4 Hz, 1H), 5.07 (s, 2H), 2.09 (s, 3H), 1.63 (d, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.3, 169.5, 167.8, 161.3, 155.6, 149.1, 136.8, 129.2, 128.6, 128.1, 127.5, 121.4, 120.3, 117.3, 116.0, 70.5, 42.8, 23.1, 20.0; ESI-TOF-HRMS calcd for $C_{25}H_{23}N_3O_4$ (m/z) $[M+Na]^+$ 452.1581, found 452.1605.

4.1.16. *N*-(1-(3-(4-(4-Hydroxyphenoxy)phenyl)-1,2,4-oxadiazol-5-yl)ethyl)acetamide (6)

General procedure D: A white solid (91%); ^{1}H NMR (500 MHz, CD_3OD) δ 7.96 (ddd, J = 9.4, 2.3, 2.3 Hz, 2H), 6.99 (ddd, J = 9.2, 2.9,

2.9 Hz, 2H), 6.96 (ddd, J = 8.6, 2.3, 2.3 Hz, 2H), 6.85 (ddd, J = 8.6, 2.3, 2.3 Hz, 2H), 5.27 (q, J = 6.9 Hz, 1H), 2.01 (s, 3H), 1.60 (d, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 181.5, 173.0, 169.1 163.2, 155.7, 149.2, 130.1, 122.7, 121.5, 117.9, 117.4, 44.0, 22.3, 18.8; ESI-TOF-HRMS calcd for $C_{18}H_{17}N_3O_4$ (m/z) $[M+Na]^+$ 362.1111, found 362.1138.

4.1.17. *N*-(1-(3-(4-(4-Propoxyphenoxy)phenyl)-1,2,4-oxadiazol-5-yl)ethyl)acetamide (7)

To a solution of **6** (75 mg, 0.22 mmol) and Cs_2CO_3 (120 mg, 0.38 mmol) in anhydrous DMF (1.0 mL) was added 1-iodopropane (27 μL, 0.28 mmol). The mixture was heated at 80 °C for 5 h, and then cooled to room temperature. The reaction was quenched by addition of water and the resulting mixture was extracted with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/AcOEt = 3:2) to afford the product **7** (79 mg, 94%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (ddd, J = 9.2, 2.3, 2.3 Hz, 2H), 7.02–6.91 (m, 4H), 6.90–6.94 (m, 2H), 6.16 (d, J = 7.4 Hz, 1H), 5.49–5.44 (m 1H), 3.93 (t, J = 6.6 Hz, 2H), 2.09 (s, 3H), 1.86–1.79 (m, 2H), 1.64 (d, J = 6.9 Hz, 3H), 1.06 (t, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.3, 169.4, 167.8, 161.4, 156.1, 148.7, 129.2, 121.4, 120.2, 117.2, 115.6, 70.0, 42.8, 23.2, 22.6, 20.0, 10.5; ESI-TOF-HRMS calcd for $C_{21}H_{23}N_3O_4$ (m/z) $[M-H]^-$ 380.1605, found 380.1609.

4.1.18. 2-Chloro-5-cyano-1-((2-(trimethylsilyl)ethoxy)methyl)benzimidazole and 2-chloro-6-cyano-1-((2-(trimethylsilyl)ethoxy)methyl)benzimidazole (17)

To a solution of 2-chloro-5-cyanobenzimidazole (1.4 g, 8.1 mmol) and triethylamine (3.4 mL, 24 mmol) in anhydrous DMF (30 mL) was added 2-(trimethylsilyl)ethoxymethyl chloride (2.2 mL, 12 mmol), and the mixture was stirred at room temperature for 2.5 h. Triethylamine (1.7 mL, 16 mmol) was further added, and stirring was continued overnight. The reaction was then quenched by addition of water, and the resulting mixture was extracted with AcOEt. The combined organic layer was washed with 2 N aq HCl and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (n-hexane/AcOEt = 3:1) to afford the product **17** (2.2 g, 87%, 1:1 *regioisomeric mixture*) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (s, 1/2H), 7.81 (d, J = 1.1 Hz, 1/2H), 7.77 (d, J = 8.6 Hz, 1/2H), 7.61–7.55 (m, 3/2H), 5.59 (s, 2H), 3.60–3.55 (m, 2H), 0.95–0.89 (m, 2H), −0.03 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.6, 144.1, 143.3, 141.3, 137.6, 134.5, 127.2, 126.9, 124.3, 120.5, 119.2, 115.0, 111.3, 73.6, 73.5, 67.2, 17.7, −1.5; MS (FAB) m/z 308 $(M+H)^+$.

4.1.19. 5-Cyano-2-(4-isopropoxyphenoxy)-1-((2-(trimethylsilyl)ethoxy)methyl)benzimidazole and 6-cyano-2-(4-isopropoxyphenoxy)-1-((2-(trimethylsilyl)ethoxy)methyl)benzimidazole (18)

To a solution of **17** (0.60 g, 2.0 mmol) and 4-isopropoxyphenol (0.46 g, 3.0 mmol) in DMF (10 mL) was added Cs_2CO_3 (1.9 g, 5.9 mmol), and the mixture was stirred at room temperature overnight. The reaction was quenched by addition of water, and the resulting mixture was extracted with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (n-hexane/AcOEt = 3:1) to afford the product **18** (0.83 g, 99%, 1:1 *regioisomeric mixture*) as a pink oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.83 (s, 1/2H), 7.68 (d, J = 1.1 Hz, 1/2H), 7.59 (d, J = 8.0 Hz, 1/2H), 7.51–7.47 (m, 1H), 7.44 (d, J = 8.0 Hz, 1/2H), 7.26–7.23 (m, 2H), 6.96–6.93 m, 2H), 5.56 (s, 1H), 5.56 (s, 1H), 4.56–4.51 (m, 1H), 3.68–3.64 (m, 2H), 1.36 (d, J = 6.0 Hz, 6H), 0.99–0.93 (m, 2H), −0.01 to −0.04 (m, 9H); ESI-TOF-HRMS calcd for $C_{23}H_{29}N_3O_3Si$ (m/z) $[M+Na]^+$ 446.1870, found 446.1870.

4.1.20. 5-Cyano-2-phenoxy-1-((2-(trimethylsilyl)ethoxy)methyl)benzimidazole and 6-cyano-2-phenoxy-1-((2-(trimethylsilyl)ethoxy)methyl)benzimidazole (19)

To a solution of **17** (0.5 g, 1.6 mmol) and phenol (230 mg, 2.4 mmol) in anhydrous DMF (5.4 mL) was added Cs_2CO_3 (1.6 g, 4.9 mmol), and the mixture was stirred at room temperature for 3 h. The reaction was then quenched by addition of water, and the resulting mixture was extracted with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (n-hexane/AcOEt = 3:1) to afford the product **19** (0.59 g, 99%, 1:1 *regioisomeric mixture*) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 (s, 1/2H), 7.70 (d, J = 1.1 Hz, 1/2H), 7.59 (d, J = 8.0 Hz, 1/2H), 7.50–7.46 (m, 7/2H), 7.37–7.31 (m, 3H), 5.58 (s, 1H), 5.58 (s, 1H), 3.69–3.64 (m, 2H), 0.99–0.93 (m, 2H), −0.02 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.9, 157.3, 152.9, 143.4, 139.8, 136.3, 133.0, 130.1, 129.6, 126.7, 126.4, 126.4, 126.0, 123.0, 120.3, 120.2, 119.9, 119.4, 115.3, 113.7, 110.2, 105.8, 104.8, 71.8, 71.8, 67.0, 17.7, −1.5; MS (FAB) m/z 366 $(M+H)^+$.

4.1.21. 2-(4-Isopropoxyphenoxy)-1-((2-(trimethylsilyl)ethoxy)methyl)benzimidazole-5-amidoxime and 2-(4-isopropoxyphenoxy)-1-((2-(trimethylsilyl)ethoxy)methyl)benzimidazole-6-amidoxime (20)

General procedure B: A white solid (87%, 1:1 *regioisomeric mixture*); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.88 (dd, J = 5.4, 1.4 Hz, 1/2H), 7.70 (d, J = 1.7 Hz, 1/2H), 7.57–7.53 (m, 1H), 7.48 (dd, J = 8.3, 1.4 Hz, 1/2H), 7.37 (d, J = 8.0 Hz, 1/2H), 7.27–7.23 (m, 2H), 6.96–6.91 (m, 2H), 5.54 (s, 1H), 5.54 (s, 1H), 4.93 (s, 1H), 4.86 (s, 1H), 4.56–4.49 (m, 1H), 3.68–3.64 (m, 2H), 1.35 (d, J = 6.3 Hz, 3H), 1.35 (d, J = 6.3 Hz, 3H), 0.95 (t, J = 6.9 Hz, 1H), 0.95 (t, J = 6.9 Hz, 1H), −0.03 (s, 9/2H), −0.03 (s, 9/2H); ESI-TOF-HRMS calcd for $C_{23}H_{32}N_4O_4Si$ (m/z) $[M+Na]^+$ 457.2266, found 457.2291.

4.1.22. 2-Phenoxy-1-((2-(trimethylsilyl)ethoxy)methyl)benzimidazole-5-amidoxime and 2-phenoxy-1-((2-(trimethylsilyl)ethoxy)methyl)benzimidazole-6-amidoxime (21)

General procedure B: A white solid (93%, 1:1 *regioisomeric mixture*); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 (d, J = 1.1 Hz, 1/2H), 7.75 (d, J = 1.1 Hz, 1/2H), 7.60 (dd, J = 8.6, 2.6 Hz, 1H), 7.54–7.48 (m, 5/2H), 7.44–7.40 (m, 5/2H), 7.33–7.30 (m, 1H), 5.60 (s, 1H), 5.60 (s, 1H), 4.98 (s, 1H), 4.91 (s, 1H), 3.72–3.68 (m, 2H), 1.01–0.97 (m, 2H), 0.00 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.6, 156.3, 153.2, 141.3, 139.7, 134.4, 133.2, 129.9, 127.3, 126.6, 125.9, 120.4, 120.2, 120.2, 118.6, 116.4, 109.4, 107.3, 71.6, 71.5, 66.7, 66.6, 17.7, 17.7, −1.4; MS (FAB) m/z 399 $(M+H)^+$.

4.1.23. *N*-((3-(2-(4-Isopropoxyphenoxy)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol-5-yl)-1,2,4-oxadiazol-5-yl)methyl)acetamide and *N*-((3-(2-(4-isopropoxyphenoxy)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol-6-yl)-1,2,4-oxadiazol-5-yl)methyl)acetamide (22)

General procedure C: A colorless oil (quant., 1:1 *regioisomeric mixture*); ^{1}H NMR (500 MHz, CD_3OD) δ 8.18 (d, J = 1.7 Hz, 1/2H), 8.10 (d, J = 1.1 Hz, 1/2H), 7.97–7.92 (m, 1H), 7.61 (d, J = 8.6 Hz, 1H), 7.50 (d, J = 8.6 Hz, 1H), 7.29–7.25 (m, 2H), 7.02 (d, J = 9.0 Hz, 2H), 5.67 (s, 1H), 5.65 (s, 1H), 4.69 (s, 1H), 4.67 (s, 1H), 4.65–4.58 (m, 1H), 3.76–3.72 (m, 2H), 2.07 (s, 3/2H), 2.05 (s, 3/2H), 1.34 (d, J = 6.3 Hz, 6H), 0.96 (t, J = 7.9, 1H), 0.94 (t, J = 7.9 Hz, 1H), −0.05 (s, 9H).

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4.1.24. *N*-(1-(3-(2-Phenoxy-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol-5-yl)-1,2,4-oxadiazol-5-yl)ethyl) acetamide and *N*-(1-(3-(2-phenoxy-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol-6-yl)-1,2,4-oxadiazol-5-yl)ethyl)acetamide (23)

General procedure C: A yellow oil (quant. 1:1 *regioisomeric mixture*); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.32 (s, 1/2H), 8.16 (s, 1/2H), 7.99 (dd, J = 8.3, 1.4 Hz, 1H), 7.66 (d, J = 8.6 Hz, 1/2H), 7.51–7.49 (m, 5/2H), 7.44–7.41 (m, 2H), 7.35 (dd, J = 7.4, 6.9 Hz, 1H), 6.34 (d, J = 5.2 Hz, 1H), 5.65 (s, 1H), 5.62 (s, 1H), 5.55–5.47 (m, 1H), 3.75–3.70 (m, 2H), 2.14 (s, 3/2H), 2.12 (s, 3/2H), 1.69 (d, J = 6.9 Hz, 3/2H), 1.67 (d, J = 6.9 Hz, 3/2H), 1.03–0.97 (m, 1H), 0.02 (s, 9H); MS (FAB) m/z 494 $(M+H)^+$.

4.1.25. *N*-(1-(3-(2-(4-Isopropoxyphenoxy)-1*H*-benzo[*d*]imidazol-5-yl)-1,2,4-oxadiazol-5-yl)ethyl)acetamide (8)

General procedure E: A white solid (13%); ^{1}H NMR (500 MHz, CD_3OD) δ 8.05 (s, 1H), 7.87 (dd, J = 8.0, 1.1 Hz, 1H), 7.45 (d, J = 8.0 Hz, 1H), 7.26–7.22 (m, 2H), 7.02–6.98 (m, 2H), 4.67 (s, 2H), 4.65–4.58 (m, 1H), 2.06 (s, 3H), 1.34 (d, J = 6.3 Hz, 6H); ESI-TOF-HRMS calcd for $C_{21}H_{21}N_5O_4$ (m/z) [M] 407.1588, found 407.1563.

4.1.26. *N*-(1-(3-(2-Phenoxy-1*H*-benzo[*d*]imidazol-5-yl)-1,2,4-oxadiazol-5-yl)ethyl)acetamide (9)

General procedure E: A white solid (22%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.73 (d, J = 7.4 Hz, 1H), 8.31 (s, 1H), 7.93 (d, J = 1.1 Hz, 1H), 7.77 (dd, J = 8.0, 1.7 Hz, 1H), 7.50–7.47 (m, 3H), 7.42–7.40 (m, 2H), 7.32–7.29 (m, 1H), 5.21–5.15 (m, 1H), 1.89 (s, 3H), 1.52 (d, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 180.0, 168.8, 168.0 157.4, 153.3, 129.4, 124.8, 120.2, 119.7, 119.0, 114.5, 112.3, 41.8, 21.9, 21.8, 18.1; ESI-TOF-HRMS calcd for $C_{19}H_{17}N_5O_3$ (m/z) $[M+Na]^+$ 386.1224, found 386.1242.

4.1.27. 5-Aminomethyl-2-(4-isopropoxyphenoxy)-1-((2-(trimethylsilyl)ethoxy)methyl)benzimidazole and 6-aminomethyl-2-(4-isopropoxyphenoxy)-1-((2-(trimethylsilyl)ethoxy)methyl) benzimidazole (24)

To a solution of **18** (0.21 g, 0.50 mmol) in 25% aq NH_3 (1.6 mL) and EtOH (16 mL) was added Raney Ni (1.6 mL, in water). The mixture was stirred for 4 h under a hydrogen atmosphere, then filtered through a Celite pad, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 8:1) to afford the product **24** (0.20 g, 97%, 1:1 *regioisomeric* mixture) as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51 (s, 1/2H), 7.48 (d, J = 8.6 Hz, 1/2H), 7.43 (s, 1/2H), 7.31 (d, J = 8.0 Hz, 1/2H), 7.24–7.20 (m, 2H), 7.19–7.14 (m, 1H), 6.95–6.90 (m, 2H), 5.50 (s, 1H), 5.46 (s, 1H), 4.55–4.48 (m, 1H), 3.97 (s, 1H), 3.92 (s, 1H), 3.64 (t, J = 8.3 Hz, 1H), 3.63 (t, J = 8.0 Hz, 1H), 1.34 (d, J = 6.0 Hz, 3H), 1.34 (d, J = 6.0 Hz, 3H), 0.93 (t, J = 8.0 Hz, 2H), −0.04 (s, 9/2H), −0.05 (s, 9/2H).

4.1.28. *N*-((2-(4-Isopropoxyphenoxy)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol-5-yl)methyl)acetamide and *N*-((2-(4-isopropoxyphenoxy)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol-6-yl)methyl)acetamide (25)

To a solution of **24** (0.2 g, 0.46 mmol) in CH_2Cl_2 (4.0 mL) was added triethylamine (0.13 mL, 0.92 mmol) and acetic anhydride (87 μL, 0.92 mmol). The mixture was stirred at room temperature for 1 h, then diluted with CH_2Cl_2 and aq $NaHCO_3$, and the organic layer was washed with aq $NaHCO_3$. After drying over Na_2SO_4, the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 10:1) to afford product **25** (0.18 g, 85%, 1:1 *regioisomeric* mixture) as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50 (d, J = 8.0 Hz, 1/2H), 7.45 (d, J = 1.1 Hz, 1/2H), 7.33 (d, J = 8.0 Hz, 1/2H), 7.31 (d, J = 1.1 Hz, 1/2H), 7.25–7.21 (m, 2H), 7.15 (dd, J = 8.0, 2.0 Hz, 1/2H), 7.11 (dd, J = 8.0, 1.5 Hz, 1/2H), 6.95–6.91 (m, 2H), 5.76 (br s, 1/2H), 5.72 (br s, 1/2H), 5.52 (s, 1H), 5.52 (s, 1H), 4.54–4.50 (m, 2H), 4.50–4.47 (m, 1H), 3.66 (t, J = 8.3 Hz, 1H), 3.64 (t, J = 8.3 Hz, 1H), 2.03 (s, 3/2H), 2.00 (s, 3/2H), 1.35 (d, J = 6.6 Hz, 6H), 0.95 (t, J = 8.8 Hz, 1H), 0.94 (t, J = 8.5 Hz, 1H), −0.03 (s, 9H); ESI-TOF-HRMS calcd for $C_{25}H_{35}N_3O_4Si$ (m/z) $[M+Na]^+$ 492.2289, found 492.2265.

4.1.29. *N*-((2-(4-Isopropoxyphenoxy)-1*H*-benzo[*d*]imidazol-5-yl)methyl)acetamide (10)

General procedure E: A white solid (55%); ^{1}H NMR (500 MHz, CD_3OD) δ 7.30–7.27 (m, 2H), 7.19 (d, J = 6.4 Hz, 2H), 7.08 (d, J = 8.0 Hz, 1H), 6.97 (d, J = 6.4 Hz, 2H), 4.61–4.56 (m, 1H), 4.40 (s, 2H), 1.97 (s, 3H), 1.32 (d, J = 5.7 Hz, 6H); ESI-TOF-HRMS calcd for $C_{19}H_{21}N_3O_3$ (m/z) $[M+Na]^+$ 362.1475, found 362.1481.

4.1.30. Phenyl (4-cyanophenyl)carbamate (26)

To a solution of 4-aminobenzonitrile (440 mg, 3.7 mmol) and pyridine (0.60 mL, 7.5 mmol) in THF (4.4 mL) was added phenylchlorocarbonate (0.52 mL, 4.2 mmol) at 0 °C. The mixture was stirred at room temperature for 2 h, then diluted with water, and extracted with CH_2Cl_2. The combined organic layer was washed with 1 N aq HCl and dried over Na_2SO_4. The solvent was removed under reduced pressure to afford the product **26** (880 mg, 98%).

^{1}H NMR (500 MHz, CD_3OD) δ 7.71–7.66 (m, 4H), 7.41 (dd, J = 8.0, 8.0 Hz, 2H), 7.26 (t, J = 7.0 Hz, 1H), 7.20 (d, J = 8.0 Hz, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 151.3, 150.3, 141.7, 133.6, 129.7, 126.3, 121.6, 118.9, 118.7, 107.0; MS (FAB) m/z 239 $(M+H)^+$.

4.1.31. Phenyl (4-(*N*′-hydroxycarbamimidoyl)phenyl)carbamate (27)

General procedure B: A white solid (29%); ^{1}H NMR (500 MHz, CD_3OD) δ 7.60 (d, J = 8.6 Hz, 2H), 7.54 (d, J = 8.6 Hz, 2H), 7.40 (dd, J = 8.0, 8.0 Hz, 2H), 7.24 (t, J = 7.4 Hz, 1H), 7.19 (d, J = 8.0 Hz, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 155.2, 154.0, 152.3, 141.3, 130.4, 129.0, 128.0, 126.7, 122.9, 119.4; MS (FAB) m/z 272 $(M+H)^+$.

4.1.32. Phenyl (4-(5-(1-acetamidoethyl)-1,2,4-oxadiazol-3-yl) phenyl)carbamate (11)

General procedure C: A white solid (78%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.59 (s, 1H), 8.72 (d, J = 6.9 Hz, 1H), 7.95 (d, J = 8.6 Hz, 2H), 7.69 (d, J = 8.6 Hz, 2H), 7.43 (ddd, J = 8.6, 8.6, 2.3 Hz, 2H), 7.29–7.23 (m, 3H), 5.17 (dq, J = 7.4, 6.9 Hz, 1H), 1.88 (s, 3H), 1.51 (d, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 180.7, 169.2, 167.2, 151.6, 150.4, 141.7, 129.5, 128.0, 125.6, 122.0, 120.4, 118.6, 42.0, 22.3, 18.4; ESI-TOF-HRMS calcd for $C_{19}H_{18}N_4O_4$ (m/z) $[M+Na]^+$ 389.1220, found 389.1192.

4.1.33. *N*-(1-(3-(4-(4-Methoxyphenoxy)phenyl)-1,2,4-oxadiazol-5-yl)ethyl)acetamide (28)

To a solution of **6** (41 mg, 0.12 mmol) and Me_2SO_4 (30 μL 0.31 mmol) in anhydrous DMF (0.80 mL) was added K_2CO_3 (36 mg, 0.26 mmol). The mixture was stirred at 90 °C for 4.5 h and then cooled to room temperature. The reaction was quenched by addition of water and the resulting mixture was extracted with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 10:1) to afford the product **28** (10 mg, 23%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (d, J = 5.7 Hz, 2H), 7.05–6.92 (m, 6H), 6.13 (br s, 1H), 5.50–5.44 (m, 1H), 3.83 (s, 3H), 2.09 (s, 3H), 1.64 (d, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.3, 169.4, 167.8, 161.4, 156.5, 148.9, 129.2, 121.5, 120.3, 117.2,

115.0, 55.7, 42.8, 23.2, 20.1; ESI-TOF-HRMS calcd for $C_{19}H_{19}N_3O_4$ (m/z) $[M+Na]^+$ 376.1268, found 376.1286.

4.1.34. *N*-(1-(3-(4-(4-Ethoxyphenoxy)phenyl)-1,2,4-oxadiazol-5-yl)ethyl)acetamide (29)

To a solution of **6** (83 mg, 0.24 mmol) and Cs_2CO_3 (140 mg, 0.43 mmol) in DMF (1.3 mL) was added iodoethane (25 μL, 0.31 mmol). The mixture was stirred at 80 °C for 5 h, and then cooled to room temperature. The reaction was quenched by addition of water, and the resulting mixture was extracted with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/AcOEt = 3:2) to afford the product **29** (74 mg, 83%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (dd, J = 9.4, 2.4 Hz, 2H), 7.03–6.98 (m, 4H), 6.93–6.90 (m, 2H), 6.14 (d, J = 7.4 Hz, 1H), 5.49–5.44 (m, 1H), 4.04 (q, J = 6.9 Hz, 2H), 2.09 (s, 3H), 1.64 (d, J = 6.9 Hz, 3H), 1.43 (t, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.3, 169.4, 167.8, 161.4, 155.8, 148.8, 129.2, 121.4, 120.2, 117.2, 115.6, 63.9, 42.8, 23.2, 20.0, 14.9; ESI-TOF-HRMS calcd for $C_{20}H_{21}N_3O_4$ (m/z) $[M-H]^-$ 366.1448 found 366.1432.

4.1.35. 4-(4-Hexyloxyphenoxy)benzonitrile (33)

General procedure A: A white solid (99%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57 (ddd, J = 9.4, 2.3, 2.3 Hz, 2H), 6.99 (ddd, J = 9.2, 2.3, 2.3 Hz, 2H), 6.95 (ddd, J = 9.2, 2.3, 2.3 Hz, 2H), 6.92 (ddd, J = 9.2, 2.3, 2.3 Hz, 2H), 3.96 (t, J = 6.6 Hz, 2H), 1.82–1.76 (m, 2H), 1.49–1.46 (m, 2H), 1.40–1.32 (m, 4H), 0.93–0,90 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.7, 156.7, 147.8, 134.2, 121.9, 119.1, 117.2, 115.9, 105.3, 68.6, 31.7, 29.4, 25.9, 22.7, 14.2; MS (FAB) m/z 296 $(M+H)^+$.

4.1.36. 4-(4-Phenoxyphenoxy)benzonitrile (34)

General procedure A: A light yellow solid (81%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.83 (ddd, J = 9.2, 2.3, 2.3 Hz, 2H), 7.42–7.38 (m, 2H), 7.17 (dd, J = 6.9, 2.3 Hz, 2H), 7.14 (t, J = 7.4 Hz, 1H), 7.09 (ddd, J = 8.6, 2.3, 2.3 Hz, 4H), 7.04 (dd, J = 8.6, 1.1 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.5, 156.8, 153.6, 149.8, 134.6, 130.1, 123.5, 122.1, 120.5, 118.8, 118.5, 117.6, 104.9; ESI-TOF-HRMS calcd for $C_{19}H_{13}NO_2$ (m/z) $[M+Na]^+$ 310.0838, found 310.0838.

4.1.37. 4-(4-Hexyloxyphenoxy)benzamidoxime (35)

General procedure B: A white solid (73%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.31 (br s, 1H), 7.55 (ddd, J = 9.4, 2.3, 2.3 Hz, 2H), 6.98 (ddd, J = 9.2, 2.3, 2.3 Hz, 2H), 6.93 (ddd, J = 9.2, 2.3, 2.3 Hz, 2H), 6.89 (ddd, J = 9.2, 2.3, 2.3 Hz, 2H), 4.84 (s, 2H), 3.94 (t, J = 6.6 Hz, 2H), 1.81–1.76 (m, 2H), 1.49–1.44 (m, 2H), 1.38–1.31 (m, 4H), 0.93–0.90 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.4, 156.0, 152.5, 149.3, 127.5, 126.5, 121.3, 117.3, 115.7, 68.6, 31.7, 29.4, 25.9, 22.8, 14.2; MS (FAB) m/z 329 $(M+H)^+$.

4.1.38. 4-(4-Phenoxyphenoxy)benzamidoxime (36)

General procedure B: A white solid (79%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 (br s, 1H), 7.59 (ddd, J = 9.2, 2.3, 2.3, 2H), 7.36–7.32 (m, 2H), 7.10 (t, J = 7.4 Hz, 1H), 7.02–6.98 (m, 8H), 4.85 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.5, 157.5, 153.3, 152.2, 151.7, 129.8, 127.4, 127.0, 123.2, 121.0, 120.4, 118.5, 117.8; ESI-TOF-HRMS calcd for $C_{19}H_{16}N_2O_3$ (m/z) $[M+H]^+$ 321.1234, found 321.1264.

4.1.39. 4-Phenoxybenzamidoxime (37)

General procedure B: A white solid (96%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.32 (br s, 1H), 7.59 (ddd, J = 8.6, 2.9, 1.7, 2H), 7.36 (dd, J = 9.6, 9.6 Hz, 2H), 7.15 (t, J = 7.4 Hz, 1H), 7.05–6.99 (m, 4H), 4.86 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.0, 156.4, 152.2, 129.9, 127.4, 127.1, 123.9, 119.4, 118.4; ESI-TOF-HRMS calcd for $C_{13}H_{12}N_2O_2$ (m/z) $[M+H]^+$ 229.0972, found 229.0999.

4.1.40. *N*-(1-(3-(4-(4-Hexyloxyphenoxy)phenyl)-1,2,4-oxadiazol-5-yl)ethyl)acetamide (30)

General procedure C: A white solid (47%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (ddd, J = 9.4, 2.3, 2.3 Hz, 2H), 7.03–6.98 (m, 4H), 6.91 (ddd, J = 9.2, 2.3, 2.3 Hz, 2H), 6.14 (d, J = 7.4 Hz, 1H), 5.47 (dq, J = 7.4, 6.9 Hz, 1H), 3.96 (t, J = 6.6 Hz, 2H), 2.09 (s, 3H), 1.82–1.76 (m, 2H), 1.64 (d, J = 6.9 Hz, 3H), 1.49–1.46 (m, 2H), 1.38–1.34 (m, 4H), 0.93–0.90 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.5, 169.6, 168.0, 161.6, 156.2, 148.9, 129.3, 121.6, 120.4, 117.3, 115.8, 68.6, 43.0, 31.7, 29.4, 25.9, 23.3, 22.8, 20,1, 14.2; ESI-TOF-HRMS calcd for $C_{24}H_{29}N_3O_4$ (m/z) $[M+Na]^+$ 446.2050, found 446.2066.

4.1.41. *N*-(1-(3-(4-(4-Phenoxyphenoxy)phenyl)-1,2,4-oxadiazol-5-yl)ethyl)acetamide (31)

General procedure C: A white solid (92%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (ddd, J = 8.6, 2.3, 2.3 Hz, 2H), 7.38–7.33 (m, 2H), 7.11 (t, J = 7.4 Hz, 1H), 7.07–7.02 (m, 8H), 6.13 (d, J = 8.0 Hz, 1H), 5.50–5.44 (m, 1H), 2.09 (s, 3H), 1.64 (d, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.4, 169.4, 167.8, 160.7, 157.4, 153.6, 151.3, 129.8, 129.3, 123.3, 121.3, 120.8, 120.4, 118.6, 117.8, 42.9, 23.2, 20.0; ESI-TOF-HRMS calcd for $C_{24}H_{21}N_3O_4$ (m/z) $[M+Na]^+$ 438.1424, found 438.1450.

4.1.42. *N*-(1-(3-(4-Phenoxyphenyl)-1,2,4-oxadiazol-5-yl)ethyl) acetamide (32)

General procedure C: A white solid (87%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (d, J = 9.2 Hz, 2H), 7.39 (dd, J = 7.4, 7.4 Hz, 2H), 7.18 (t, J = 7.4 Hz, 1H), 7.08–7.05 (m, 4H), 6.17 (d, J = 7.4 Hz, 1H), 5.50–5.44 (m, 1H), 2.09 (s, 3H), 1.64 (d, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.4, 169.5, 167.8, 160.3, 155.9, 130.0, 129.2, 124.2, 120.9, 119.8, 118.3, 42.8, 23.1, 19.9; ESI-TOF-HRMS calcd for $C_{18}H_{17}N_3O_3$ (m/z) $[M+Na]^+$ 346.1162, found 346.1179.

4.1.43. *N*-((3-(4-(4-Benzyloxyphenoxy)phenyl)-1,2,4-oxadiazol-5-yl)methyl)acetamide (40)

General procedure C: A white solid (76%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (ddd, J = 9.2, 2.4, 2.4 Hz, 2H), 7.47–7.43 (m, 2H), 7.42–7.39 (m, 2H), 7.36–7.33 (m, 1H), 7.03–6.98 (m, 6H), 6.20 (br s, 1H), 5.07 (s, 2H), 4.75 (d, J = 5.7 Hz, 2H), 2.13 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.7, 170.2, 167.9, 161.3, 155.7, 149.1, 136.8, 129.2, 128.6, 128.1, 127.5, 121.4, 120.2, 117.3, 116.0, 70.5, 35.9, 23.0; ESI-TOF-HRMS calcd for $C_{24}H_{21}N_3O_4$ (m/z) $[M+Na]^+$ 438.1424, found 438.1427.

4.1.44. *N*-((3-(4-(4-Hydroxyphenoxy)phenyl)-1,2,4-oxadiazol-5-yl)methyl)acetamide (38)

General procedure D: A white solid (92%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (d, J = 9.0 Hz, 2H), 7.00 (d, J = 8.5 Hz, 2H), 6.97 (d, J = 9.0 Hz, 2H), 6.86 (d, J = 9.0 Hz, 2H), 6.17 (br s, 1H), 5.04 (br s, 1H), 4.76–4.74 (m, 2H), 2.13 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.7, 170.5, 167.9, 161.5, 152.8, 148.7, 129.2, 121.7, 120.0, 117.1, 116.5, 35.9, 23.0; ESI-TOF-HRMS calcd for $C_{17}H_{15}N_3O_4$ (m/z) $[M+Na]^+$ 348.0955, found 348.0947.

4.1.45. *N*-((3-(4-Phenoxyphenyl)-1,2,4-oxadiazol-5-yl)methyl) acetamide (39)

General procedure C: A white solid (62%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (ddd, J = 8.0, 2.5, 2.5 Hz, 2H), 7.39 (dd, J = 8.0, 8.0 Hz, 2H), 7.19 (t, J = 8.0 Hz, 1H), 7.10–7.05 (m, 4H), 6.15 (br s, 1H), 4.75 (d, J = 6.0 Hz, 2H), 2.13 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 177.7, 169.9, 167.1, 159.8, 155.3, 130.3, 129.1, 124.6, 120.6, 119.8, 118.2, 35.2, 22.2; ESI-TOF-HRMS calcd for $C_{17}H_{15}N_3O_3$ (m/z) $[M+Na]^+$ 332.1006, found 332.1029.

4.1.46. *tert*-Butyl (*R*)-(1-(3-(4-(4-phenoxyphenoxy)phenyl)-1,2,4-oxadiazol-5-yl)ethyl)carbamate ((*R*)-41)

General procedure C: A white solid (65%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (ddd, J = 9.4, 2.4, 2.4 Hz, 2H), 7.37–7.33 (m, 2H), 7.13–7.10 (m, 1H), 7.06–7.02 (m, 8H), 5.17 (br s, 2H), 1.62 (d, J = 6.9 Hz, 3H), 1.46 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.9, 167.8, 160.7, 157.4, 154.8, 153.6, 151.3, 129.8, 129.3, 123.3, 121.3, 121.0, 120.4, 118.6, 117.8, 80.5, 44.3, 28.3, 20.1; ESI-TOF-HRMS calcd for $C_{27}H_{27}N_3O_5$ (m/z) $[M-H]^-$ 472.1867, found 472.1871.

4.1.47. *tert*-Butyl (*R*)-(1-(3-(4-phenoxyphenyl)-1,2,4-oxadiazol-5-yl)ethyl)carbamate ((*R*)-42)

General procedure C: A white solid (69%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (ddd, J = 9.4, 2.3, 2.3 Hz, 2H), 7.41–7.37 (m, 2H), 7.20–7.16 (m, 1H), 7.08–7.05 (m, 4H) 5.18 (br s, 2H), 1.63 (d, J = 6.6 Hz, 3H), 1.46 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.9, 167.8, 160.2, 156.0, 154.8, 130.0, 129.3, 124.2, 121.2, 119.8, 118.3, 80.5, 44.3, 28.3, 20.1; ESI-TOF-HRMS calcd for $C_{21}H_{23}N_3O_4$ (m/z) $[M+H]^+$ 404.1581, found 404.1578.

4.1.48. (*R*)-1-(3-(4-(4-Phenoxyphenoxy)phenyl)-1,2,4-oxadiazol-5-yl)ethan-1-amine ((*R*)-43)

General procedure F: A white solid (quant.); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (ddd, J = 9.4, 2.4, 2.4 Hz, 2H), 7.36–7.32 (m, 2H), 7.12–7.09 (m, 1H), 7.05–6.99 (m, 8H), 4.85 (q, J = 7.1 Hz, 1H), 1.83 (d, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.9, 168.1, 161.3, 157.4, 153.8, 150.9, 129.8, 129.4, 123.3, 121.5, 120.4, 119.5, 118.6, 117.7, 44.8, 17.3; ESI-TOF-HRMS calcd for $C_{22}H_{19}N_3O_3$ (m/z) $[M+H]^+$ 374.1499, found 374.1485.

4.1.49. (*R*)-1-(3-(4-Phenoxyphenyl)-1,2,4-oxadiazol-5-yl)ethan-1-amine ((*R*)-44)

General procedure F: A white solid (90%); ^{1}H NMR (500 MHz, CD_3OD) δ 8.07 (ddd, J = 9.4, 2.3, 2.3 Hz, 2H), 7.44–7.40 (m, 2H), 7.21 (t, J = 7.4 Hz, 1H), 7.10–7.06 (m, 4H), 4.95 (q, J = 6.9 Hz, 1H), 1.78 (d, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 177.5, 169.3, 162.3, 157.2, 131.2, 130.4, 125.6, 121.7, 121.0, 119.2, 45.2, 17.4; ESI-TOF-HRMS calcd for $C_{16}H_{15}N_3O_2$ (m/z) $[M+H]^+$ 282.1237, found 282.1221.

4.1.50. (*R*)-*N*-(1-(3-(4-(4-Phenoxyphenoxy)phenyl)-1,2,4-oxadiazol-5-yl)ethyl)acetamide ((*R*)-31)

General procedure G: A white solid (73%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (ddd, J = 8.6, 2.4, 2.4 Hz, 2H), 7.38–7.33 (m, 2H), 7.11 (t, J = 7.4 Hz, 1H), 7.07–7.02 (m, 8H), 6.15 (d, J = 8.0 Hz, 1H), 5.50–5.44 (m, 1H), 2.09 (s, 3H), 1.64 (d, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.4, 169.4, 167.8, 160.7, 157.4, 153.6, 151.2, 129.8, 129.3, 123.3, 121.3, 120.8, 120.4, 118.6, 117.8, 42.8, 23.2, 20.0; ESI-TOF-HRMS calcd for $C_{24}H_{21}N_3O_4$ (m/z) $[M-H]^-$ 414.1448, found 414.1443.

4.1.51. (*R*)-*N*-(1-(3-(4-Phenoxyphenyl)-1,2,4-oxadiazol-5-yl)ethyl) acetamide ((*R*)-32)

General procedure G: A white solid (90%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (ddd, J = 9.4, 2.4, 2.4 Hz, 2H), 7.41–7.37 (m, 2H), 7.20–7.16 (m, 1H), 7.08–7.05 (m, 4H), 6.20 (d, J = 7.4 Hz, 1H), 5.50–5.44 (m, 1H), 2.09 (s, 3H), 1.64 (d, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.4, 169.5, 167.8, 160.3, 155.9, 130.0, 129.2, 124.2, 120.9, 119.8, 118.3, 42.8, 23.1, 19.9; ESI-TOF-HRMS calcd for $C_{18}H_{17}N_3O_3$ (m/z) $[M+H]^+$ 346.1162, found 346.1152.

4.1.52. *tert*-Butyl (*S*)-(1-(3-(4-(4-phenoxyphenoxy)phenyl)-1,2,4-oxadiazol-5-yl)ethyl)carbamate ((*S*)-41)

General procedure C: A white solid (69%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (ddd, J = 9.4, 2.4, 2.4 Hz, 2H), 7.37–7.33 (m, 2H), 7.13–7.09 (m, 1H), 7.06–7.02 (m, 8H), 5.18 (br s, 2H), 1.62 (d, J = 6.9 Hz, 3H), 1.46 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.9, 167.8, 160.7, 157.4, 154.8, 153.6, 151.3, 129.8, 129.3, 123.3, 121.3, 121.0, 120.4, 118.6, 117.8, 80.5, 44.3, 28.3, 20.1; ESI-TOF-HRMS calcd for $C_{27}H_{27}N_3O_5$ (m/z) $[M-H]^-$ 472.1867, found 472.1892.

4.1.53. *tert*-Butyl (*S*)-(1-(3-(4-phenoxyphenyl)-1,2,4-oxadiazol-5-yl)ethyl)carbamate ((*S*)-42)

General procedure C: A white solid (61%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (ddd, J = 9.4, 2.3, 2.3 Hz, 2H), 7.41–7.37 (m, 2H), 7.20–7.16 (s, 1H), 7.08–7.05 (m, 4H), 5.18 (br s, 2H), 1.63 (d, J = 6.6 Hz, 3H), 1.46 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.9, 167.8, 160.2, 156.0, 154.8, 130.0, 129.3, 124.2, 121.2, 119.8, 118.3, 80.5, 44.3, 28.3, 20.1; ESI-TOF-HRMS calcd for $C_{21}H_{23}N_3O_4$ (m/z) $[M+H]^+$ 404.1581, found 404.1580.

4.1.54. (*S*)-1-(3-(4-(4-Phenoxyphenoxy)phenyl)-1,2,4-oxadiazol-5-yl)ethan-1-amine ((*S*)-43)

General procedure F: A white solid (quant.); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (ddd, J = 9.4, 2.4, 2.4 Hz, 2H), 7.36–7.32 (m, 2H), 7.12–7.09 (m, 1H), 7.05–6.99 (m, 8H), 4.85 (q, J = 7.1 Hz, 1H), 1.83 (d, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.9, 168.1, 161.3, 157.4, 153.8, 150.9, 129.8, 129.4, 123.3, 121.5, 120.4, 119.5, 118.6, 117.7, 44.8, 17.3; ESI-TOF-HRMS calcd for $C_{22}H_{19}N_3O_3$ (m/z) $[M+H]^+$ 374.1499, found 374.1470.

4.1.55. (*S*)-1-(3-(4-Phenoxyphenyl)-1,2,4-oxadiazol-5-yl)ethan-1-amine ((*S*)-44)

General procedure F: A white solid (98%); ^{1}H NMR (500 MHz, CD_3OD) δ 8.07 (ddd, J = 9.4, 2.3, 2.3 Hz, 2H), 7.44–7.40 (m, 2H), 7.21 (t, J = 7.4 Hz, 1H), 7.10–7.06 (m, 4H), 4.95 (q, J = 6.9 Hz, 1H), 1.78 (d, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 177.5, 169.3, 162.3, 157.2, 131.2, 130.4, 125.6, 121.7, 121.0, 119.2, 45.2, 17.4; ESI-TOF-HRMS calcd for $C_{16}H_{15}N_3O_2$ (m/z) $[M+H]^+$ 282.1237, found 282.1207.

4.1.56. (*S*)-*N*-(1-(3-(4-(4-Phenoxyphenoxy)phenyl)-1,2,4-oxadiazol-5-yl)ethyl)acetamide ((*S*)-31)

General procedure G: A white solid (74%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (ddd, J = 8.6, 2.4, 2.4 Hz, 2H), 7.38–7.33 (m, 2H), 7.11 (t, J = 7.4 Hz, 1H), 7.07–7.02 (m, 8H), 6.15 (d, J = 7.4 Hz, 1H), 5.50–5.44 (m, 1H), 2.09 (s, 3H), 1.64 (d, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.4, 169.4, 167.8, 160.7, 157.4, 153.6, 151.2, 129.8, 129.3, 123.3, 121.3, 120.8, 120.4, 118.6, 117.8, 42.8, 23.2, 20.0; ESI-TOF-HRMS calcd for $C_{24}H_{21}N_3O_4$ (m/z) $[M-H]^-$ 414.1448, found 414.1442.

4.1.57. (*S*)-*N*-(1-(3-(4-Phenoxyphenyl)-1,2,4-oxadiazol-5-yl)ethyl) acetamide ((*S*)-32)

General procedure G: A white solid (79%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (ddd, J = 9.4, 2.3, 2.3 Hz, 2H), 7.41–7.37 (m, 2H), 7.20–7.16 (m, 1H), 7.08–7.05 (m, 4H), 6.19 (d, J = 7.4 Hz, 1H), 5.50–5.44 (m, 1H), 2.09 (s, 3H), 1.64 (d, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.4, 169.5, 167.8, 160.3, 160.0, 130.0, 129.2, 124.2, 120.9, 119.8, 118.3, 42.8, 23.1, 19.9; ESI-TOF-HRMS calcd for $C_{18}H_{17}N_3O_3$ (m/z) $[M+H]^+$ 346.1162, found 346.1154.

4.2. Biology

4.2.1. Cell culture

Human embryonic kidney 293 (HEK293) cells were cultured in DMEM supplemented with 10% FBS and penicillin/streptomycin mixture at 37 °C in a humidified incubator (5% CO_2).

4.2.2. ACC2 inhibitory activity assay

The enzymatic activity of ACC2 was determined by means of an ATP consumption assay.[19] The assay was performed using 30 mM HEPES buffer (pH 7.5), 18 mM $NaHCO_3$, 1.0 mg/mL BSA, 0.5 μM ATP, 1 mM DTT, 4 mM $MgCl_2$, 2 mM sodium citrate, 0.5 mM acetyl-CoA, 1% DMSO, and 50 ng of enzyme (human ACC2, recombinant, C-terminal His-tag, BPS Bioscience) per well (96-well half plate). The ACC inhibitor was added at concentrations between 0 and 100 μM. The reaction was started by addition of enzyme (50 ng/well) followed by incubation at 37 °C for 90 min. Unconverted ATP was determined using ATP monitoring reagent (Lonza, ViaLight plus kit) according to the manufacturer's instructions. Some of synthetic compounds exhibited partial ACC2-inhibitory activity. Thus, IC_{50} values were calculated from the dose–response curves, and the values of percent inhibition at 30 μM were determined relative to that of a positive control.

4.2.3. PPAR reporter gene assay[23]

HEK293 cells were seeded at a density of 20% confluence (96-well culture plate) the day before transfection. Cells were then co-transfected with 30 ng of CMX-GAL4N-hPPAR LBD expression plasmid (CMX-GAL4N-hPPARα LBD, CMX-GAL4N-hPPARγ LBD or CMX-GAL4N-hPPARδ LBD), 100 ng of TK-MH100x4-Luc luciferase reporter, and 10 ng of CMX-β-galactosidase expression vector. The transfection was performed by the calcium phosphate co-precipitation method. After 24 h, the cells were treated with DMSO or DMSO solution of a test compound for 24 h (final: 0.5% DMSO). The luciferase activity of each well was measured using a luminometer and normalized by the level of β-galactosidase activity: 2-nitrophenyl-β-D-galactopyranoside was added, and the absorbance was measured (at 405 nm). Each sample was tested in triplicate.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2016.08.045.

References and notes

1. Eckel, R. H.; Grundy, S. M.; Zimmet, P. Z. *Lancet* **2005**, *365*, 1415.
2. Samuel, V.; Shulman, G. I. *Cell* **2012**, *148*, 852.
3. Unger, R. H.; Clark, G. O.; Scherer, P. E.; Orci, L. *Biochim. Biophys. Acta* **2010**, *1801*, 209.
4. Savage, D. B.; Petersen, K. F.; Shulman, G. I. *Physiol. Rev.* **2007**, *87*, 507.
5. Kusunoki, J.; Kanatani, A.; Moller, D. E. *Endocrine* **2006**, *29*, 91.
6. Bourbeau, M. P.; Bartberger, M. D. *J. Med. Chem.* **2015**, *58*, 525.
7. Savage, D. B.; Choi, C. S.; Samuel, V. T.; Liu, Z.-X.; Zhang, D.; Wang, A.; Zhang, X.-M.; Cline, G. W.; Yu, X. X.; Geisler, J. G.; Bhanot, S.; Monia, B. P.; Shulman, G. I. *J. Clin. Invest.* **2006**, *116*, 817.
8. Schreurs, M.; van Dijk, T. H.; Gerding, A.; Havinga, R.; Reijngoud, D.-J.; Kuipers, F. *Diabetes Obes. Metab.* **2009**, *11*, 987.
9. Pirat, C.; Farce, A.; Lebègue, N.; Renault, N.; Furman, C.; Millet, R.; Yous, S.; Speca, S.; Berthelot, P.; Desreumaux, P.; Chavatte, P. *J. Med. Chem.* **2012**, *55*, 4027.
10. Lee, C. H.; Olson, P.; Hevener, A.; Mehl, I.; Chong, L. W.; Olefsky, J. M.; Gonzalez, F. J.; Ham, J.; Kang, H.; Peters, J. M.; Evans, R. M. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 3444.
11. Csermely, P.; Àgoston, V.; Pongor, S. *Trends Pharmacol. Sci.* **2005**, *26*, 178.
12. Roth, B. L.; Sheffler, D. J.; Kroeze, W. K. *Nat. Rev. Drug Disc.* **2004**, *3*, 353.
13. Hopkins, A. L. *Nat. Chem. Biol.* **2008**, *4*, 682.
14. Blöcher, R.; Lamers, C.; Wittmann, S. K.; Diehl, O.; Hanke, T.; Merk, D.; Steinhilber, D.; Schubert-Zsilavecz, M.; Kahnt, A. S.; Proschak, E. *Med. Chem. Commun.* **2016**, *7*, 1209.
15. Blöcher, R.; Lamers, C.; Wittmann, S. K.; Merk, D.; Hartmann, M.; Weizel, L.; Diehl, O.; Brüggerhoff, A.; Boß, M.; Kaiser, A.; Schader, T.; Göbel, T.; Grundmann, M.; Angioni, C.; Heering, J.; Geisslinger, G.; Wurglics, M.; Kostenis, E.; Brüne, B.; Steinhilber, D.; Schubert-Zsilavecz, M.; Kahnt, A. S.; Proschak, E. *J. Med. Chem.* **2016**, *59*, 61.
16. Casimiro-Garcia, A.; Filzen, G. F.; Flynn, D.; Bigge, C. F.; Chen, J.; Davis, J. A.; Dudley, D. A.; Edmunds, J. J.; Esmaeil, N.; Geyer, A.; Heemstra, R. J.; Jalaie, M.; Ohren, J. F.; Ostroski, R.; Ellis, T.; Schaum, R. P.; Stoner, C. *J. Med. Chem.* **2011**, *54*, 4219.
17. Fruchart, J.-C. *Cardiovasc. Diabetol.* **2013**, *12*, 82.
18. Gu, Y. G.; Weitzberg, M.; Clark, R. F.; Xu, X.; Li, Q.; Zhang, T.; Hansen, T. M.; Liu, G.; Xin, Z.; Wang, X.; Wang, R.; McNally, T.; Camp, H.; Beutel, B. A.; Sham, H. L. *J. Med. Chem.* **2006**, *49*, 3770.
19. Keil, S.; Müller, M.; Zoller, G.; Haschke, G.; Schroeter, K.; Glien, M.; Ruf, S.; Focken, I.; Herling, A. W.; Schmoll, D. *J. Med. Chem.* **2010**, *53*, 8679.
20. Nolte, R. T.; Wisely, G. B.; Westin, S.; Cobb, J. E.; Lambert, M. H.; Kurokawa, R.; Rosenfeld, M. G.; Willson, T. M.; Glass, C. K.; Millburn, M. V. *Nature* **1998**, *395*, 137.
21. Gu, Y. G.; Weitzberg, M.; Clark, R. F.; Xu, X.; Li, Q.; Lubbers, N. L.; Yang, Y.; Beno, D. W. A.; Widomski, D. L.; Zhang, T.; Hansen, T. M.; Keyes, R. F.; Waring, J. F.; Carroll, S. L.; Wang, X.; Wang, R.; Healan-Greenberg, C. H.; Blomme, E. A.; Beutel, B. A.; Sham, H. L.; Camp, H. S. *J. Med. Chem.* **2007**, *50*, 1078.
22. Yoshida, T.; Akahoshi, F.; Sakashita, H.; Sonda, S.; Takeuchi, M.; Tanaka, Y.; Nabeno, M.; Kishida, H.; Miyaguchi, I.; Hayashi, Y. *Bioorg. Med. Chem.* **2012**, *20*, 5033.
23. Kasuga, J.; Yamasaki, D.; Araya, Y.; Nakagawa, A.; Makishima, M.; Doi, T.; Hashimoto, Y.; Miyachi, H. *Bioorg. Med. Chem.* **2006**, *14*, 8405.
24. Haque, T. S.; Liang, N.; Golla, R.; Seethala, R.; Ma, Z.; Ewing, W. R.; Cooper, C. B.; Pelleymounter, M. A.; Poss, M. A.; Cheng, D. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5872.
25. Perry, R. J.; Samuel, V. T.; Petersen, K. F.; Shulman, G. I. *Nature* **2014**, *510*, 84.