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Synthesis and biological evaluation of N-alkylated 8-oxybenz[*c*]azepine derivatives as selective PPARδ agonists

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ABSTRACT

We describe the discovery of small molecule benzazepine derivatives as agonists of human peroxisome proliferator-activated receptor δ (PPARδ) that displayed excellent selectivity over the PPARα and PPARγ subtypes. Compound **8** displayed good PK in the rat and efficacy in upregulation of pyruvate dehydrogenase kinase, isozyme 4 (PDK4) mRNA in human primary myotubes, a biomarker for increased fatty acid oxidation.

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The peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear hormone receptor superfamily and comprise three subtypes, namely PPARα (OMIM170998), PPARγ (OMIM601487) and PPARδ (or β, OMIM600409). PPARs have been implicated in processes that regulate plasma levels of lipoproteins and triglycerides, and PPARα and PPARγ agonists have been developed as drugs for treatment of metabolic disorders such as dyslipidemia and type II diabetes.[1,2]

PPARδ is widely expressed, with highest levels in tissues with high lipid metabolism such as adipose tissue, skeletal muscle and intestine. It is the major PPAR subtype in skeletal muscle, where its expression is associated with oxidative fibres. Activation of PPARδ increases fatty acid oxidation, and thus represents a potentially attractive mechanism for treatment of dyslipidemia. There are currently no marketed drugs identified as targeting PPARδ, although a PPARδ agonist (GW501516, compound **1** in Fig. 1) has been demonstrated to reduce plasma triglycerides and LDL cholesterol in moderately obese men in a small scale clinical study.[3]

In the past few years, a number of companies have identified selective PPARδ ligands and considerable structural and structure–activity data have been published.[4–23] Many of the reported PPAR ligands (α, γ and δ) are appreciably lipophilic, as illustrated by their *c* log *P* lipophilicity values.[24] Four PPARδ-selective agonists (**1**–**4**) are shown in Figure 1 with their reported PPARδ agonist activity and selectivity over the other two PPAR subtypes. The GSK compound, GW501516 (**1**), has provided a clearer understanding of the pharmacological role of PPARδ, demonstrating efficacy in a number of animal models. For example, in studies with insulin-resistant middle-aged rhesus monkeys, PPARδ compounds caused a dramatic dose-dependent rise in serum HDLc whilst lowering LDLc levels.[4,5] Compound **1** shows about 1000-fold selectivity versus the PPARα and γ subtypes. Within the benzazepine compound (**4**) reported by Johnson & Johnson is a lipophilic heterocycle-aryl side chain, a similar motif to the aryl-substituted thiazole present in compound **1**.

During our hit identification programme a low lipophilicity benzazepine compound **5** (*c* log *P* = 1.5) was synthesized that combined the anilide portion from compound **2** with the benzazepine scaffold contained within compound **4** (Fig. 2). The resulting compound **5** displayed only weak activity at PPARδ, however, with an EC_{50} value of 3 μM. Compound potency was assessed using a human PPARδ reporter assay.[25]

It became evident that a small structural change could afford more potent PPARδ agonists. The nitrogen-transposed compound **6** (8-oxybenz[*c*]azepine scaffold) was synthesized (*c* log *P* = 1.6) and surprisingly was found to be a potent PPARδ agonist, with an EC_{50} value of 25 nM (Fig. 3).

Performing the corresponding switch with the aryl thiophene methyl moiety to give compound **7** (regioisomer of the Johnson & Johnson compound **4**) resulted in a considerable drop in PPARδ potency. In short, the arylthienylmethyl group is more active with the 7-oxybenz[*d*]azepine scaffold and the acetanilide is more active in the 8-oxybenz[*c*]azepine.

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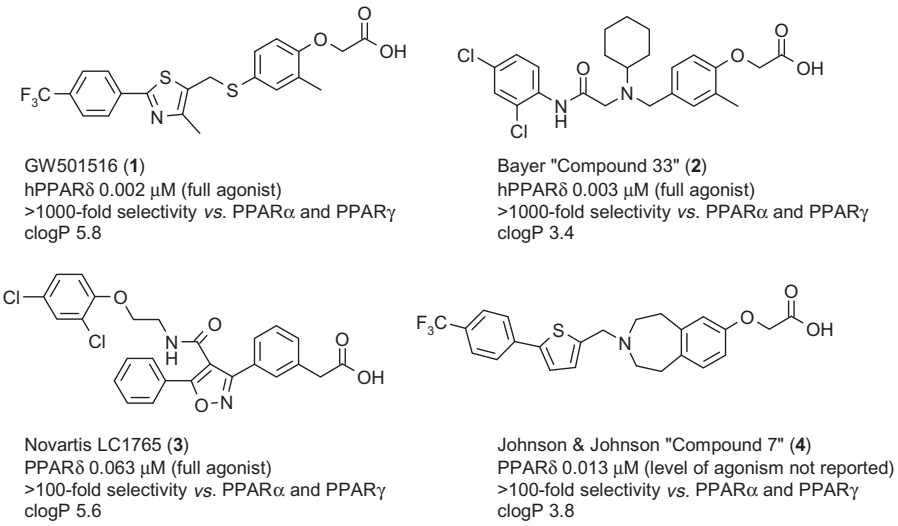
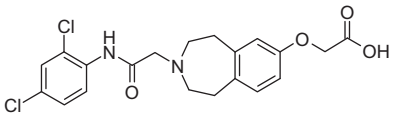
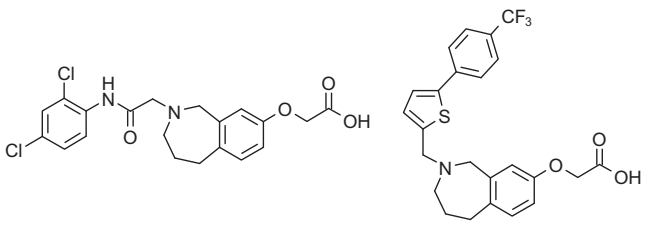


Figure 1. Four selective PPARδ agonists with reported PPARδ agonist activity (EC_{50}) and subtype selectivity.



5 PPARδ EC_{50} = 3.2 μM (full agonist)

Figure 2. Compound **5**, combining the anilide fragment from compound **2** with the benzazepine moiety from compound **4**.



6, PPARδ EC_{50} = 0.025 μM (full agonist) **7**, PPARδ EC_{50} = 0.31 μM (full)

Figure 3. Two 8-oxybenz[*c*]azepine compounds.

Structure–activity relationships within the series (EC_{50} agonism values are shown in Fig. 4) were explored through variation of the anilide substituents (Table 1, entries **6**, **11**–**15**) and through incorporation of a chiral alkyl group in the chain adjacent to the benzazepine nitrogen (entries **8**–**10**).

These data show that changes to the 2,4-dichlorophenyl substitution on the terminal aryl ring result in a dramatic reduction in potency, whereas incorporation of a simple side chain adjacent to the weakly basic nitrogen affords a significant improvement in potency. In some ways this mirrors the structure–activity relationships seen in the J&J compounds where an ethyl side chain affords a log unit improvement in potency.[12] Most of the potency is present in one of the two isomers; compound **8**, with a PPARδ EC_{50} of 3 nM, was found to be 40-fold more potent than its enantiomer **9**.

The synthesis of compounds **5** and **6** is illustrated in Scheme 1.[26] Initially, in order to access both of the regioisomeric benzazepines, 7-methoxy-2-tetralone **19** was treated with sodium azide in sulfuric acid via Schmidt rearrangement to give a 1:1 ratio of benzazepinone regioisomers **20** and **21**.

The isomeric mixture was elaborated through borane-mediated amide reduction, demethylation, *tert*-butoxycarbonyl protection, and alkylation of the phenol to furnish isomers **26** and **27**. Selective removal of the carbamate protecting group gave the amines **28** and **29** that were then separated by crystallisation of the oxalate salt. Alkylation prior to final *tert*-butyl ester deprotection yielded the final compounds **5** and **6**. All compounds gave satisfactory spectral and analytical data.[27]

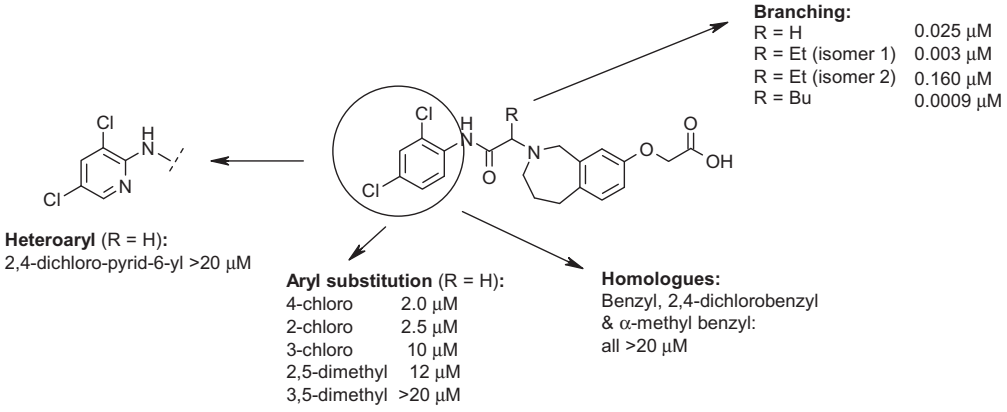
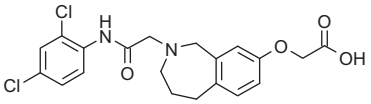
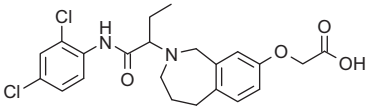
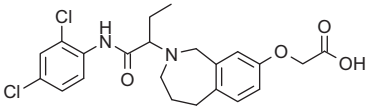
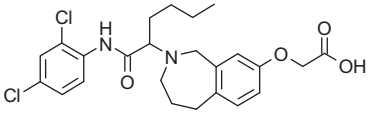
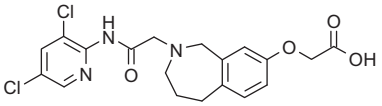
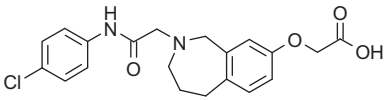
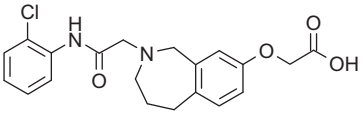
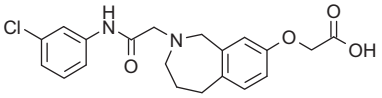
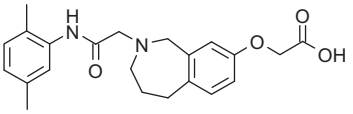
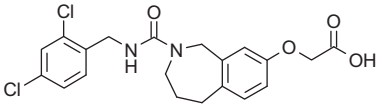
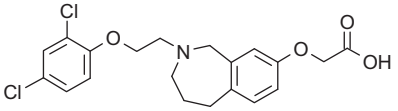
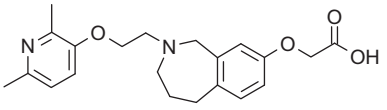


Figure 4. Summary of structure–PPARδ activity relationships for the 8-oxybenz[*c*]azepine compounds. All compounds were full agonists versus PPARδ reference compound **1**.

Table 1
PPARδ, PPARα and PPARγ activity of the 8-oxybenz[*c*]azepine compounds

Entry	Structure	*c* log *P*	PPARδ EC_{50}[a] (μM)	PPARα EC_{50}[a] (μM)	PPARγ EC_{50}[a] (μM)
6		1.6	0.025 (1.0)	3.2 (0.8)	>10 (0.4)
8 (enantiomer 1)		2.3	0.003 (1.0)	6.0 (1.3)	>20 (>0.5)
9 (enantiomer 2)		2.3	0.16 (1.0)	NT	NT
10		3.3	0.0009 (1.2)	4.2 (1.0)	>20 (>0.6)
11		0.7	>20 (>0.2)	NT	NT
12		1.6	2.0 (1.0)	NT	NT
13		0.8	2.5 (0.8)	NT	NT
14		1.6	10.0 (0.9)	NT	NT
15		0.9	12.6 (1.2)	NT	NT
16		4.3	15.8 (1.1)	NT	NT
17		3.0	1.6 (1.2)	7.9 (0.2)	IA
18		1.4	5.0 (0.9)	IA	IA

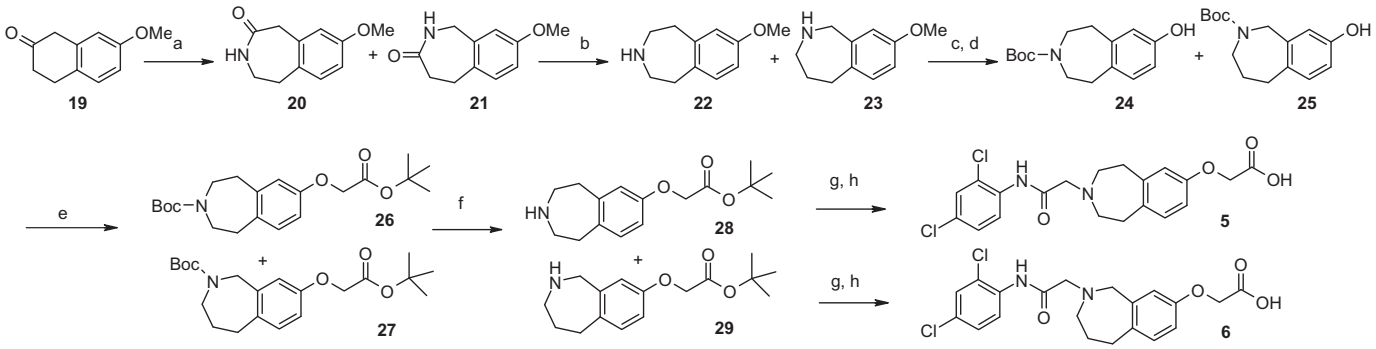
[a] Efficacy ratio (in parentheses) compared to a standard PPARδ, PPARα or PPARγ agonist, respectively.

As it became apparent that compounds based upon the 8-oxybenz[*c*]azepine scaffold were more active at PPARδ, the core **31** was synthesized from 7-methoxy-1-tetralone **30** in an improved yield, as shown in Scheme 2.

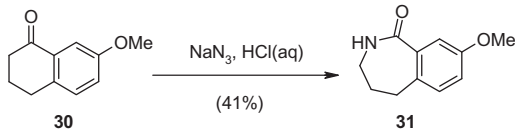
The synthesis of chiral ethyl compounds **8** and **9** is shown in Scheme 3. Racemic compound **10** with a butyl side chain was prepared in a similar fashion. The individual enantiomers of the racemic ethyl compound methyl ester were separated by HPLC using a chiral column,[28] then hydrolysed to give compounds **8** and **9**.

In order to determine the absolute stereochemical configurations of compounds **8** and **9** both the (*R*)- and (*S*)-configurations of the structure were docked into the PPARδ crystal structure (pdb code 2J14).[14] This involved preparing the crystal structure where hydrogen atoms were added and minimised.[29] The three-dimensional low energy ligand structures were then prepared using LigPrep,[30] and subsequently Glide[31–34] was used to dock both stereochemical configurations into the active site of PPARδ.

The docking of the (*R*)-configuration of this compound in Figure 5A shows that the PPARδ cavity lined by residues Phe327, Leu330, Val334, Leu339, Ile364 and Lys367 is occupied by the chiral ethyl group (circled), and so this is likely to be the configuration of the more active compound **8**. For the (*S*)-configuration the ethyl group does not sit in this pocket, and clashes with the protein (Fig. 5B). Therefore the (*S*)-configuration is likely to be the less active compound **9**. This cavity is filled by the isoxazole 5-phenyl substituent in the Novartis isoxazole compound **3**.[13] In PPARα and PPARγ, the



Scheme 1. Synthesis of benzazepine compounds **5** and **6**. Reagents and conditions: (a) NaN_3, H_2SO_4 (29%, 1:1 mixture of **20** and **21**); (b) BH_3, THF, reflux 18 h; (c) HBr, H_2O; (d) $(BOC)_2O$; (e) *tert*-butyl bromoacetate, Cs_2CO_3, MeCN (85% over three steps); (f) (i) TFA, CH_2Cl_2, 10 °C, 18 h; (ii) work-up 10% Na_2CO_3 (aq)/CH_2Cl_2; (iii) oxalic acid (1.5 equiv), EtOAc, recrystallize (70%); (g) 2-bromo-*N*-(2,4-dichlorophenyl)acetamide, K_2CO_3, NaI, DMF, 100 °C, 18 h (82%); (h) 1:2 TFA/CH_2Cl_2, rt (81%).



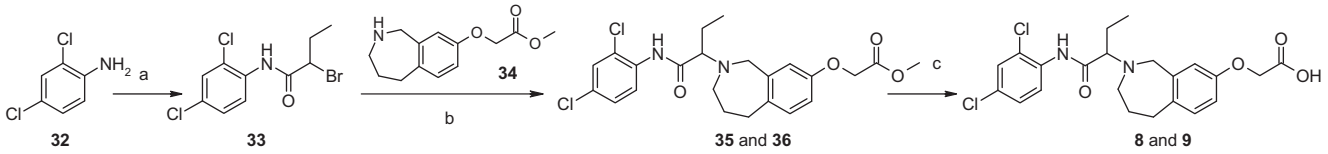
Scheme 2. Synthesis of the 8-oxybenz[*c*]azepine scaffold from 7-methoxy-1-tetralone.

corresponding cavity does not exist, which might explain the observed enhanced PPARδ potency and selectivity of compounds **8** and **10**.

Selectivity at PPARδ over the other PPAR subtypes was thought to be important in order to understand the in vivo effects of PPARδ agonism. Compound **8** satisfied these selectivity criteria, being 2000-fold selective against PPARα and over 6000-fold selective over PPARγ in the reporter gene agonist assays (see Table 1). Furthermore, selectivity against these PPAR subtypes is viewed as essential with respect to safety.

Compound **8**, with a PPARδ EC_{50} of 3 nM, displayed reasonable in vitro intrinsic clearance (CL_{int}) values (rat hepatocytes 23 μL/min/1E6 cells, human liver microsomes 24 μL/min/mg). This compared favourably to compound **2**, for example, with corresponding values of 43 μL/min/1E6 cells and 100 μL/min/mg. In addition, compound **8** had a measurable unbound fraction in the presence of plasma protein, with a human free fraction of 1.2%, and 0.6% in the rat. This compound also displayed high aqueous solubility (2.9 mM), and a measured dissociation coefficient (log $D_{7.4}$) value of 1.0. The base pK_a was measured at 5.7 and the acid moiety at 3.2.

Compound **8** displayed promising pharmacokinetics in the rat. An iv dose (1 mg/kg) of **8** gave a volume of distribution (V_{ss}) of 1.7 L/kg, likely due to the weakly zwitterionic nature of the compound. Coupled with a low plasma clearance value of 6 mL/min/kg (scaling from the rat hepatocyte value of 23 μL/min/1E6 cells using the well-stirred model had predicted an in vivo clearance of 4 mL/min/kg), this translated into an iv half-life of 4.1 h. Incubation of **8** with rat hepatocytes (1.5 h incubation at 10 μM) revealed six metabolites (analysed by LC–MS/MS) together representing



Scheme 3. Synthesis of benzazepine compounds **8** and **9**. Reagents and conditions: (a) 2-bromobutanoyl bromide, Hünig's base, CH_2Cl_2, 18 h, rt (86%); (b) (i) K_2CO_3, NaI, DMF, 100 °C, 18 h; (ii) separation by HPLC using a Chiralcel OD column: **35** (22%) and **36** (25%); (c) 1:2 TFA/CH_2Cl_2, rt: **8** (57%) and **9** (46%).

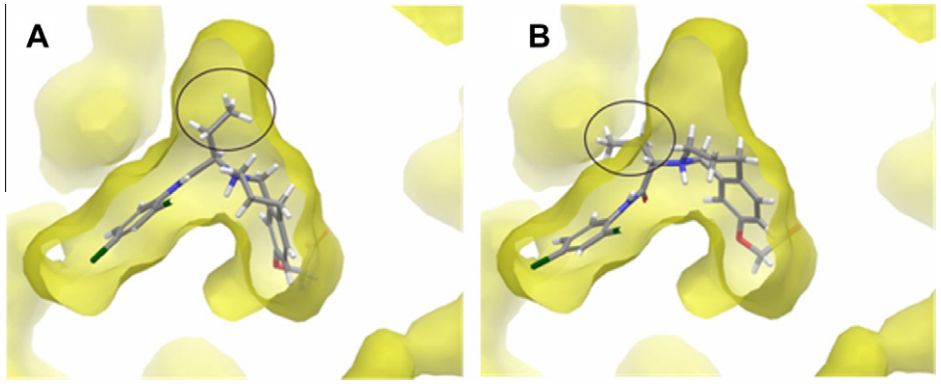


Figure 5. Docked conformations for (A) (*R*)-configuration, and (B) (*S*)-configuration. The PPARδ protein surface is shown in yellow, and has been clipped to show the ligand-binding site. The ligands are shown as capped sticks.

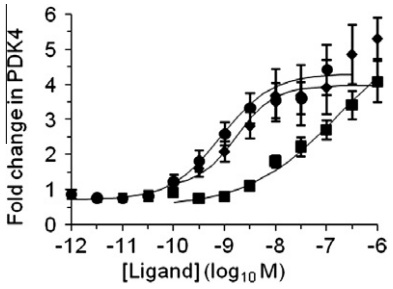


Figure 6. Dose–response curve for compounds **6** and **8** in the PDK4 assay showing effect of compound concentration on upregulation of PDK4 mRNA. ● GW501516 (**1**), ■ compound **6**, ♦ compound **8**.

about 10% of the total judged by LC–MS response, leaving over 80% unchanged parent. All of these six metabolites were derived from chemical modification of the azepine ring, three with loss of 2H, and three hydroxylated derivatives, one of which was the glucuronide. Furthermore, the compounds showed no cardiac QT liability in vitro and exhibited excellent glutathione stability (pH 7.4), chemical (pH 2, 7.4 and 11) and plasma stability.

Compound **10**, slightly higher in lipophilicity, was yet more potent in the PPARδ agonist assay, with an EC_{50} value of 0.9 nM. Additionally, this showed excellent PPAR selectivity: 4500-fold selectivity against PPARα and over 20,000-fold over PPARγ.

Activity in a relevant human biological effect assay (primary human myotubes)[35] has been determined for two key compounds in this series, **6** and **8**, with GW501516 (**1**) included as a reference compound (Fig. 6). This assay was configured to measure upregulation of pyruvate dehydrogenase kinase, isozyme 4 (PDK4) mRNA. PDK4 is a mitochondrial enzyme, which inhibits glucose oxidation via inactivation of the pyruvate dehydrogenase complex, thereby increasing fatty acid oxidation. For compound **6**, potency in this assay (EC_{50} = 69 nM) was consistent with the potency determined in the hPPARδ reporter assay (EC_{50} = 25 nM). Similarly, compound **8**, displayed a potency of 2 nM in the biological effect assay, and 3.2 nM in the reporter assay.

Some exploratory chemistry was conducted around modifications to the acetamide linker, but generally proved unsuccessful in delivering compound improvements. For example, the urea functionality in **16** (Table 1) was not tolerated and the ether compounds **17** and **18** were only weak agonists at PPARδ, showing that the acetamide linker is intolerant of changes.

In summary, a series of small molecule PPARδ agonists has been found that displays excellent selectivity over the PPARα and PPARγ subtypes. Compounds **6** and **8** demonstrated efficacy in upregulation of PDK4 in human primary myotubes, whilst compound **8** showed a good pharmacokinetic profile in the rat.

Acknowledgement

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24. *c* log *P* 4.3 is a predicted octanol/water partition coefficient from Daylight Chemical Information Systems and BioByte Corp.
25. HEK293 cells were stably transfected with a construct comprising the ligand binding domain of human PPARδ fused to the GAL4 DNA binding domain. The primary PPARδ screening assay contained the most common sequence of PPARD (NM_006238). The cell stably expresses a luciferase gene under the control of a GAL4 promoter. Compounds which bind and activate the PPARδ/GAL4 fusion promote luciferase production, which was quantified by measuring luminescence generated from a luciferase substrate. GW501516 **1** was used as reference compound and the intrinsic activity of **1** was set to 100%.
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27. *Selected experimental and analytical information*: *Compound* **5**: (2-(3-(2-(2,4-dichlorophenylamino)-2-oxoethyl)-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-7-yloxy)acetic acid): To a stirred solution of *tert*-butyl-2-(3-(2-(2,4-dichlorophenylamino)-2-oxoethyl)-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-7-yloxy) acetic acid (280 mg, 0.58 mmol) in dichloromethane (3 mL) at rt was added TFA (1.5 mL), and the solution was stirred at rt for 2 h. The mixture was concentrated in vacuo and was subjected to mass-directed reverse-phase HPLC purification (0.1% NH_3(aq)/MeOH) on a Gemini® column NX C18 (30 × 100 mm) to provide **5** (58 mg, 24%) as a white solid. Purity 98.3% @ 220 nm; LRMS (APCI) *m*/*z* [M+H]+ = 423, 425; [M−H]− = 421, 423; HRMS *m*/*z* [M+H]+ = 423.0855, calcd 423.0873, (err. 4.25 ppm); ^{1}H NMR (399.8 MHz, DMSO) δ 10.18 (s, 1H), 8.33 (d, *J* = 9.0 Hz, 1H), 7.71 (d, *J* = 2.3 Hz, 1H), 7.46 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.98 (d, *J* = 8.2 Hz, 1H), 6.65 (d, *J* = 2.6 Hz, 1H), 6.56 (dd, *J* = 8.2, 2.6 Hz, 1H), 4.30 (s, 2H), 3.27 (s, 2H), 2.91–2.84 (m, 4H), 2.74–2.65 (m, 4H), acid OH resonance absent; ^{13}C NMR (125.8 MHz, DMSO) δ 170.4, 169.0, 156.7, 142.3, 133.6, 133.1, 129.5, 128.6, 127.9, 127.7, 123.5, 121.9, 115.2, 111.2, 65.9, 62.1, 55.9, 55.5, 36.3, 35.2; melting point 205–210 °C (dec). Elemental Anal. Calcd for $C_{20}H_{20}Cl_2N_2O_4 \cdot 0.55NH_3, 0.45H_2O$: C, 54.50; H, 5.16; N 8.10. Found: C, 54.47; H, 5.06; N, 8.12.
Compound (R)-**8**: (2-(2-(1-(2,4-dichlorophenylamino)-1-oxobutan-2-yl)-2,3,4,5-tetrahydro-1*H*-benzo[*c*]azepin-8-yloxy)acetic acid): To a stirred solution of **35** (methyl 2-(2-(1-(2,4-dichlorophenylamino)-1-oxobutan-2-yl)-2,3,4,5-tetrahydro-1*H*-benzo[*c*]azepin-8-yloxy)acetate, 63 mg, 0.14 mmol)[28] in THF (3 mL) and water (1.5 mL) was added lithium hydroxide (16 mg, 0.68 mmol). This solution was stirred at rt for 18 h. Acetic acid (six drops) was added to the

mixture, which was subjected to mass-directed reverse-phase HPLC purification (0.1% NH_3(aq)/MeOH) on a Gemini® column NX C18 (30 × 100 mm) to furnish **8** (35 mg, 55%) as an off-white solid. Purity 99.9% @ 220 nm; LRMS (APCI) *m*/*z* [M+H]+ = 451, 453; HRMS *m*/*z* [M+H]+ = 451.1180, calcd 451.1180, (err. 1.33 ppm); $[\alpha]_D^{23}$ +29 (*c* 1.71, MeOH); ^{1}H NMR (400 MHz, DMSO) δ 9.83 (s, 1H), 8.02 (d, *J* = 8.7 Hz, 1H), 7.60 (d, *J* = 2.6 Hz, 1H), 7.41 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.98 (d, *J* = 8.2 Hz, 1H), 6.58 (d, *J* = 2.6 Hz, 1H), 6.55 (dd, *J* = 8.2, 2.6 Hz, 1H), 4.11 (s, 2H), 3.87 (d, *J* = 14.6 Hz, 1H), 3.79 (d, *J* = 14.6 Hz, 1H), 3.35 (t, *J* = 6.9 Hz, 1H), 3.08–3.01 (m, 2H), 2.81–2.75 (m, 2H), 1.88–1.78 (m, 1H), 1.77–1.64 (m, 3H), 0.95 (t, *J* = 7.4 Hz, 3H), acid OH resonance absent; ^{13}C NMR (125.8 MHz, DMSO) δ 171.4, 169.7, 157.0, 140.5, 133.8, 133.2, 129.2, 128.6, 128.3, 127.6, 125.4, 124.2, 115.5, 111.8; melting point 151–155 °C (dec). Elemental Anal. Calcd for $C_{22}H_{24}Cl_2N_2O_4 \cdot 1.8H_2O$: C, 54.62; H, 5.75; N 5.79. Found: C, 54.02; H, 5.14; N, 5.85.

Compound (rac)-**10**: (2-(2-(1-(2,4-dichlorophenylamino)-1-oxohexan-2-yl)-2,3,4,5-tetrahydro-1*H*-benzo[*c*]azepin-8-yloxy)acetic acid): the title compound was prepared analogously to compound **8** to provide an off-white solid (42 mg, 46%). Purity 99.2% @ 220 nm; LRMS (APCI) *m*/*z* [M+H]+ = 479, 481; [M−H]− = 477, 479; HRMS *m*/*z* [M+H]+ = 479.1499, calcd 479.1499; ^{1}H NMR (400 MHz, DMSO) δ 9.87 (s, 1H), 8.03 (d, *J* = 8.7 Hz, 1H), 7.61 (d, *J* = 2.6 Hz, 1H), 7.41 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.97 (d, *J* = 8.2 Hz, 1H), 6.58–6.51 (m, 2H), 4.01 (s, 2H), 3.85 (d, *J* = 14.6 Hz, 1H), 3.77 (d, *J* = 14.4 Hz, 1H), 3.44 (t, *J* = 6.9 Hz, 1H), 3.07–3.01 (m, 2H), 2.80–2.74 (m, 2H), 1.85–1.77 (m, 1H), 1.75–1.64 (m, 3H), 1.38–1.29 (m, 4H), 0.91–0.85 (m, 3H), acid OH resonance absent; ^{13}C NMR (125.8 MHz, DMSO) δ 171.5, 169.8, 156.8, 140.6, 133.8, 133.4, 129.2, 128.6, 128.2, 127.6, 125.2, 124.2, 115.4, 111.8, 68.0, 67.3, 57.9, 56.2, 33.7, 28.6, 28.5, 27.5, 22.2, 13.8; melting point 137–142 °C (dec). Elemental Anal. Calcd for $C_{24}H_{28}Cl_2N_2O_4 \cdot 0.1NH_3$, $0.85H_2O$: C, 58.07; H, 6.09; N 5.93. Found: C, 57.86; H, 5.82; N, 6.20.

28. Compounds **35** and **36** were isolated by preparative HPLC on a Chiralcel OD 10 x 250 mm column, eluent 50:50 EtOH/MeOH, flow rate 2 mL/min, at rt, uv detection 220 nm, run time 27 min.
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35. Human skeletal muscle myoblast cells were isolated by enzymatic digestion from the *vastus lateralis*. The myoblasts were differentiated for 7 days in 2% horse serum. Cells were treated with compound for 24 h before measuring fold induction of PDK4 mRNA.