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Design, Synthesis and Pharmacological Evaluation of 4-Hydroxyphenylglycine and 4-Hydroxyphenylglycinol Derivatives as GPR88 Agonists

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Abstract

The orphan receptor GPR88 is an attractive therapeutic target because of its implications in a number of basal ganglia-associated disorders. To date, pharmacological characterization of GPR88 has been limited due to the lack of potent and selective agonists and antagonists appropriate for CNS investigations. We have previously reported that GPR88 couples to $G\alpha_i$ proteins and modulates cAMP levels upon treatment with a small molecule agonist 2-PCCA. Recently, another chemotype of GPR88 agonist, represented by 2-AMPP [(2*S*)-*N*-((1*R*)-2-amino-1-(4-(2-methylpentyloxy)-phenyl)ethyl)-2-phenylpropanamide], has also been discovered. In this report, a new series of 2-AMPP structurally related 4-hydroxyphenylglycine and 4-hydroxyphenylglycinol derivatives have been designed and evaluated for agonist activity at GPR88. The structure-activity relationship (SAR) studies suggest that the amine group in 2-AMPP can be replaced by hydroxyl, ester and amide groups, resulting in analogues with good to moderate potency, whereas the phenyl group on the amide cap is essential for activity and has limited size, shape and electronic tolerance.

TOC images

X = CH₂NH₂, CH₂OH, CO₂Me, CONMe₂

R = alkyl and phenyl substitutions

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Keywords

GPR88; 2-PCCA; 2-AMPP; agonist; SAR

1. Introduction

The G protein-coupled receptors (GPCRs) represent the largest and most diverse group of membrane proteins. GPCRs have attracted significant attention for drug discovery due to their numerous physiological and pathological roles in mediating cellular responses to hormones and neurotransmitters.¹ Within the large GPCR family, 140 GPCRs, excluding olfactory receptors, are classified as orphan receptors because their endogenous ligands are still unknown.² The key to discovering the therapeutic potential of orphan GPCRs is to identify highly potent and selective ligands thereby allowing the elucidation of their biological functions in both physiological and pathological states.³

GPR88 is an orphan GPCR and has recently attracted considerable interest in studying its biological functions, mainly through genetic interference. GPR88 exhibits high expression in the brain, with particularly robust expression in both striatal D₁ and D₂-expressing GABAergic neurons, suggesting the receptor may play a role in regulating dopaminergic activity.^{4–8} Genetically-modified mice that lack GPR88 expression exhibit enhanced response to dopaminergic agonists and altered performance in models relevant to schizophrenia and anxiety, indicating that GPR88 may have a potential therapeutic role in treating these CNS disorders.^{9–11}

With the aim of developing potent and selective small molecular ligands to probe GPR88 functions, we previously reported that (1*R*,2*R*)-2-PCCA [(1*R*,2*R*)-2-(pyridin-2-yl)cyclopropane carboxylic acid ((2*S*,3*S*)-2-amino-3-methylpentyl)-(4'-propylbiphenyl-4-yl)amide (**1**); Figure 1] was able to activate GPR88 through a Gα_i-coupled pathway.¹² (1*R*,2*R*)-2-PCCA had an EC₅₀ value in the range of 50–100 nM in Lance cAMP assays using stable PPLS-HA-GPR88 CHO cells.¹³ Although 2-PCCA is useful in the characterization of signaling pathways of GPR88, much research remains to be done before the pharmacology of GPR88 is fully understood. Structurally different chemotypes showing potent and selective GPR88 agonism/antagonism will be particularly helpful. Recently, another chemotype of GPR88 agonist, 2-AMPP [(2*S*)-*N*((1*R*)-2-amino-1-(4-(2-methylpentyl)oxy)-phenyl)ethyl)-2-phenylpropanamide (**2**)], was reported to activate GPR88 with an EC₅₀ value of 90 nM in the HTRF cAMP assay.¹⁴ Later, **2** was shown to elicit strong [³⁵S]-GTPγS binding (EC₅₀ = 940 nM) in mice striatal membranes but was ineffective in the samples from the GPR88 knockout mice, demonstrating the compound is GPR88-specific in the striatum.¹¹ Early structure-activity relationship (SAR) studies of **2** suggest that the amine group can be replaced by a hydroxyl group to give phenylglycinol **3** without altering GPR88 potency, whereas the substitutions on the amide cap seem to have a profound effect on the activity. In this paper, we designed and synthesized a new series of 4-hydroxyphenylglycine and 4-hydroxyphenylglycinol derivatives with structural modifications at sites A and B (Figure 1) in order to determine receptor tolerances at each site and enhance the potency for GPR88.

2. Chemistry

Synthesis of the designed compounds **2**, **3** and **11a–c** is outlined in Scheme 1. Boc protection of the amine group in (*R*)-2-phenylglycine methyl ester (**4**) afforded **5** in quantitative yield. O-alkylation of **5** with 2-methylpentanol under Mitsunobu conditions gave **6** in 78% yield. The methyl ester was reduced to the hydroxyl group, which was then reacted with triphenylphosphine and carbon tetrabromide to produce the bromo intermediate **7b** in 76% yield over two steps. Treatment of **7b** with sodium azide yielded **7c** quantitatively. Removal of the Boc protecting group in **7a** and **7c** with TFA led to amines **8a,b**. Amine **8a** was coupled with (*S*)-2-phenylpropionic acid using HBTU to give the target compound **3** in 53% yield. Following the same conditions, amine **8b** was coupled with (*S*)-2-phenylpropionic acid to afford azide **9** in 72% yield. Reduction of azide under hydrogenation provided 2-AMPP (**2**) in 66% yield. Boc deprotection of **6** led to amine **10**, which was then coupled with (*S*)-2-phenylpropionic acid to give **11a** in 94% yield. Saponification of the ester group afforded acid **11b** in 77% yield. Coupling of the acid with dimethylamine using EDC hydrochloride and HOBt gave amide **11c** in 45% yield.

Synthesis of target compounds **12a–d** and **14** was accomplished in 48–98% yield by coupling of amine **8a** or **10** with an appropriate acid (Scheme 2). A library of compounds with general structure **13** were synthesized by acylation of **10** with commercially available carboxylic acids using HBTU as the coupling agent, which provided clean products as judged by TLC analyses. The identity of library compounds was confirmed by mass spectroscopy (MS). The library compounds, used without purification, were screened at 1 μ M to evaluate their agonist activity at GPR88. Eight of the library compounds (**13a**, **13e–g**, **13m**, **13p**, **13z** and **13aw**) were resynthesized in pure form to determine their potency. All purified target compounds were characterized by ^1H NMR, ^{13}C NMR and MS, and the data were in agreement with the assigned structures.

3. Results and discussion

Recently, 2-AMPP was discovered as a GPR88 agonist by high throughput screening, followed by hit-to-lead optimization.¹⁴ Given its favorable calculated physiochemical properties (clogP = 4.53, TPSA = 64.35, logBB = -0.12)^{15–17} and GPR88-specific activity in the striatum,¹¹ 2-AMPP is a promising lead for further optimization to probe GPR88 *in vivo* functions. In order to gain the SAR information of 2-AMPP for high potency, we have synthesized a series of analogues with structural modifications at sites A and B (Figure 1) and evaluated their agonist activity at GPR88. In our Lance cAMP assay, 2-AMPP had an EC₅₀ of 414 nM (Table 1). Exchange of the amine group (site A) with a hydroxyl (**3**) led to a 2-fold increase of potency. The phenylglycinol **3** possessed an EC₅₀ of 194 nM, which was in line with the EC₅₀ value (110 nM) obtained in the HTRF assay.¹⁴ Interestingly, an azide group at site A gave the most potent compound (**9**, EC₅₀ = 134 nM) in the series. However, a carboxylic acid group resulted in a completely inactive compound **11b**. The methyl ester **11a** and amide **11c** were moderately active, with EC₅₀ value of 538 nM and 616 nM, respectively. These findings suggested that a hydrogen bonding/electrostatic interaction between site A and the receptor might contribute to the agonist activity, but a carboxylic acid was not favorable.

Substituent effects on the amide cap (site B) of **3** were next investigated and the results are summarized in Table 2. We first examined the linkage requirement between the phenyl and amide carbonyl group for receptor activity. The benzyl analogue **12a** was approximately 2-fold less potent than **3** (487 nM vs. 194 nM). Substitution with a phenethyl group led to **12b**, which also possessed a moderate potency ($EC_{50} = 345$ nM). However, moving the phenyl group farther away from the amide carbonyl using a propyl link resulted in deteriorated activity. The phenyl substitution on the amide cap is essential for activity, possibly forming an aromatic stacking interaction with the binding pocket, as the cyclohexyl analogue **12d** was completely inactive compared to the moderately active **11a** ($EC_{50} = 538$ nM).

To further evaluate the substituent effects of size, polarity, lipophilicity, and electronic tolerance on the amide cap, a library of 54 analogues with general structure **13** was synthesized and screened for agonist activity at a final concentration of 1 μ M using the Lance cAMP assay. Although the methyl ester **11a** was approximately 2.5-fold less potent than the hydroxyl analogue **3** (Table 1), the methyl ester at site A was chosen for library synthesis because of its synthetic feasibility. We initially attempted to synthesize a library of analogues of **3**, but the coupling reaction of amine **8a** with carboxylic acid gave a mixture of crude products, which was not suitable for screening without purification. The 1 μ M concentration was used for screening based on the activity of the parent analogue **11a**. The structures of the compounds synthesized are given in Table 3. The agonist activity is presented in Figure 2 as the percentage of TR-FRET signal of the control compound **11a**. The screening results revealed that **13ay** (compound #52), the crude form of **11a**, had an activity similar to **11a**. The (*R*)-isomer **13az** with a benzylic methyl group was less potent than the (*S*)-isomer (compound #53 vs #52, Figure 2). The benzylic position could also tolerate an ethyl group (compound #50), but other bulky groups (compounds #45–49 and #51) at this position led to decreased activity compared to **11a**. None of the benzyl derivatives bearing a variety of substitutions on the phenyl ring had a significantly improved activity compared to the unsubstituted analogue (compounds #3–44 vs #2). In view of these results, eight of the library compounds (**13a**, **13e–g**, **13m**, **13p**, **13z** and **13aw**) were resynthesized and characterized by ^1H NMR, ^{13}C NMR and MS for biological testing (Table 2). In general, the SAR trend observed in the library screen was in agreement with that of the purified compounds. The benzyl analogue **13a**, lacking a benzylic methyl group, was 5-fold less potent than **11a** (2630 nM vs 538 nM). All the substitutions on the phenyl ring led to detrimental activity, possibly due to the limited steric tolerance, as both electron donating (**13p**) and electron withdrawing (**13z**) groups were inactive. The ethyl analogue **13aw** was equipotent with **11a** and was 4.5-fold more potent than **13a**, validating the screening results. Finally, we synthesized the phenylglycinol analogue **14**, which had an EC_{50} value of 145 nM.

4. Conclusions

In summary, we have designed and synthesized a series of 4-hydroxyphenylglycine and 4-hydroxyphenylglycinol analogues to determine SAR requirements at GPR88. The target compounds were evaluated in a Lance cAMP assay using stable PPLS-HA-GPR88 CHO cells. SAR studies suggest that the amine group in 2-AMPP can be replaced by azide,

hydroxyl, ester and amide groups, resulting in analogues with good to moderate potency. The phenyl group on the amide cap is essential for activity, possibly due to the formation of an aromatic stacking interaction with the receptor. However, the substitutions on the phenyl ring have limited size, shape and electronic tolerance.

Recently, we reported a homology model of GPR88 and docking study of (1*R*,2*R*)-PCCA, which suggested the presence of a small hydrophobic binding pocket housing the aromatic group on the amide cap.¹³ Superposition of (1*R*,2*R*)-PCCA with 2-AMPP, as well as the similar SAR trends¹⁸ observed in the region of the amide cap for both scaffolds, suggests that the amide cap in 2-AMPP may position in the same binding pocket as that of (1*R*,2*R*)-PCCA. Docking of 2-AMPP analogues will help refine the model and explore structural features for optimization. Studies including chemical modifications of compounds **3**, **9** and **14** to further improve the potency and evaluation of receptor specificity are currently under investigation.

5. Experimental procedures

5.1. Chemistry

5.1.1. General—Reagents and starting materials were obtained from commercial suppliers and were used without purification. All moisture- and air-sensitive reactions and reagent transfers were carried out under dry nitrogen. Nuclear magnetic resonance (¹H NMR and ¹³C NMR) spectra were obtained on a Bruker Avance DPX-300 MHz NMR spectrometer. Chemical shifts are reported in parts per million (ppm) with reference to internal solvent. Mass spectra (MS) were run on a Waters Alliance HT/Micromass ZQ system (ESI). Analytical thin-layer chromatography (TLC) was carried out using EMD silica gel 60 F₂₅₄ TLC plates. TLC visualization was achieved with a UV lamp or in an iodine chamber. Flash column chromatography was done on a CombiFlash Companion system using Isco preppacked silica gel columns.

5.1.2. (2*R*)-Methyl 2-(*tert*-butoxycarbonylamino)-2-(4-hydroxyphenyl)acetate (5**)**—To a solution of (*R*)-2-phenylglycine methyl ester hydrochloride (5 g, 23 mmol) in DCM (175 mL) at 0 °C under nitrogen was added DIPEA (12 mL, 69 mmol), followed by di-*tert*-butyldicarbonate (5.2 g, 23 mmol). The reaction mixture was stirred at room temperature overnight, and concentrated under reduced pressure. The residue was dissolved in EtOAc (200 mL), washed with 10% citric acid (3 × 50 mL), brine (3 × 50 mL) and dried (Na₂SO₄). Removal of the solvent under reduced pressure afforded the crude **5** (6.45 g, 100%) as a colorless oil. ¹H NMR (300 MHz; CDCl₃) δ 7.16 (d, *J* = 9.0 Hz, 2H), 7.13 (d, *J* = 9.0 Hz, 2H), 6.44 (s, 1H), 5.60 (br d, *J* = 6.0 Hz, 1H), 5.22 (d, *J* = 6.0 Hz, 1H), 3.71 (s, 3H), 1.44 (s, 9H); ¹³C NMR (75 MHz; CDCl₃) δ 172.0, 156.3, 155.0, 128.5, 128.4, 115.8, 80.5, 57.1, 52.7, 28.3; MS (ESI) *m/z* 282.3 [M + H]⁺.

5.1.3. (2*R*)-Methyl 2-(*tert*-butoxycarbonylamino)-2-[4-(2-methylpentylloxy)phenyl]acetate (6**)**—To a solution of **5** (2.81 g, 10 mmol), 2-methylpentanol (2.48 mL, 20 mmol), and PPh₃ (4.45 g, 17 mmol) in THF (70 mL) at room temperature under nitrogen was slowly added DEAD (7.8 mL, 17 mmol) dropwise, while keeping the reaction temperature below 35 °C. After addition, the reaction mixture was

stirred at room temperature overnight and quenched with H₂O (50 mL). The layers were separated and the aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (3 × 50 mL), dried (Na₂SO₄) and concentrated under reduced pressure. Flash column chromatography of the crude product on silica gel using 0–20% EtOAc in hexanes afforded **6** (2.85 g, 78%) as a colorless oil. ¹H NMR (300 MHz; CDCl₃) δ 7.26 (d, *J* = 9.0 Hz, 2H), 6.87 (d, *J* = 9.0 Hz, 2H), 5.46 (br d, *J* = 6.0 Hz, 1H), 5.23 (d, *J* = 6.0 Hz, 1H), 3.85–3.65 (m, 2H), 3.71 (s, 3H), 2.00–1.88 (m, 1H), 1.50–1.15 (m, 4H), 1.43 (s, 9H), 1.01 (d, *J* = 6.0 Hz, 3H), 0.92 (t, *J* = 7.5 Hz, 3H); ¹³C NMR (75 MHz; CDCl₃) δ 171.9, 159.5, 154.8, 128.7, 128.3, 114.9, 80.0, 73.3, 57.1, 52.5, 35.7, 32.9, 28.3, 20.0, 17.0, 14.3; MS (ESI) *m/z* 388.6 [M + Na]⁺.

5.1.4. *tert*-Butyl (1*R*)-2-hydroxy-1-[4-(2-methylpentylloxy)phenyl]ethylcarbamate (7a**)**—To a suspension of NaBH₄ (0.62 g, 16.4 mmol) in EtOH (23 mL) at 0 °C under nitrogen was added LiCl (0.7 g, 16.4 mmol). After stirring at 0 °C for 10 min, a solution of **6** (2.3 g, 6.3 mmol) in THF (23 mL) was added. The reaction mixture was stirred at room temperature for 3 h and quenched with saturated NH₄Cl solution (10 mL), followed by addition of H₂O (50 mL). The mixture was extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with brine (50 mL), dried (Na₂SO₄) and concentrated under reduced pressure. Flash column chromatography of the crude product on silica gel using 0–50% EtOAc in hexanes afforded **7a** (1.93 g, 91%) as a white semisolid. ¹H NMR (300 MHz; CDCl₃) δ 7.20 (d, *J* = 9.0 Hz, 2H), 6.87 (d, *J* = 9.0 Hz, 2H), 5.17 (br d, *J* = 6.0 Hz, 1H), 4.71 (br s, 1H), 3.86–3.72 (m, 3H), 3.72–3.65 (m, 1H), 2.48 (br s, 1H), 2.00–1.88 (m, 1H), 1.54–1.13 (m, 4H), 1.43 (s, 9H), 1.01 (d, *J* = 6.0 Hz, 3H), 0.92 (t, *J* = 7.5 Hz, 3H); ¹³C NMR (75 MHz; CDCl₃) δ 159.2, 156.4, 131.5, 127.9, 115.0, 80.2, 73.5, 67.2, 56.8, 36.0, 33.1, 28.6, 20.3, 17.2, 14.5; MS (ESI) *m/z* 338.6 [M + H]⁺.

5.1.5. *tert*-Butyl (1*R*)-2-bromo-1-[4-(2-methylpentylloxy)phenyl]ethylcarbamate (7b**)**—A solution of **7a** (1.05 g, 3.12 mmol), Ph₃P (1.25 g, 4.67 mmol) and CBr₄ (1.55 g, 4.67 mmol) in THF (20 mL) was stirred at room temperature under nitrogen for 3 h. The mixture was filtered and the filtrate was concentrated under reduced pressure. Flash column chromatography of the crude product on silica gel using 0–30% EtOAc in hexanes afforded **7b** (1.03 g, 83%) as a white solid. ¹H NMR (300 MHz; CDCl₃) δ 7.20 (d, *J* = 9.0 Hz, 2H), 6.88 (d, *J* = 9.0 Hz, 2H), 5.08 (br s, *J* = 6.0 Hz, 1H), 4.92 (br s, 1H), 3.85–3.76 (m, 1H), 3.75–3.60 (m, 3H), 2.02–1.90 (m, 1H), 1.60–1.12 (m, 4H), 1.44 (s, 9H), 1.02 (d, *J* = 6.0 Hz, 3H), 0.92 (t, *J* = 7.5 Hz, 3H); ¹³C NMR (75 MHz; CDCl₃) δ 159.1, 155.0, 131.3, 127.5, 114.7, 80.0, 73.3, 54.5, 37.2, 35.8, 32.9, 28.3, 20.0, 17.0, 14.3; MS (ESI) *m/z* 400.01 [M + H]⁺ (⁷⁹Br), 402.3 [M + H]⁺ (⁸¹Br).

5.1.6. *tert*-Butyl (1*R*)-2-azido-1-[4-(2-methylpentylloxy)phenyl]ethylcarbamate (7c**)**—To a solution of **7b** (0.9 g, 2.25 mmol) in DMF (10 mL) at room temperature under nitrogen was added NaN₃ (0.59 g, 9 mmol). After stirring for 6 h, the reaction was quenched by H₂O (30 mL) and extracted with Et₂O (3 × 30 mL). The combined organic layers were washed with brine (50 mL) and dried (Na₂SO₄). Removal of the solvent under reduced pressure afforded the crude **7c** (0.81 g, 100%) as a white semi-solid. ¹H NMR (300 MHz; CDCl₃) δ 7.21 (d, *J* = 9.0 Hz, 2H), 6.90 (d, *J* = 9.0 Hz, 2H), 4.98 (br s, 1H), 4.81 (br s, 1H),

3.86–3.78 (m, 1H), 3.78–3.55 (m, 3H), 2.00–1.90 (m, 1H), 1.55–1.13 (m, 4H), 1.43 (s, 9H), 1.01 (d, $J = 6.0$ Hz, 3H), 0.92 (t, $J = 7.5$ Hz, 3H); MS (ESI) m/z 363.2 $[M + H]^+$.

5.1.7. (2R)-2-Amino-2-[4-(2-methylpentylloxy)phenyl]ethanol hydrochloride (8a)

—To a solution of **7a** (400 mg, 1.2 mmol) in DCM (2 mL) at room temperature under nitrogen was added TFA (2 mL). After stirring for 1 h, the reaction was quenched with saturated NaHCO_3 (10 mL). The mixture was extracted with DCM (3×20 mL). The combined organic layers were washed with brine (20 mL) and dried (Na_2SO_4). Removal of the solvent under reduced pressure afforded the desired amine, which was then converted into the hydrochloride salt **8a** (325 mg, 100%) as a yellow foamy solid. ^1H NMR (300 MHz; CDCl_3) δ 8.32 (s, 3H), 7.39 (d, $J = 9.0$ Hz, 2H), 6.88 (d, $J = 9.0$ Hz, 2H), 4.44 (br s, 1H), 3.98–3.82 (m, 1H), 3.80–3.58 (m, 3H), 2.21 (br s, 1H), 2.00–1.80 (m, 1H), 1.52–1.12 (m, 4H), 0.99 (d, $J = 6.0$ Hz, 3H), 0.92 (t, $J = 7.5$ Hz, 3H); ^{13}C NMR (75 MHz; CDCl_3) δ 159.0, 129.0, 128.0, 115.2, 73.6, 68.2, 56.9, 36.0, 32.5, 20.3, 17.2, 14.5; MS (ESI) m/z 238.4 $[M + H]^+$.

5.1.8. (1R)-2-Azido-1-[4-(2-methylpentylloxy)phenyl]ethanamine (8b)—The procedure for **8a** was followed using 810 mg (2.2 mmol) of **7c** to give 575 mg (100%) of **8b** as a yellow oil. ^1H NMR (300 MHz; CDCl_3) δ 7.26 (d, $J = 9.0$ Hz, 2H), 6.88 (d, $J = 9.0$ Hz, 2H), 4.14–4.05 (m, 1H), 3.90–3.68 (m, 2H), 3.56–3.32 (m, 2H), 2.26 (br s, 2H), 2.02–1.86 (m, 1H), 1.54–1.13 (m, 4H), 1.01 (d, $J = 6.0$ Hz, 3H), 0.92 (t, $J = 7.5$ Hz, 3H); ^{13}C NMR (75 MHz; CDCl_3) δ 159.0, 133.5, 127.7, 114.9, 73.5, 59.2, 55.1, 35.9, 33.1, 20.2, 17.2, 14.5; MS (ESI) m/z 263.1 $[M + H]^+$.

5.1.9. (2S)-N-((1R)-2-Hydroxy-1-[4-(2-methylpentylloxy)phenyl]ethyl)-2-phenylpropanamide (3)

—To a solution of **8a** (90 mg, 0.33 mmol) in MeCN (10 mL) at room temperature under nitrogen was added TEA (0.14 mL, 0.99 mmol), followed by (S)-2-phenylpropionic acid (50 mg, 0.33 mmol) and HBTU (152 mg, 0.4 mmol). After stirring for 5 h, the reaction was quenched by H_2O (5 mL), followed by addition of EtOAc (50 mL). The layers were separated. The organic layer was washed with saturated NaHCO_3 (10 mL), brine (10 mL), dried (Na_2SO_4) and concentrated under reduced pressure. Flash column chromatography of the crude product on silica gel using 0–30% EtOAc in hexanes afforded **3** (65 mg, 53%) as an off-white solid. ^1H NMR (300 MHz; CDCl_3) δ 7.40–7.22 (m, 5H), 6.96 (d, $J = 9.0$ Hz, 2H), 6.78 (d, $J = 9.0$ Hz, 2H), 5.97 (d, $J = 6.0$ Hz, 1H), 5.02–4.93 (m, 1H), 3.85–3.72 (m, 3H), 3.70–3.60 (m, 2H), 2.60 (br s, 1H), 1.98–1.86 (m, 1H), 1.52 (d, $J = 6.0$ Hz, 3H), 1.50–1.12 (m, 4H), 0.99 (d, $J = 6.0$ Hz, 3H), 0.91 (t, $J = 7.5$ Hz, 3H); ^{13}C NMR (75 MHz; CDCl_3) δ 174.6, 158.9, 141.3, 130.6, 128.9, 127.6, 127.5, 127.3, 114.8, 73.3, 66.7, 55.4, 47.1, 35.8, 32.9, 20.0, 18.5, 17.0, 14.3; MS (ESI) m/z 370.5 $[M + H]^+$.

5.1.10. (2S)-N-((1R)-2-Azido-1-[4-(2-methylpentylloxy)phenyl]ethyl)-2-phenylpropanamide (9)

—The procedure for **3** was followed using 291 mg (1.1 mmol) of **8b** to give 326 mg (72%) of **9** as an off-white semi-solid. ^1H NMR (300 MHz; CDCl_3) δ 7.42–7.26 (m, 5H), 6.97 (d, $J = 9.0$ Hz, 2H), 6.79 (d, $J = 9.0$ Hz, 2H), 5.76 (d, $J = 6.0$ Hz, 1H), 5.15–5.06 (m, 1H), 3.82–3.75 (m, 1H), 3.75–3.50 (m, 4H), 2.00–1.88 (m, 1H), 1.53 (d, $J = 6.0$ Hz, 3H), 1.50–1.15 (m, 4H), 0.99 (d, $J = 6.0$ Hz, 3H), 0.91 (t, $J = 7.5$ Hz, 3H); ^{13}C

NMR (75 MHz; CDCl₃) δ 172.2, 157.6, 139.6, 128.9, 127.6, 126.2, 126.1, 126.0, 113.3, 71.8, 53.7, 50.6, 45.7, 34.3, 31.5, 18.6, 16.9, 15.6, 12.9; MS (ESI) m/z 395.5 [M + H]⁺.

5.1.11. (2S)-N-((1R)-2-Amino-1-[4-(2-methylpentylloxy)phenyl]ethyl)-2-phenylpropanamide hydrochloride (2)—A mixture of **9** (316 mg, 0.8 mmol) and 10% Pd/C (80 mg) in EtOH (5 mL) was hydrogenated under 40 psi for 1 h. The mixture was filtered through a short pad of Celite and the filtrate was concentrated. Flash column chromatography of the crude product on silica gel using 0–10% MeOH in DCM afforded 195 mg of a semi-solid, which was then treated with 2 M HCl in Et₂O to give the hydrochloride salt **2** (210 mg, 65%) as an off-white solid. Free base: ¹H NMR (300 MHz; CDCl₃) δ 7.40–7.15 (m, 5H), 6.92 (d, J = 9.0 Hz, 2H), 6.76 (d, J = 9.0 Hz, 2H), 6.30 (d, J = 6.0 Hz, 1H), 4.96–4.82 (m, 1H), 3.81–3.58 (m, 3H), 3.12–2.82 (m, 2H), 2.02–1.86 (m, 1H), 1.51 (d, J = 6.0 Hz, 3H), 1.50–1.12 (m, 6H), 0.99 (d, J = 6.0 Hz, 3H), 0.91 (t, J = 7.5 Hz, 3H); ¹³C NMR (75 MHz; CDCl₃) δ 173.6, 158.5, 141.6, 132.0, 128.8, 127.6, 127.3, 127.2, 114.6, 73.2, 54.3, 47.1, 46.8, 35.8, 32.9, 20.0, 18.5, 17.0, 14.3; MS (ESI) m/z 369.3 [M + H]⁺.

5.1.12. Methyl (2R)-2-amino-2-[4-(2-methylpentylloxy)phenyl]acetate hydrochloride (10)—The procedure for **8a** was followed using 3.86 g (10.5 mmol) of **6** to give 3.15 g (100%) of **10** as an off-white foamy solid. ¹H NMR (300 MHz; CDCl₃) δ 9.02 (s, 3H), 7.48 (d, J = 9.0 Hz, 2H), 6.86 (d, J = 9.0 Hz, 2H), 5.22–5.12 (m, 1H), 3.80–3.60 (m, 2H), 3.66 (s, 3H), 2.00–1.85 (m, 1H), 1.52–1.12 (m, 4H), 1.00 (d, J = 6.0 Hz, 3H), 0.90 (t, J = 7.5 Hz, 3H); ¹³C NMR (75 MHz; CDCl₃) δ 169.0, 160.3, 130.2, 123.2, 115.0, 73.2, 56.6, 53.3, 35.7, 32.8, 20.0, 17.0, 14.3; MS (ESI) m/z 266.3 [M + H]⁺.

5.1.13. Methyl (2R)-2-[4-(2-methylpentylloxy)phenyl]-2-[(S)-2-phenylpropanamido]acetate (11a)—The procedure for **3** was followed using 512 mg (2 mmol) of **10** to give 750 mg (94%) of **11a** as a white solid. ¹H NMR (300 MHz; CDCl₃) δ 7.32–7.20 (m, 5H), 7.09 (d, J = 9.0 Hz, 2H), 6.78 (d, J = 9.0 Hz, 2H), 6.46 (d, J = 6.0 Hz, 1H), 5.44 (d, J = 6.0 Hz, 1H), 3.78–3.58 (m, 3H), 3.65 (s, 3H), 1.98–1.86 (m, 1H), 1.50–1.16 (m, 4H), 1.48 (d, J = 6.0 Hz, 3H), 0.97 (d, J = 6.0 Hz, 3H), 0.91 (t, J = 7.5 Hz, 3H); ¹³C NMR (75 MHz; CDCl₃) δ 173.4, 171.6, 159.4, 141.1, 128.8, 128.3, 128.1, 127.6, 127.2, 114.8, 73.2, 56.0, 52.6, 46.7, 35.7, 32.9, 20.0, 18.5, 17.0, 14.3; MS (ESI) m/z 398.4 [M + H]⁺.

5.1.14. (2R)-2-[4-(2-Methylpentylloxy)phenyl]-2-[(S)-2-phenylpropanamido]acetic acid (11b)—A solution of **11a** (550 mg, 1.39 mmol) and 1 N NaOH (2 mL) in 1:1 THF-MeOH (5 mL) was stirred at room temperature for 1 h. The mixture was acidified with 1 N HCl and extracted with EtOAc (3 $\times$ 30 mL). The combined organic layers were washed with brine (10 mL), dried (Na₂SO₄) and concentrated under reduced pressure. Crystallization of the crude product from EtOAc/hexanes afforded **11b** (410 mg, 77%) as a white solid. ¹H NMR (300 MHz; CDCl₃) δ 8.91 (br s, 1H), 7.40–7.20 (m, 5H), 7.10 (d, J = 9.0 Hz, 2H), 6.78 (d, J = 9.0 Hz, 2H), 6.32 (d, J = 6.0 Hz, 1H), 5.43 (d, J = 6.0 Hz, 1H), 3.80–3.60 (m, 3H), 2.00–1.86 (m, 1H), 1.50–1.15 (m, 4H), 1.47 (d, J = 6.0 Hz, 3H), 0.98 (d, J = 6.0 Hz, 3H), 0.91 (t, J = 7.5 Hz, 3H); ¹³C NMR (75 MHz; CDCl₃) δ

174.4, 174.1, 159.6, 140.7, 128.9, 128.3, 127.6, 127.4, 127.3, 114.9, 73.3, 56.1, 46.7, 35.7, 32.9, 20.0, 18.3, 17.0, 14.3; MS (ESI) m/z 384.4 $[M + H]^+$.

5.1.15. *N*-Dimethyl (2*R*)-2-[4-(2-methylpentylloxy)phenyl]-2-[(*S*)-2-phenylpropanamido]- acetamide (11c)—To a solution of **11b** (115 mg, 0.3 mmol) in DMF (5 mL) under nitrogen were added EDC hydrochloride (63 mg, 0.33 mmol), HOBT (45 mg, 0.33 mmol) and DIPEA (104 μ L, 0.6 mmol). After cooling to 0 °C, dimethylamine hydrochloride (27 mg, 0.33 mmol) was added and the reaction mixture was stirred at room temperature overnight. The reaction was quenched with 10% citric acid solution (5 mL) and extracted with EtOAc (3 $\times$ 20 mL). The combined organic layers were washed with saturated NaHCO₃ (10 mL) and brine (10 mL), dried (Na₂SO₄) and concentrated under reduced pressure. Flash column chromatography of the crude product on silica gel using 0–50% EtOAc in hexanes afforded **11c** (55 mg, 45%) as a colorless oil. ¹H NMR (300 MHz; CDCl₃) δ 7.32–7.10 (m, 7H), 6.98 (d, J = 9.0 Hz, 1H), 6.78 (d, J = 9.0 Hz, 2H), 5.75 (d, J = 9.0 Hz, 1H), 3.80–3.55 (m, 3H), 2.94 (s, 3H), 2.88 (s, 3H), 1.98–1.86 (m, 1H), 1.52–1.10 (m, 4H), 1.48 (d, J = 6.0 Hz, 3H), 0.99 (d, J = 6.0 Hz, 3H), 0.91 (t, J = 7.5 Hz, 3H); ¹³C NMR (75 MHz; CDCl₃) δ 173.0, 170.0, 159.1, 141.2, 129.1, 128.9, 128.7, 127.5, 127.0, 114.7, 73.2, 53.4, 46.8, 36.8, 35.9, 35.7, 32.9, 20.0, 18.5, 17.0, 14.3; MS (ESI) m/z 411.6 $[M + H]^+$.

5.1.16. *N*-{(1*R*)-2-Hydroxy-1-[4-(2-methylpentylloxy)phenyl]ethyl}-2-phenylacetamide (12a)—The procedure for **3** was followed using 68 mg (0.25 mmol) of **8a** to give 45 mg (51%) of **12a** as a white solid. ¹H NMR (300 MHz; CDCl₃) δ 7.38–7.20 (m, 5H), 7.05 (d, J = 9.0 Hz, 2H), 6.81 (d, J = 9.0 Hz, 2H), 6.23 (d, J = 9.0 Hz, 1H), 5.01–4.93 (m, 1H), 3.80–3.62 (m, 4H), 3.55 (s, 2H), 2.98 (br s, 1H), 1.98–1.85 (m, 1H), 1.52–1.12 (m, 4H), 0.99 (d, J = 6.0 Hz, 3H), 0.91 (t, J = 7.5 Hz, 3H); ¹³C NMR (75 MHz; CDCl₃) δ 171.6, 158.9, 134.8, 130.6, 129.3, 129.0, 127.6, 127.4, 114.8, 73.3, 66.5, 55.5, 43.7, 35.8, 32.9, 20.0, 17.0, 14.3; MS (ESI) m/z 356.3 $[M + H]^+$.

5.1.17. *N*-{(1*R*)-2-Hydroxy-1-[4-(2-methylpentylloxy)phenyl]ethyl}-3-phenylpropanamide (12b)—The procedure for **3** was followed using 68 mg (0.25 mmol) of **8a** to give 47 mg (51%) of **12b** as a white solid. ¹H NMR (300 MHz; CDCl₃) δ 7.32–7.12 (m, 5H), 7.04 (d, J = 9.0 Hz, 2H), 6.82 (d, J = 9.0 Hz, 2H), 6.10 (d, J = 6.0 Hz, 1H), 4.98–4.88 (m, 1H), 3.80–3.65 (m, 4H), 2.95 (t, J = 7.5 Hz, 3H), 2.51 (t, J = 7.5 Hz, 2H), 2.00–1.88 (m, 1H), 1.52–1.12 (m, 4H), 1.00 (d, J = 6.0 Hz, 3H), 0.92 (t, J = 7.5 Hz, 3H); ¹³C NMR (75 MHz; CDCl₃) δ 172.7, 159.0, 140.6, 130.6, 128.6, 128.4, 127.8, 126.3, 114.8, 73.3, 66.5, 55.4, 18.5, 35.8, 32.9, 31.7, 20.0, 17.0, 14.3; MS (ESI) m/z 370.3 $[M + H]^+$.

5.1.18. *N*-{(1*R*)-2-Hydroxy-1-[4-(2-methylpentylloxy)phenyl]ethyl}-4-phenylbutanamide (12c)—The procedure for **3** was followed using 68 mg (0.25 mmol) of **8a** to give 55 mg (57%) of **12c** as a white solid. ¹H NMR (300 MHz; CDCl₃) δ 7.30–7.10 (m, 7H), 6.85 (d, J = 9.0 Hz, 2H), 6.22 (d, J = 6.0 Hz, 1H), 5.01–4.92 (m, 1H), 3.82–3.65 (m, 3H), 3.24 (br s, 1H), 2.62 (t, J = 7.5 Hz, 3H), 2.19 (t, J = 7.5 Hz, 2H), 2.00–1.87 (m, 3H), 1.52–1.12 (m, 4H), 0.99 (d, J = 6.0 Hz, 3H), 0.91 (t, J = 7.5 Hz, 3H); ¹³C NMR (75

MHz; CDCl₃) δ 173.4, 159.0, 141.4, 130.8, 128.5, 128.4, 127.9, 126.0, 114.9, 73.3, 66.6, 55.5, 35.8, 35.7, 35.1, 32.9, 27.0, 20.0, 17.0, 14.3; MS (ESI) *m/z* 384.5 [M + H]⁺.

5.1.19. Methyl (2*R*)-2-[4-(2-methylpentyloxy)phenyl]-2-[(*R,S*)-2-cyclohexylpropanamido]-acetate (12d)—

The procedure for **3** was followed using 60 mg (0.2 mmol) of **10** to give 61 mg (76%) of **12d** as a white semi-solid. ¹H NMR (300 MHz; CDCl₃) δ 7.26 (d, *J* = 9.0 Hz, 2H), 6.87 (d, *J* = 9.0 Hz, 2H), 6.30 (d, *J* = 9.0 Hz, 1H), 5.49 (d, *J* = 6.0 Hz, 1H), 3.82–3.66 (m, 2H), 3.72 (s, 3H), 2.05–1.88 (m, 2H), 1.82–1.58 (m, 5H), 1.56–1.00 (m, 10H), 1.08 (d, *J* = 6.0 Hz, 3H), 0.98 (d, *J* = 6.0 Hz, 3H), 0.91 (t, *J* = 7.5 Hz, 3H); ¹³C NMR (75 MHz; CDCl₃) δ 175.6, 171.8, 159.5, 128.4, 114.9, 73.3, 55.7, 52.6, 47.3, 40.9, 35.7, 32.9, 31.3, 29.9, 26.4, 26.3, 20.0, 17.0, 14.7, 14.3; MS (ESI) *m/z* 404.3 [M + H]⁺.

5.1.20. Synthesis of library compounds 13—Amine **10** (0.02 mmol × the number of derivatives being prepared) was dissolved in anhydrous THF (0.5 mL × the number of derivatives being prepared). HBTU (0.024 mmol × the number of derivatives being prepared) was dissolved in anhydrous MeCN (1 mL × the number of derivatives being prepared). To the prelabeled 20 mL scintillation vial containing a stir bar was added one of the chosen carboxylic acid (0.022 mmol) solution in MeCN (0.5 mL). To this was added the appropriate fraction of amine solution (0.5 mL) followed by TEA (0.006 mL, 0.04 mmol) and the appropriate fraction of HBTU solution (1 mL). The vial was then capped with a Teflon-lined lid and stirred at room temperature. After TLC analysis indicated no amine left, Et₂O (4 mL) and H₂O (2 mL) were added to the vial. After the vial was shaken and the layers were allowed to settle, the aqueous layer was withdrawn with a pipet. Next, saturated NaHCO₃ (2 × 2 mL) was added and the procedure repeated. This was followed by washing with brine (2 × 2 mL). Na₂SO₄ was added to the vial, and after drying, the mixture was pipetted into a preweighed, prelabeled 20 mL scintillation vials using a 6 in. Pasteur pipet containing a small cotton plug. Following this, Et₂O (2 mL) was added to the drying agent and the vial was shaken, after which the Et₂O rinse was filtered as above. The vials were placed under a nitrogen outlet and allowed to evaporate. Once the solvent was removed, the vials were placed in a high vacuum desiccator and allowed to remain overnight. The vials were reweighed, and the crude yield was determined by difference. The products were characterized by MS to confirm the identity and were used without further purification.

5.1.21. Methyl (2*R*)-2-[4-(2-methylpentyloxy)phenyl]-2-(2-phenylacetamido)acetate (13a)—

The procedure for **3** was followed using 60 mg (0.2 mmol) of **10** to give 72 mg (94%) of **13a** as a white solid. ¹H NMR (300 MHz; CDCl₃) δ 7.48–7.20 (m, 5H), 7.16 (d, *J* = 9.0 Hz, 2H), 6.82 (d, *J* = 9.0 Hz, 2H), 6.46 (d, *J* = 6.0 Hz, 1H), 5.45 (d, *J* = 9.0 Hz, 1H), 3.82–3.62 (m, 2H), 3.67 (s, 3H), 3.58 (s, 2H), 1.98–1.85 (m, 1H), 1.52–1.12 (m, 4H), 0.99 (d, *J* = 6.0 Hz, 3H), 0.91 (t, *J* = 7.5 Hz, 3H); ¹³C NMR (75 MHz; CDCl₃) δ 171.5, 170.3, 159.5, 134.5, 129.4, 129.0, 128.4, 128.1, 127.4, 114.9, 73.3, 56.0, 52.7, 43.4, 35.7, 32.9, 20.0, 17.0, 14.3; MS (ESI) *m/z* 384.4 [M + H]⁺.

5.1.22. Methyl (2*R*)-2-[4-(2-methylpentyloxy)phenyl]-2-[2-(2-fluorophenyl)acetamido]acetate (13e)—

The procedure for **3** was followed using 60

mg (0.2 mmol) of **10** to give 75 mg (93%) of **13e** as a white solid. ¹H NMR (300 MHz; CDCl₃) δ 7.26–7.16 (m, 2H), 7.12 (d, *J* = 9.0 Hz, 2H), 7.06–6.95 (m, 2H), 6.76 (d, *J* = 9.0 Hz, 2H), 6.50 (d, *J* = 6.0 Hz, 1H), 5.40 (d, *J* = 6.0 Hz, 1H), 3.72–3.56 (m, 2H), 3.60 (s, 3H), 3.53 (s, 2H), 1.90–1.78 (m, 1H), 1.45–1.05 (m, 4H), 0.91 (d, *J* = 6.0 Hz, 3H), 0.83 (t, *J* = 7.5 Hz, 3H); ¹³C NMR (75 MHz; CDCl₃) δ 170.4, 168.1, 159.9 (d, *J*_{C-F} = 245 Hz), 158.5, 130.6 (d, *J*_{C-F} = 3.8 Hz), 128.3 (d, *J*_{C-F} = 8.3 Hz), 127.3, 127.1, 123.5 (d, *J*_{C-F} = 3.3 Hz), 120.8 (d, *J*_{C-F} = 15.8 Hz), 114.6 (d, *J*_{C-F} = 21.8 Hz), 113.9, 72.2, 55.0, 51.6, 35.4 (d, *J*_{C-F} = 2.3 Hz), 34.7, 31.8, 19.0, 16.0, 13.3; MS (ESI) *m/z* 402.0 [M + H]⁺.

5.1.23. Methyl (2*R*)-2-[4-(2-methylpentylloxy)phenyl]-2-[2-(3-fluorophenyl)acetamido]acetate (13f**)**—The procedure for **3** was followed using 60

mg (0.2 mmol) of **10** to give 73 mg (92%) of **13f** as a white solid. ¹H NMR (300 MHz; CDCl₃) δ 7.34–7.22 (m, 1H), 7.18 (d, *J* = 9.0 Hz, 2H), 7.06–6.90 (m, 3H), 6.83 (d, *J* = 9.0 Hz, 2H), 6.61 (d, *J* = 6.0 Hz, 1H), 5.47 (d, *J* = 6.0 Hz, 1H), 3.80–3.62 (m, 2H), 3.68 (s, 3H), 3.55 (s, 2H), 1.98–1.88 (m, 1H), 1.52–1.10 (m, 4H), 0.99 (d, *J* = 6.0 Hz, 3H), 0.91 (t, *J* = 7.5 Hz, 3H); ¹³C NMR (75 MHz; CDCl₃) δ 171.5, 169.6, 162.9 (d, *J*_{C-F} = 245 Hz), 159.6, 136.9 (d, *J*_{C-F} = 7.5 Hz), 130.3 (d, *J*_{C-F} = 8.3 Hz), 128.4, 128.0, 125.0 (d, *J*_{C-F} = 3.0 Hz), 116.3 (d, *J*_{C-F} = 21.8 Hz), 114.9, 114.2 (d, *J*_{C-F} = 21.0 Hz), 73.3, 56.1, 52.7, 42.9 (d, *J*_{C-F} = 1.5 Hz), 35.7, 32.9, 20.0, 17.0, 14.3; MS (ESI) *m/z* 402.2 [M + H]⁺.

5.1.24. Methyl (2*R*)-2-[4-(2-methylpentylloxy)phenyl]-2-[2-(4-fluorophenyl)acetamido]acetate (13g**)**—The procedure for **3** was followed using 60

mg (0.2 mmol) of **10** to give 75 mg (93%) of **13g** as a white solid. ¹H NMR (300 MHz; CDCl₃) δ 7.28–7.12 (m, 4H), 7.05–6.96 (m, 2H), 6.83 (d, *J* = 9.0 Hz, 2H), 6.54 (d, *J* = 6.0 Hz, 1H), 5.47 (d, *J* = 6.0 Hz, 1H), 3.80–3.62 (m, 2H), 3.68 (s, 3H), 3.53 (s, 2H), 1.98–1.87 (m, 1H), 1.52–1.14 (m, 4H), 0.99 (d, *J* = 6.0 Hz, 3H), 0.91 (t, *J* = 7.5 Hz, 3H); ¹³C NMR (75 MHz; CDCl₃) δ 171.5, 170.4, 162.1 (d, *J*_{C-F} = 245 Hz), 159.5, 130.9 (d, *J*_{C-F} = 8.3 Hz), 130.3 (d, *J*_{C-F} = 3.0 Hz), 128.4, 128.0, 115.8 (d, *J*_{C-F} = 21.0 Hz), 114.9, 73.3, 56.0, 52.7, 42.4, 35.7, 32.9, 20.0, 17.0, 14.3; MS (ESI) *m/z* 402.2 [M + H]⁺.

5.1.25. Methyl (2*R*)-2-[4-(2-methylpentylloxy)phenyl]-2-[2-(4-methylphenyl)acetamido]acetate (13m**)**—The procedure for **3** was followed using 60

mg (0.2 mmol) of **10** to give 72 mg (91%) of **13m** as a white solid. ¹H NMR (300 MHz; CDCl₃) δ 7.21–7.10 (m, 6H), 6.82 (d, *J* = 9.0 Hz, 2H), 6.50 (d, *J* = 9.0 Hz, 1H), 5.47 (d, *J* = 6.0 Hz, 1H), 3.80–3.62 (m, 2H), 3.66 (s, 3H), 3.53 (s, 2H), 2.33 (s, 3H), 1.96–1.85 (m, 1H), 1.52–1.12 (m, 4H), 0.99 (d, *J* = 6.0 Hz, 3H), 0.93 (t, *J* = 7.5 Hz, 3H); ¹³C NMR (75 MHz; CDCl₃) δ 171.6, 170.5, 159.5, 136.9, 131.4, 129.6, 129.3, 128.4, 128.2, 114.9, 73.2, 55.9, 52.6, 43.0, 35.7, 32.9, 21.1, 20.3, 17.0, 14.3; MS (ESI) *m/z* 398.3 [M + H]⁺.

5.1.26. Methyl (2*R*)-2-[4-(2-methylpentylloxy)phenyl]-2-[2-(4-methoxyphenyl)acetamido]acetate (13p**)**—The procedure for **3** was followed using

60 mg (0.2 mmol) of **10** to give 78 mg (94%) of **13p** as a white solid. ¹H NMR (300 MHz; CDCl₃) δ 7.22–7.12 (m, 4H), 6.92–6.78 (m, 4H), 6.46 (d, *J* = 6.0 Hz, 1H), 5.47 (d, *J* = 6.0 Hz, 1H), 3.81–3.62 (m, 2H), 3.79 (s, 3H), 3.67 (s, 3H), 3.52 (s, 2H), 1.98–1.88 (m, 1H), 1.52–1.12 (m, 4H), 0.99 (d, *J* = 6.0 Hz, 3H), 0.91 (t, *J* = 7.5 Hz, 3H); ¹³C NMR (75 MHz;

CDCl₃) δ 171.6, 170.7, 159.5, 159.9, 130.5, 128.4, 128.2, 126.5, 114.9, 114.4, 73.2, 55.9, 55.3, 52.6, 42.5, 35.7, 32.9, 20.0, 17.0, 14.3; MS (ESI) *m/z* 414.4 [M + H]⁺.

5.1.27. Methyl (2*R*)-2-[4-(2-methylpentylloxy)phenyl]-2-[2-(4-cyanophenyl)acetamido]acetate (13z)—The procedure for **3** was followed using 60 mg (0.2 mmol) of **10** to give 80 mg (98%) of **13z** as a white solid. ¹H NMR (300 MHz; CDCl₃) δ 7.62 (d, *J* = 9.0 Hz, 2H), 7.39 (d, *J* = 9.0 Hz, 2H), 7.19 (d, *J* = 9.0 Hz, 2H), 6.85 (d, *J* = 9.0 Hz, 2H), 6.48 (d, *J* = 6.0 Hz, 1H), 5.47 (d, *J* = 6.0 Hz, 1H), 3.86–3.55 (m, 2H), 3.71 (s, 3H), 3.63 (s, 2H), 2.00–1.88 (m, 1H), 1.52–1.12 (m, 4H), 1.00 (d, *J* = 6.0 Hz, 3H), 0.91 (t, *J* = 7.5 Hz, 3H); ¹³C NMR (75 MHz; CDCl₃) δ 171.5, 168.7, 159.6, 140.0, 132.4, 130.1, 128.4, 127.8, 118.7, 115.0, 111.2, 73.3, 56.1, 52.8, 43.0, 35.7, 32.9, 20.0, 17.0, 14.3; MS (ESI) *m/z* 409.6 [M + H]⁺.

5.1.28. Methyl (2*R*)-2-[4-(2-methylpentylloxy)phenyl]-2-[(*S*)-2-phenylbutanamido]acetate (13aw)—The procedure for **3** was followed using 60 mg (0.2 mmol) of **10** to give 78 mg (95%) of **13aw** as a white solid. ¹H NMR (300 MHz; CDCl₃) δ 7.35–7.20 (m, 5H), 7.09 (d, *J* = 9.0 Hz, 2H), 6.78 (d, *J* = 9.0 Hz, 2H), 6.34 (d, *J* = 6.0 Hz, 1H), 5.42 (d, *J* = 6.0 Hz, 1H), 3.80–3.62 (m, 2H), 3.68 (s, 3H), 3.31 (t, *J* = 7.5 Hz, 1H), 2.22–2.08 (m, 1H), 1.98–1.70 (m, 2H), 1.52–1.15 (m, 4H), 0.98 (d, *J* = 6.0 Hz, 3H), 0.95–0.85 (m, 6H); ¹³C NMR (75 MHz; CDCl₃) δ 172.8, 171.6, 159.4, 139.7, 128.7, 128.2, 128.0, 127.2, 114.8, 73.2, 56.0, 54.8, 52.6, 35.7, 32.9, 26.4, 20.0, 17.0, 14.3, 12.2; MS (ESI) *m/z* 412.3 [M + H]⁺.

5.1.29. (2*S*)-*N*-{(1*R*)-2-Hydroxy-1-[4-(2-methylpentylloxy)phenyl]ethyl}-2-phenylbutanamide (14)—The procedure for **3** was followed using 68 mg (0.25 mmol) of **8a** to give 46 mg (48%) of **14** as a white solid. ¹H NMR (300 MHz; CDCl₃) δ 7.35–7.20 (m, 5H), 6.94 (d, *J* = 9.0 Hz, 2H), 6.75 (d, *J* = 9.0 Hz, 2H), 6.22 (d, *J* = 6.0 Hz, 1H), 5.00–4.90 (m, 1H), 3.80–3.70 (m, 3H), 3.68–3.60 (m, 1H), 3.30 (t, *J* = 7.5 Hz, 1H), 2.97 (br s, 1H), 2.22–2.10 (m, 1H), 1.98–1.72 (m, 2H), 1.52–1.14 (m, 4H), 0.98 (d, *J* = 6.0 Hz, 3H), 0.97–0.85 (m, 6H); ¹³C NMR (75 MHz; CDCl₃) δ 174.1, 158.8, 139.8, 130.6, 128.8, 128.0, 127.5, 127.2, 114.7, 73.3, 66.6, 55.4, 55.1, 35.8, 32.9, 26.3, 20.0, 17.0, 14.3, 12.3; MS (ESI) *m/z* 384.4 [M + H]⁺.

5.2. Pharmacology

5.2.1. Materials—Cell culture materials were purchased from Fisher SSI. Forskolin was purchased from Sigma-Aldrich. The Lance Ultra kit (TRF0262) was purchased from PerkinElmer.

5.2.2. Lance™ cAMP assay using stable PPLS-HA-GPR88 CHO cells—Stimulation buffer containing 1X Hank's Balanced Salt Solution (HBSS), 5 mM HEPES, 0.1% BSA stabilizer, and 0.5 mM final IBMX was prepared and titrated to pH 7.4 at room temperature. Serial dilutions of the test compounds (5 μL) and 300 nM forskolin (5 μL), both prepared at 4× the desired final concentration in 2% DMSO/stimulation buffer, were added to a 96-well white ½ area microplate (PerkinElmer). A cAMP standard curve was prepared at 4× the desired final concentration in stimulation buffer and 5 μL was added to the assay

plate. Stable PPLS-HA-GPR88 CHO cells were lifted with versene and spun at 270g for 10 minutes. The cell pellet was resuspended in stimulation buffer and 4,000 cells (10 μ L) were added to each well except wells containing the cAMP standard curve. After incubating for 30 min at RT, Eu-cAMP tracer and u*LIGHT-anti-cAMP* working solutions were added per the manufacturer's instructions. After incubation at RT for 1 hour, the TR-FRET signal (ex 337 nm) was read on a CLARIOstar multimode plate reader (BMG Biotech, Cary, NC). For the screen, the same procedure was followed except that instead of adding serial dilutions of the test compounds, a single concentration (1 μ M, prepared at 4 $\times$ final) of each test compound was added to the assay plates.

5.2.3. Data analysis—The TR-FRET signal (665 nm) was converted to fmol cAMP by interpolating from the standard cAMP curve. Fmol cAMP was plotted against the log of compound concentration and data were fit to a three-parameter logistic curve to generate EC₅₀ values (Prism, version 6.0, GraphPad Software, Inc., San Diego, CA). For the screen, the raw TR-FRET signal was expressed as percent of control (**11a**) signal.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

2-PCCA	(1 <i>R</i> ,2 <i>R</i>)-2-(pyridin-2-yl)cyclopropane carboxylic acid ((2 <i>S</i> ,3 <i>S</i>)-2-amino-3-methylpentyl)-(4'-propylbiphenyl-4-yl)amide
2-AMPP	(2 <i>S</i>)- <i>N</i> -((1 <i>R</i>)-2-amino-1-(4-(2-methylpentylloxy)phenyl)ethyl)-2-phenylpropanamide
SAR	structure activity relationship
PPLS	pre-prolactin leader sequence
HA	human influenza hemagglutinin
HTRF	homogeneous time resolved fluorescence
MS	mass spectroscopy
TLC	thin layer chromatography
DIPEA	<i>N,N</i> -diisopropylethylamine
DCM	dichloromethane
DEAD	diethyl azodicarboxylate

THF	tetrahydrofuran
DMF	N,N-dimethylformamide
TEA	triethylamine
HBTU	2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
TFA	trifluoroacetic acid
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
HOBt	hydroxybenzotriazole

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Figure 1.
Structures of (1*R*,2*R*)-2-PCCA (**1**), **2** and **3**, and sites of chemical modification explored.

Figure 2.

Screening of library compounds. The library compounds were screened at 1 μ M final. **11a** was used as control (compound #1) and its TR-FRET signal set as 100%. Representative data from two separate experiments are demonstrated. Compounds with red columns were resynthesized to determine the potency.

Scheme 1.

Synthesis of target compounds **2**, **3**, **9** and **11a–c**. Reagents and conditions: (a) Boc₂O, DIPEA, DCM, rt, overnight, 100%; (b) PPh₃, DEAD, 2-methylpentanol, THF, rt, overnight, 78%; (c) NaBH₄, LiCl, THF-EtOH (1:1), rt, 3 h, 91%; (d) PPh₃, CBr₄, THF, rt, 3 h, 83%; (e) NaN₃, DMF, rt, 6 h, 100%; (f) TFA, DCM, rt, 1 h, 100%; (g) (*S*)-2-phenylpropionic acid, HBTU, TEA, MeCN, rt, 5 h, 53–94%; (h) 10% Pd/C, H₂, EtOH, 40 psi, 1 h, 66%; (i) 1 N NaOH, THF-MeOH (1:1), rt, 1 h, 77%; (j) EDC hydrochloride, HOBT, DIPEA, Me₂N•HCl, DMF, rt, overnight, 45%.

Scheme 2.

Synthesis of target compounds **12a–d**, **14** and library compounds **13**. Reagents and conditions: (a) carboxylic acid, HBTU, TEA, MeCN, rt, 5 h, 48–98%.

Table 1

Structural modification at site A.

Compd	R	pEC ₅₀ (EC ₅₀ , nM) ^b
2^a	CH ₂ NH ₂	6.38 ± 0.05 (414)
3	CH ₂ OH	6.71 ± 0.09 (194)
9	CH ₂ N ₃	6.87 ± 0.05 (134)
11a	CO ₂ Me	6.27 ± 0.09 (538)
11b	COOH	NA ^c
11c	CONMe ₂	6.21 ± 0.08 (616)

^aThe compound was tested as the HCl salt.^bpEC₅₀ values are means ± standard error of at least three independent experiments performed in duplicate.^cEC₅₀ > 10 μM, mean of two independent experiments.

Table 2

Structural modification at site B.

Compd	X	R	pEC ₅₀ (EC ₅₀ , nM) ^a
12a	CH ₂ OH	Bn	6.31 ± 0.04 (487)
12b	CH ₂ OH	Ph(CH ₂) ₂	6.46 ± 0.11 (345)
12c	CH ₂ OH	Ph(CH ₂) ₃	5.84 ± 0.02 (1440)
12d	CO ₂ Me		NA ^b
13a	CO ₂ Me	Bn	5.58 ± 0.03 (2630)
13e	CO ₂ Me	2-F-Bn	5.10 ± 0.06 (7870)
13f	CO ₂ Me	3-F-Bn	5.41 ± 0.07 (3910)
13g	CO ₂ Me	4-F-Bn	5.23 ± 0.03 (5860)
13m	CO ₂ Me	4-Me-Bn	5.43 ± 0.07 (3700)
13p	CO ₂ Me	4-MeO-Bn	5.02 ± 0.07 (9570)
13z	CO ₂ Me	4-CN-Bn	NA ^b
13aw	CO ₂ Me		6.23 ± 0.09 (583)
14	CH ₂ OH		6.84 ± 0.09 (145)

^apEC₅₀ values are means ± standard error of at least three independent experiments performed in duplicate.^bEC₅₀ > 10 μM, mean of two independent experiments.

Table 3

Structures of library compounds **13**.

#	Compd	R ¹	R ²	X	#	Compd	R ¹	R ²	X
1	11a	Control			29	13ab	H	H	3,5-diF
2	13a	H	H	H	30	13ac	H	H	2,5-diF
3	13b	H	H	2-Cl	31	13ad	H	H	2,4-diF
4	13c	H	H	3-Cl	32	13ae	H	H	2,3-diF
5	13d	H	H	4-Cl	33	13af	H	H	2,6-diF
6	13e	H	H	2-F	34	13ag	H	H	2,4-diCl
7	13f	H	H	3-F	35	13ah	H	H	2,6-diCl
8	13g	H	H	4-F	36	13ai	H	H	3,4-diCl
9	13h	H	H	2-Br	37	13aj	H	H	2,3-diMeO
10	13i	H	H	3-Br	38	13ak	H	H	2,4-diMeO
11	13j	H	H	4-Br	39	13al	H	H	2,5-diMeO
12	13k	H	H	2-Me	40	13am	H	H	3,4-diMeO
13	13l	H	H	3-Me	41	13an	H	H	3,4-MD
14	13m	H	H	4-Me	42	13ao	H	H	3,4,5-triMeO
15	13n	H	H	2-MeO	43	13ap	H	H	2,5-diMe
16	13o	H	H	3-MeO	44	13aq	H	H	2,4,6-triMe
17	13p	H	H	4-MeO	45	13ar	Ph	H	H
18	13q	H	H	2-NO ₂	46	13as	Me	Me	H
19	13r	H	H	3-NO ₂	47	13at		Cyclopropyl	H
20	13s	H	H	4-NO ₂	48	13au		Cyclobutyl	H
21	13t	H	H	2-CF ₃	49	13av		Cyclohexyl	H
22	13u	H	H	3-CF ₃	50	13aw	(S)-Et	H	H

#	Compd	R ¹	R ²	X	#	Compd	R ¹	R ²	X
23	13v	H	H	4-CF ₃	51	13ax	(<i>S</i>)-MeO	H	H
24	13w	H	H	4-EtO	52	13ay	(<i>S</i>)-Me	H	H
25	13x	H	H	4-BuO	53	13az	(<i>R</i>)-Me	H	H
26	13y	H	H	4-Me ₂ N	54	13ba
27	13z	H	H	4-CN	55	13bb
28	13aa	H	H	3,4-diF					