SUPPORTING INFORMATION **Yau et al**

Derivatives of GB88 as PAR2 Modulators

Mei-Kwan Yau[‡,†], *Ligong Liu*[‡,†], *Jacky Y. Suen*[†], *Junxian Lim*[†], *Rink-Jan Lohman*[†], *Yuhong Jiang*[†], *Adam J. Cotterell*[†], *Grant D. Barry*[†], *Jeffrey Y. W. Mak*[†], *David A. Vesey*[§], *Robert C. Reid*[†] *and David P. Fairlie*[†,*]

[†] Division of Chemistry and Structural Biology, Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland 4072, Australia.

[§] Centre for Kidney Research, Department of Medicine, The University of Queensland, Princess Alexandra Hospital, Brisbane, Queensland 4102, Australia.

[‡] These authors contributed equally.

Correspondence to: d.fairlie@imb.uq.edu.au

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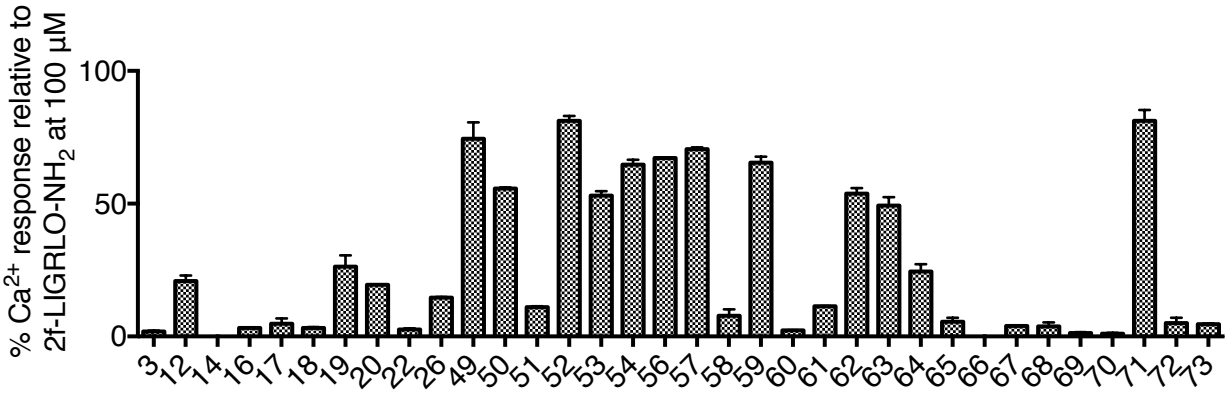


Figure S1. Activation of the calcium efflux in HT29 cells induced by representative compounds at 10 µM relative to 100% induced by 100 µM of 2f-LIGRLO-NH_2 in HT29 cells (data at 100 µM were not shown). Data represent the average of at least two measurements with SEM.

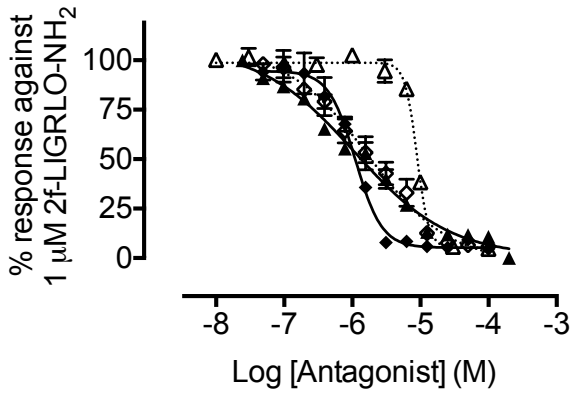


Figure S2. PAR2 selective antagonists. Concentration dependent curves of **17** (◆), **18** (△), **65** (▲), versus **3** (◇) based on the inhibition of iCa^{2+} release in HT29 cells induced by 1 µM 2f-LIGRLO-NH_2. Each data point represents mean ± SEM (n ≥ 3).

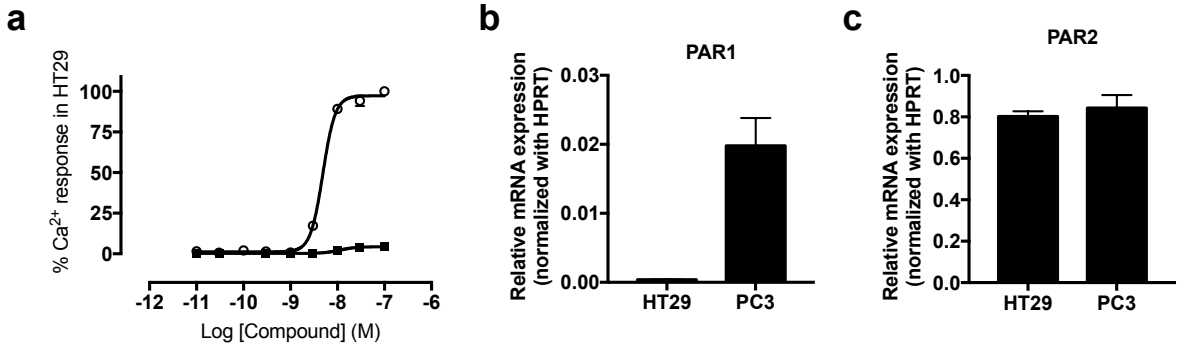


Figure S3. Comparison of PAR1 and PAR2 in HT29 and PC3 cells. (a) Trypsin- (○) versus thrombin- (■) induced iCa^{2+} release in HT29 cells, indicating PAR2 agonist induced a concentration-dependent response, while PAR1 agonist induced a negligible response even at the maximum concentration of 100 nM. (b) PAR1 mRNA expression in HT29 versus PC3 cells, indicating PAR1 expression in HT29 cells was very low. (c) Similar level of PAR2 mRNA expression in HT29 and PC3 cells. Each data point represents mean ± SEM (n ≥ 3).

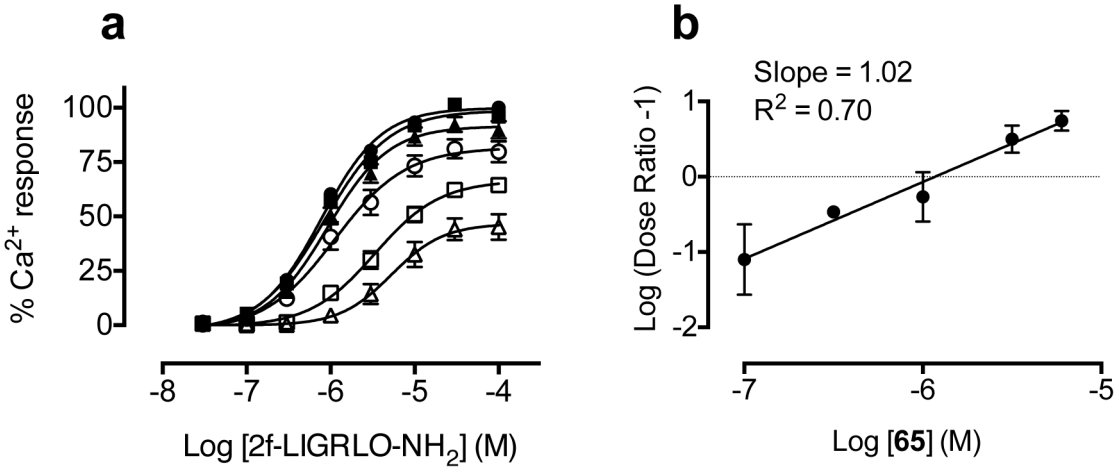


Figure S4. Mechanism of PAR2 antagonism in iCa^{2+} function. (a) **65** is a competitive yet insurmountable PAR2 antagonist against 2f-LIGRLO-NH_2 showing concentration-dependent (zero, ●; 0.1 μM, ■; 0.3 μM, ▲; 1 μM, ○; 3 μM, □; 6 μM, △) inhibition of iCa^{2+} release in HT29 cells induced by varying concentrations of PAR2 agonist 2f-LIGRLO-NH_2. (b) Schild analysis of **65** against 2f-LIGRLO-NH_2 showing competitive mode. Calculated pA2 values for **65** were 5.9 ± 0.3 against 2f-LIGRLO-NH_2 (6.3 ± 0.3 for **3**, competitive and surmountable). Each data point represents mean ± SEM (n = 3).

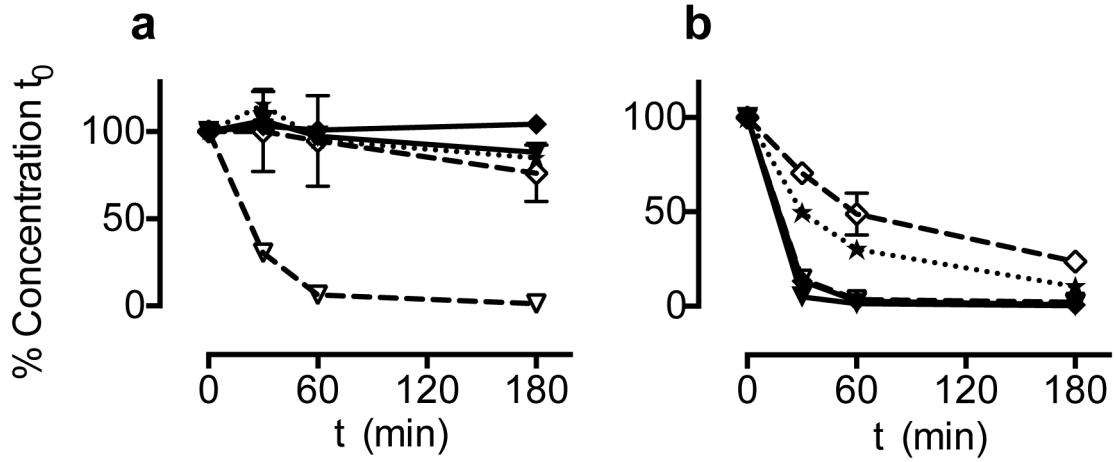


Figure S5. Stability of selective PAR2 ligands in rat plasma (a) and rat liver homogenate (b) at 37 ºC over 3 h. SLIGRL-NH_2 (▽), GB88 (◇), **17** (◆) and **65** (★). Data represent mean ± SEM (n = 3).

Table S1. Pharmacokinetic data of new antagonist **65** in comparison with GB88 after oral dose (10 mg/kg in olive oil, male Wistar rat)[a]

Compound	**3** (n = 7)	**65** (n = 4)
T_{max} (min)	167 ± 18	173 ± 31
C_{max} (μM)	1.97± 0.38	1.45 ± 0.39
AUC_{6h} (ng•h/mL)	3420	1964

[a] Data represent mean ± SEM (n = 3).

Experimental Section

All amino acid derivatives were purchased from Novabiochem and used as received. DMF, TFA, and diisopropylethylamine (DIEA) were "peptide grade" obtained from Auspep Pty Ltd., Australia. Other chemicals were obtained from Sigma-Aldrich and used as received. LCMS was performed using an Agilent 6110 Quadrupole LC/MS (API-ES) equipped with an Agilent 1100 Series ELS and a single wavelength (UV230 nm) detector with a Phenomenex Luna 5 μm, C18 250 × 4.60 mm column. Five standard conditions were used for all compounds unless otherwise indicated at a flow rate of 1 mL/min. Method I: 20% to 100% B linear gradient over 10 min followed by a further 10 min at 100% B; Method II: 50% to 100% B linear gradient over 10 min followed by a further 10 min at 100% B; Method III: 90% B isocratic for 10 min; Method IV: 20% to 100% B linear gradient over 15 min followed by a further 10 min at 100% B; Method V: 5% to 100% B linear gradient over 15 min followed by a further 10 min at 100% B; where solvent B was MeCN + 0.1% formic acid and solvent A was H_2O + 0.1% formic acid. HPLC was performed using an Agilent 1200 Series with a diode-array detector on a Phenomenex Luna 5 μm, C18 or C8 250 × 4.60 mm column. The solvent gradient was the same as LCMS except 0.1% TFA was used instead of 0.1% formic acid. Preparative-scale reverse-phase HPLC

separations were performed on a 15 μm Phenomenex Luna C18 250 × 21.2 mm column, using a Waters 600 series HPLC. Standard conditions were used for all compounds unless otherwise indicated at a flow rate of 20 mL/min: 50% to 100% B linear gradient over 10 min followed by a further 10 min at 100% B where solvent B was 90% MeCN, 10% H_2O + 0.1% TFA and solvent A was H_2O + 0.1% TFA. All synthesized compounds were ≥ 95% pure by HPLC.

Mass spectra were obtained on a triple quadrupole mass spectrometer (PE SCIEX API III) using electrospray ionization (ESI-MS) from solutions in 75% MeCN + 25% H_2O + 0.1% formic acid. Electrospray ionization high-resolution mass spectra (ESI-HRMS) measurements were obtained on a Bruker micrOTOF mass spectrometer equipped with an Agilent 1100 Series LC/MSD mass detector in positive ion mode by direct infusion in MeCN at 100 μL/h using sodium formate clusters as an internal calibrant.

^1H and ^{13}C NMR spectra were recorded on either a Varian Gemini 400 or Bruker Avance 600 spectrometers at 298 K in the solvents indicated and referenced to residual signals in the deuterated solvents (^1H at δ 7.26 for $CDCl_3$ and 2.50 for DMSO-d_6; ^{13}C at δ 77.16 for $CDCl_3$ and 39.52 for DMSO-d_6). All compounds were di-or tri-peptide like molecules (**3** and **6–76**) and contained generally two rotamers as observed in both ^1H and ^{13}C NMR. Further complication of NMR was caused by the presence of tertiary amide derived from piperidine ring, which displayed asymmetry of each ring methylene unit due to its syn- or anti-orientation relative to amide carbonyl. More distinct in ^{13}C than ^1H NMR, most of the signals were duplicated with a separation of ~0.5–2 ppm. Total assignments of proton and carbon NMR of representative compounds were determined using various one- and two-dimensional NMR experiments (JMOD, gCOSY, gHSQC, gHMBC). Characteristically, the unsymmetrical $H_{equatorial}$ and H_{axial} of *s*-syn piperidine-NCH_2 were always separated more apart than *s*-anti piperidine-NCH_2 ($\Delta\delta$ ~1.9 vs ~1.1 ppm) due to the deshielding effect of the amide carbonyl on $H_{equatorial}$ of *s*-syn methylene.

Accordingly, the corresponding methylene carbon was shielded, i.e., $\Delta\delta$ ~3.5 ppm between carbons of *s*-anti and *s*-syn piperidine-NCH_2. To distinguish the possibility of rotational isomers rather than epimers, NMR at elevated temperatures was investigated and some coalescence was observed (Supporting Information Figure S2). The presence of two rotamers rather than epimers was further evidenced by solvent-dependent ratio change of two isomers. For example, compound **75** showed two rotamers in a ratio of 1:1 in $CDCl_3$ and 3:2 in DMSO-d_6.

General procedure for the synthesis of compounds 3, 12–76. Compounds were synthesized in solution phase using Boc-protected amino acids or carboxylic acid, starting from C-terminal amine residue. The product was either purified as a final product using reversed-phase preparative HPLC or directly used for next step. If necessary, the Boc protecting group was removed and subsequent amide coupling was repeated. Each coupling reactions were monitored by ESMS, with most reactions went to completion overnight. Where required, post-coupling modification was carried out. Analytical data of representative compounds were listed below and all the rest summarized in Supporting Information.

General procedure for amide coupling. To a solution of the acid (1.2 mmol) in DMF (2 mL) was added a solution of HBTU (1.2 mmol) in DMF (2 mL) and DIPEA (1.2 mmol, or 2.4 mmol if the amine added as TFA or HCl salts). The medium was checked using pH paper to make sure pH > 8, if necessary, another aliquot of DIPEA was added. The mixture was stirred at rt for 15 min and added into a solution of the amine (1 mmol) in DMF (1 mL). The mixture was stirred at rt overnight and evaporated to dryness on rotavapor. The residue was re-dissolved in ethyl acetate (6 mL) and washed with sat. aqueous $NaHCO_3$ solution (3 × 6 mL). In parallel synthesis, the extraction was facilitated by centrifuging the mixture in a 15 mL Falcon tube at 3000 rpm for 5 min. The aqueous phase was removed using a Pasteur pipette. The organic phase was dried (anhydrous $MgSO_4$, 30 min), filtered using a small cotton ball and a pipette, and evaporated on

rotavapor to give the crude product, which was either purified as final product (yield 60–95%) or used directly for next step.

General procedure for Boc-deprotection. The substrate (1 mmol) was dissolved in a solution of TFA (0.5 mL) in dichloromethane (2 mL). The mixture was stirred at rt for 2 h and evaporated on rotavapor to dryness. The residue was either purified as final product (yield 60–99%) or co-evaporated twice with toluene (3 × 2 mL), pumped to dryness on high vacuum for 1 h, and used directly for next round of amide coupling.

*5-isoxazoyl-Cha-Ile-spiro[1H-indene-1,4'-piperidine] (**3**)*. 56% yield in six steps from **6**. ^{1}H NMR (600 MHz, DMSO-d_6) (two rotamers in a ratio of 64:36) δ 0.84–1.00 (m, 8H), 1.05–1.42 (m, 7H), 1.50-2.00 (m, 11H), 3.04 (td, J = 12.6, 1.8 Hz, 0.64H) and 3.10 (td, J = 12.6, 2.4 Hz, 0.36H, H_{ax} of *s*-syn amide piperidine NCH_2), 3.48 (t, J = 12.6 Hz, 1H, H_{ax} of *s*-anti amide piperidine NCH_2), 4.18 (d, J = 13.8 Hz, 0.36H) and 4.21 (d, J =13.8 Hz, 0.64H, H_{eq} of *s*-anti amide piperidine NCH_2), 4.44 (d, J = 13.8 Hz, 0.36 H) and 4.52 (d, J = 13.8 Hz, 0.64H, H_{eq} of *s*-syn amide piperidine NCH_2), 4.57–4.65 (m, 1H, Cha-α-CH), 4.68–4.73 (m, 1H, Ile-α-CH), 6.82 (d, J = 5.6 Hz, 1H, indene-CH), 7.07–7.38 (m, 6H), 8.16 (d, J = 9.0 Hz, 0.36H) and 8.31 (d, J = 9.0 Hz, 0.64H, Ile-NH), 8.77 (d, J = 1.8 Hz, 1H, isoxazole-3-CH), 8.91 (d, J = 8.4 Hz, 0.36H) and 9.00 (d, J = 8.4 Hz, 0.64H, Cha-NH); ^{13}C NMR (150 MHz, DMSO-d_6): 11.1/11.2 (CH_3, Ile-δ-CH_3), 15.6/15.9 (CH_3, Ile-γ-CH_3), 24.0 (CH_2, Ile-γ-CH_2), 25.7(0)/25.7(3) (CH_2, Cha-CH_2), 25.9 (CH_2, Cha-CH_2), 26.1 (CH_2, Cha-CH_2), 31.8(0)/31.8(1) (CH_2, Cha-CH_2), 33.1(6)/33.1(8) (CH_2, Cha-CH_2), 33.2(5)/33.3(1) (CH_2, *s*-syn amide piperidine N $CH_2\underline{CH_2}$), 33.7(5)/33.7(9) (CH, Cha-γ-CH), 33.9/34.1 (CH_2, *s*-anti amide piperidine N $CH_2\underline{CH_2}$), 36.3/36.6 (CH, Ile-β-CH), 38.9/39.1 (CH_2, Cha-β-CH_2), 40.3/40.5 (CH_2, *s*-syn amide piperidine NCH_2), 44.0/44.3 (CH_2, *s*-anti amide piperidine NCH_2), 51.1 (CH, Cha-α-CH), 52.0/52.1(8) (C, spiro-C), 52.1(7)/52.3 (CH, Ile-α-CH), 106.2(7)/106.3(2) (CH, isoxazole-4-CH), 121.4(0)/121.4(3) (CH), 121.4(7)/121.6(4) (CH),

125.2/125.4 (CH), 127.0(0)/127.0(4) (CH), 129.8(9)/129.9(2) (CH, indene-3-CH), 141.3(7)/141.4(7) (CH, indene-2-CH), 142.5(1)/142.5(7) (C), 151.2/151.3 (C), 151.8 (CH, isoxazole-3-CH), 155.4(7)/155.4(9) (C), 162.5 (C), 169.5/169.6 (C, Ile-CO), 171.3(0)/171.3(2) (C, Cha-CO). HRMS: calcd for $C_{32}H_{43}N_4O_4^+$ $[MH]^+$: 547.3279, found: 547.3291. LCMS t_R = 16.2 min. HPLC t_R = 15.5 min (Method IV).

General procedure for N-methylation. A solution of the amine (1 mmol) in anhydrous DCM (2 mL) was added anhydrous triethylamine (2.1 mmol) and 2-nitrobenzenesulfonyl chloride (1.05 mmol). The mixture was stirred at rt for 2 h, diluted with DCM (4 mL) and washed with water (4 mL). The DCM phase was dried ($MgSO_4$), filtered and evaporated on rotovap to dryness. The residue (pale-yellow foam) was dried under P_2O_5 under high vacuum for 1 h. The crude was dissolved in anhydrous DMF (2 mL), treated with DBU (2 mmol) and cooled in an ice-water bath while dimethyl sulfate (3 mmol) was added dropwise. After stirred at 0 ºC for 15 min, the mixture was treated with acetic acid (1.1 mmol) and evaporated under high vacuum. The residue was taken up in ethyl acetate (5 mL) and washed with sat. aqueous solution of $NaHCO_3$ (3 × 4 mL) and water (4 mL). The organic phase was dried ($MgSO_4$), filtered and evaporated. The crude was dissolved in DCM (4 mL) and treated with DBU (1.5 mmol) and 2-thioethanol (3 mmol). The mixture was stirred at rt for 30 min and treated with sat. aqueous solution of $NaHCO_3$ (4 mL). The mixture was stirred vigorously at rt for 10 min and centrifuged (250 rpm for 5 min). The organic phase was removed using a Pasteur pipette, dried ($MgSO_4$), filtered and evaporated on rotovap. The crude N-methylated amine was either purified by preparative HPLC, or co-evaporated with toluene (2 × 3 mL) and used for next coupling.

(N-Me)Cha-Ile-spiro[1H-indene-1,4'-piperidine] as TFA salt. ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.87–1.03 (m, 8H), 1.10–2.11 (m, 18H), 2.72 (s)/2.74 (s, 3H, NCH_3), 3.03–3.11 (m, 1H, H_{ax} of *s*-syn amide piperidine-NCH_2), 3.42–3.51 (m, 1H, H_{ax} of *s*-anti

amide piperidine-NCH_2), 3.94–4.01 (m, 1H, Cha-α-CH), 4.15–4.20 (m, 1H, H_{eq} of *s*-anti amide piperidine-NCH_2), 4.61–4.68 (m, 1H, H_{eq} of *s*-syn amide piperidine-NCH_2), 4.91–4.98 (m, 1H, Ile-α-CH), 6.78–6.85 (m, 2H, 2× indene-CH), 7.16–7.35 (m, 4H), 7.88–7.94 (m, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.0/11.2 (Ile-δ-CH_3), 15.6/16.0 (Ile-γ-CH_3), 24.1/24.2 (Ile-γ-CH_2), 25.7(9)/25.8(5) (CH_2), 25.9(4) (CH_2), 26.0(1) (CH_2), 31.6 (NCH_3), 32.6/32.7 (CH_2), 33.3(7) (CH_2), 33.4(3)/33.4(8), 33.7/33.8 (CH_2, *s*-syn amide piperidine $NCH_2\underline{CH_2}$), 34.2/34.3(CH_2, *s*-anti amide piperidine $NCH_2\underline{CH_2}$), 37.3/37.4 (Ile-β-CH), 37.9 (Cha-γ-CH), 41.3/41.4 (*s*-syn amide piperidine NCH_2), 44.9/45.2 (*s*-anti amide piperidine NCH_2), 51.8/51.9 (spiro-C), 53.6 (Ile-α-CH), 60.6 (Cha-α-CH), 116.3 (q, $^1J_{F-C}$ = 290 Hz, $\underline{CF_3}COO^-$), 121.4/121.5 (CH), 121.6(5)/121.7(2) (CH), 125.5/125.6 (CH), 127.3(0)/127.3(3) (CH), 130.9(7)/131.0(1) (indene-CH), 139.3/139.5 (indene-CH), 142.6/142.7 (C), 150.7 (C), 162.0 (q, $^2J_{F-C}$ = 36.7 Hz, $CF_3\underline{CO}O^-$), 167.7(7)/167.8(0) (C), 169.7/169.8 (C). HRMS: calc. for $C_{29}H_{44}N_3O_2^+$ $[MH]^+$: 466.3428, found: 466.3424. HPLC t_R = 9.3 min (Method I).

5-isoxazoyl-Cha-(N-Me)Ile-spiro[1H-indene-1,4'-piperidine]. ^{1}H NMR (400 MHz, DMSO-d_6) (two rotamers in a ratio of 70:30) δ 0.79–1.96 (m, 25H), 2.06–2.14 (m, 1H, Ile-β-CH), 2.97 (s, minor) and 3.06 (s, major, 3H, N-Me), 2.99–3.17 (m, 1H, *s*-syn amide piperidine NCH_2), 3.30–3.46 (m, 1H, *s*-anti amide piperidine NCH_2), 4.11 (d, *J* = 13.6 Hz, minor) and 4.18 (d, *J* = 13.6 Hz, manor, 1H, *s*-anti amide piperidine $NCH_2\underline{CH_2}$), 4.43 (d, *J* = 13.6 Hz, minor) and 4.56 (d, *J* = 13.6 Hz, major, 1H, *s*-syn amide piperidine NCH_2), 4.87–4.97 (m, 1H, Cha-α-CH), 5.07–5.13 (m, 1H, Ile-α-CH), 6.84 (d, *J* = 5.6 Hz, major) and 7.07 (d, *J* = 5.6 Hz, minor, 1H, indene-CH), 7.10–7.40 (m, 6H), 8.73 (d, *J* = 2.0 Hz, major) and 8.74 (d, *J* = 2.0 Hz, minor, 1H, isoxazole-CH), 9.13 (d, *J* = 9.0 Hz, minor) and 9.15 (d, *J* = 8.5 Hz, major, 1H, Cha-NH). ^{13}C NMR (100 MHz, DMSO-d_6): major isomer, 10.8 (Ile-δ-CH_3), 15.7 (Ile-γ-CH_3), 23.5 (Ile-γ-CH_2), 25.5 (Cha-CH_2), 25.7 (Cha-CH_2), 26.0 (Cha-CH_2), 29.7 (NCH_3), 31.2 (Cha-CH_2), 32.2 (Ile-β-CH), 33.2

(CH_2), 33.3 (CH_2), 33.6 (CH_2), 34.8 (CH, Ile-β-CH), 37.8 (CH_2, Cha-β-CH_2), 40.4 (*s*-syn amide piperidine NCH_2), 43.6 (*s*-anti amide piperidine NCH_2), 47.4 (Cha-α-CH), 52.1 (spiro-C), 55.8 (Ile-α-CH), 106.1 (isoxazole-4-CH), 121.4 (CH), 121.5 (CH), 125.0 (CH), 127.0(4) (CH), 129.9 (indene-3-CH), 141.2 (indene-2-CH), 142.5 (C), 151.1 (C), 151.7 (isoxazole-3-CH), 155.6 (C), 162.1 (C), 167.3 (C), 171.5 (C); minor isomer (only those not overlapped), 10.9 (Ile-δ-CH_3), 15.9 (Ile-γ-CH_3), 25.6 (Cha-CH_2), 25.8 (Cha-CH_2), 29.8 (NCH_3), 31.5 (Cha-CH_2), 32.4 (Ile-β-CH), 33.7 (CH_2), 33.9 (CH_2), 37.7 (CH_2, Cha-β-CH_2), 43.4 (*s*-anti amide piperidine NCH_2), 47.5 (Cha-α-CH), 52.0 (spiro-C), 55.9 (Ile-α-CH), 121.3 (CH), 121.8 (CH), 125.3 (CH), 127.0(0) (CH), 141.5 (indene-2-CH), 155.5 (C), 167.7 (C), 171.7 (C). HRMS: calc. for $C_{33}H_{45}N_4O_4^+$ $[MH]^+$: 561.3435, found: 561.3435. HPLC t_R = 12.5 min (Method I).

5-isoxazoyl-(N-Me)Cha-Ile-spiro[1H-indene-1,4'-piperidine]. ^{1}H NMR (DMSO-d_6) (two rotamers in a ratio of 58:42) δ 0.82–2.05 (m, 26H), 2.96 (s, minor) and 3.07 (s, major, 3H, N-Me), 3.00–3.10 (m, 1H), 3.43–3.55 (m, 1H), 4.13–4.22 (m, 1H), 4.44–4.52 (m, 1H), 4.63–4.69 (m, 1H, Ile-α-CH), 5.11–5.19 (m, 1H, Cha-α-CH), 6.83–7.36 (m, 7H), 8.22 (d, J = 8.5 Hz, minor) and 8.35 (d, J = 8.8 Hz, major, 1H, Ile-NH), 8.76–8.78 (m, 1H, isoxazole-CH). HRMS: calc. for $C_{33}H_{45}N_4O_4^+$ $[MH]^+$: 561.3435, found: 561.3435. HPLC t_R = 12.2 min (Method I).

(N,N,N-trimethyl)Cha-Ile-spiro[1H-indene-1,4'-piperidine] (7) as trifluoroacetate salt. In a microwave vial was loaded a solution of the H_2N-*Cha-Ile-spiro[1H-indene-1,4'-piperidine]* (33 mg, 0.0731 mmol) in acetone (0.4 mL), methyl iodide (27 μL, 0.439 mmol, 6 eq) and powdered Na_2CO_3 (23 mg, 0.219 mmol, 3 eq). The vial was sealed and loaded into Biotage Initiator microwave reaction system with settings at temperature = 120 ºC and reaction time = 20 min. After cooling to room temperature, the mixture was filtered and the filtrate evaporated on rotavap. The residue was dissolved in MeCN-H_2O (1:1, 4 mL) and purified by preparative rpHPLC. The product fractions were identified by ESMS (m/z 494, ammonium cation) and pure

fractions (confirmed by analytical rpHPLC) were pooled and lyophilized to give a white amorphous powder (21.3 mg, 48% yield). ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.84–1.02 (m, 8H), 1.05–1.76 (m, 13H), 1.87–2.17 (m, 5H), 3.01–3.12 (m, 1H), 3.29 (s) and 3.30 (s, 9H, 3 × Me), 3.44–3.55 (m, 1H), 4.19–4.25 (m, 1H), 4.64–4.71 (m, 2H), 4.79–4.87 (m, 1H), 6.81–6.88 (m, 2H), 7.17–7.36 (m, 4H), 9.20–9.24 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.8/11.0 (Ile-δ-CH_3), 15.6/16.1 (Ile-γ-CH_3), 24.8/24.9 (Ile-γ-CH_2), 25.6(7)/25.7(3), 26.2, 26.4/26.5, 32.1/32.2, 33.5/33.6, 34.2/34.3, 34.5, 34.6/34.7, 36.4/36.6, 41.3/41.4 (*s*-syn amide piperidine NCH_2), 45.1/45.5 (*s*-anti amide piperidine NCH_2), 52.1/52.2 (spiro-C), 52.5 ($^{+}NMe_3$), 53.8 (Ile-α-CH), 72.3/72.4 (Cha-α-CH), 116.2 (q, $^{1}J_{F-C}$ = 286 Hz, $\underline{CF_3}COO^{-}$), 121.6/121.7 (CH), 121.9 (CH), 125.6/125.7 (CH), 127.4/127.5 (CH), 131.1 (indene-CH), 139.8/139.9 (indene-CH), 142.9 (C), 150.9(8)/151.0(7) (C), 161.1 (q, $^{2}J_{F-C}$ = 37.1 Hz, $CF_3\underline{CO}O^{-}$), 166.4/166.5 (C), 169.5/169.7 (C). HRMS: calcd for $C_{31}H_{48}N_3O_2^{+}$ $[MH]^{+}$: 494.3741, found: 494.3741. HPLC t_R = 10.2 min (Method V).

*Carbamimidoyl-Cha-Ile-spiro[1H-indene-1,4'-piperidine] (**8**)*. In a 2 mL HPLC glass vial was loaded the H_2N-*Cha-Ile-spiro[1H-indene-1,4'-piperidine]* (46 mg, 0.102 mmol), 1*H*-pyrazole-1-carboxamidine HCl salt (15 mg, 0.102 mmol, 1 eq), DMF (204 μL) and DIPEA (18 μL, 0.102 mmol, 1 eq). The suspension was stirred at room temperature for 1 h. DCM (102 μL) was added to make thick precipitate turn to homogenous solution. After stirred at room temperature overnight, the mixture was transferred into a RBF and evaporated on rotavap to dryness. The crude was purified by preparative rpHPLC and the product fractions were pooled (ESMS: m/z 494, MH^{+}) and lyophilized to give the product as TFA salt (white amorphous solid, 19% yield). ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.87–1.02 (m, 8H), 1.11–1.28 (m, 4H), 1.37–1.75 (m, 11H), 1.89–2.18 (m, 3H), 3.04–3.12 (m, 1H), 3.44–3.54 (m, 1H), 4.13–4.21 (m, 2H), 4.53–4.61 (m, 1H), 4.66–4.73 (m, 1H), 6.79–6.83 (m, 2H), 7.12–7.36 (m, 7H), 8.08–

8.14 (m, 2H). HRMS: calc. for $C_{29}H_{44}N_5O_2^+$ $[MH]^+$: 494.3490, found: 494.3489. HPLC t_R = 10.1 min (Method V).

Carbamoyl-Cha-Ile-spiro[1H-indene-1,4'-piperidine] (***9***). In a 2 mL HPLC glass vial was loaded the H_2N-*Cha-Ile-spiro[1H-indene-1,4'-piperidine]* (20 mg, 0.0443 mmol), acetic acid (7.6 μL, 0.133 mmol, 3 eq), water (0.48 mL) and MeCN (0.48 mL). The mixture was sonicated to give a homogenous solution. A solution of sodium cyanate (8.6 mg, 0.133 mmol, 3 eq) in water (0.4 mL) was added to the above reaction mixture dropwise over a period of 4 h. *Note: at 2 h after addition of 0.2 mL of the sodium cyanate solution, rapid precipitation was observed and thus extra MeCN (0.4 mL) was added to make the reaction mixture homogenous*. After stirred at room temperature overnight. ESMS indicated the complete conversion (m/z 495 MH^+ and 517 MNa^+ exclusively). The reaction mixture was purified by preparative rpHPLC and the desired product fractions were pooled and lyophilized to give a white amorphous powder (9.11 mg, 42% yield). ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.88–1.03 (m, 8H), 1.10–1.30 (m, 4H), 1.32–2.14 (m, 14H), 3.06–3.12 (m, 1 H), 3.44–3.54 (m, 1H), 4.16 (m, 1H), 4.31 (br s, 1H), 4.61–4.69 (m, 1H), 4.89–4.91 (m, 1H), 6.01 (br s, 1H), 6.82–6.87 (m, 2H), 7.14–7.38 (m, 6H). HRMS: calc. for $C_{29}H_{43}N_4O_3^+$ $[MH]^+$ 495.3330, found 495.3340; HPLC t_R = 9.4 min (Method II).

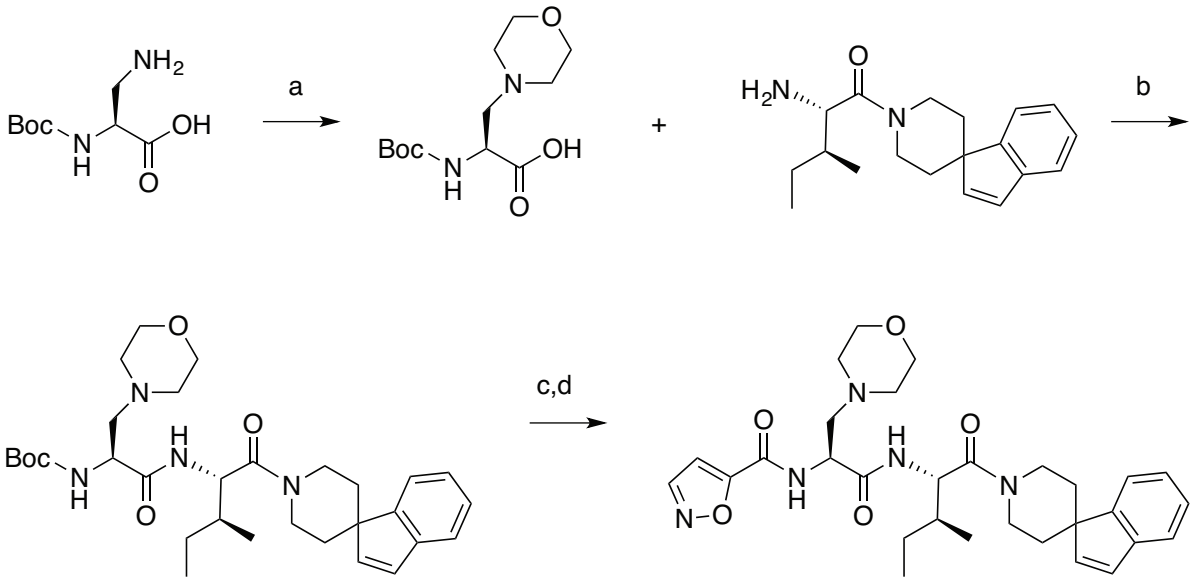
(2-Hydroxyethylcarbamoyl)-Cha-Ile-spiro[1H-indene-1,4'-piperidine] (***10***). In a 2 mL HPLC glass vial was loaded triphosgene (5.3 mg, 0.0177 mmol, 0.4 eq) and anhydrous DCM (100 μL). A solution of the H_2N-*Cha-Ile-spiro[1H-indene-1,4'-piperidine]* (20 mg, 0.0443 mmol, 1 eq) in DIPEA (17 μL, 0.0975 mmol, 2.2 eq) and anhydrous DCM (100 μL) was added dropwise. After 5 min, a solution of ethanolamine (2.9 μL, 0.0487 mmol, 1.1 eq) in anhydrous DCM (100 μL) was added in one portion. The mixture was stirred at room temperature overnight and transferred into a RBF. The mixture was evaporated on rotavap and the residue was treated with MeCN (5

mL). The mixture was heated to reflux, cooled and filtered. The filtrate was purified by preparative rpHPLC and the desired product fractions were pooled and lyophilized to give a white amorphous powder (7.05 mg, 30% yield). ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.89–1.06 (m, 8H), 1.10–1.32 (m, 4H), 1.34–1.60 (m, 5H), 1.62–1.88 (m, 7H), 1.90–2.40 (m, 4H), 3.04–3.14 (m, 1 H), 3.30–3.60 (m, 2H), 3.75 (br s, 2H), 4.12–4.30 (m, 2H), 4.58–4.70 (m, 1H), 4.84–4.94 (m, 1H), 5.66 (br s, 1 H), 6.82–6.87 (m, 2H), 7.14–7.38 (m, 5H). HRMS: calc. for $C_{31}H_{47}N_4O_4^+$ $[MH]^+$ 539.3592, found 539.3600; HPLC t_R = 9.1 min (Method II).

*Benzylcarbamoyl-Cha-Ile-spiro[1H-indene-1,4'-piperidine] (**11**)*. In a 2 mL HPLC glass vial was loaded the H_2N-*Cha-Ile-spiro[1H-indene-1,4'-piperidine]* (20 mg, 0.0443 mmol, 1 eq), DCM (150 μL), and benzyl isocyanate (5.4 μL, 0.0443 mmol, 1 eq). The mixture was stirred at rt overnight and transferred into a RBF. The mixture was evaporated on rotavap and the residue was dissolved in MeCN (5 mL) and purified by preparative rpHPLC and the desired product fractions were pooled and lyophilized to give a white amorphous powder (8.50 mg, 33% yield). ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.86–1.02 (m, 8H), 1.06–2.20 (m, 18H), 2.98–3.08 (m, 1H), 3.39–3.48 (m, 1H), 4.07–4.15 (m, 1H), 4.30–4.46 (m, 3H), 4.56–4.67 (m, 1H), 4.84–4.91 (m, 1H), 5.25 (br s, 1 H), 6.80–7.37 (m, 12H). HRMS: calc. for $C_{36}H_{49}N_4O_3^+$ $[MH]^+$ 585.3799, found 585.3807; HPLC t_R = 12.3 min (Method II).

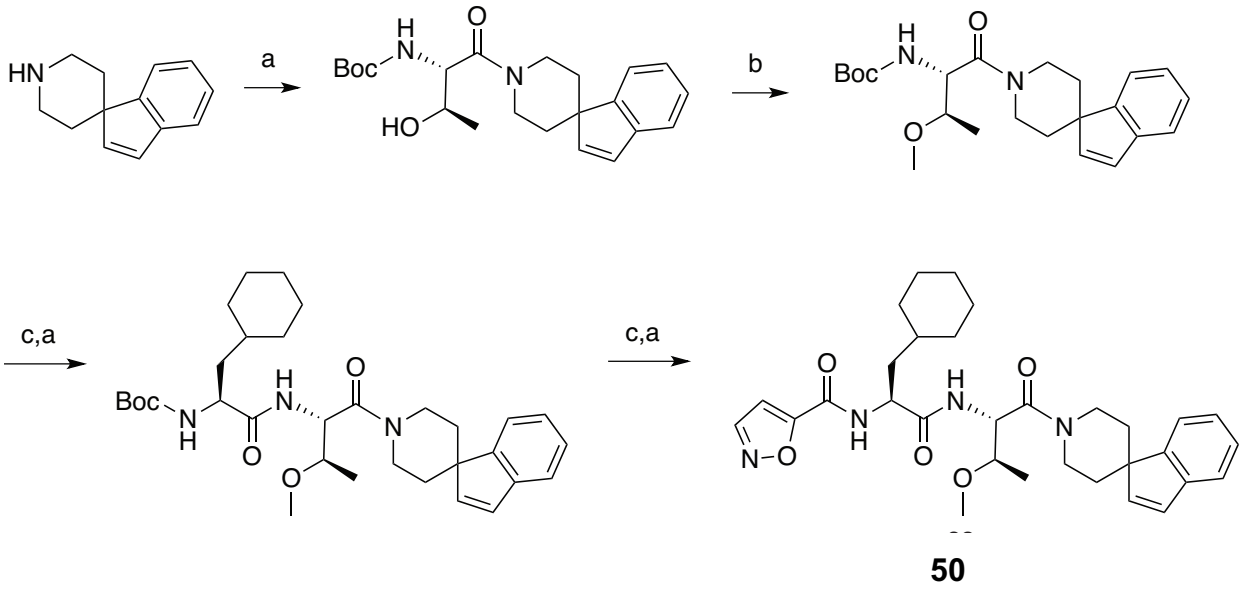
*5-Isoxazoyl-Cha-Ile-(4-phenylpiperidine) (**65**)*. 46% yield in five steps. ^{1}H NMR (600 MHz, DMSO-d_6) (two rotamers in a ratio of 60:40) δ 0.78–0.95 (m, 8H), 0.99–1.21 (m, 4H), 1.25–1.44 (m, 2H), 1.44–1.90 (m, 12H), 2.59–2.68 (m, 1H, H_{ax} of *s*-syn amide piperidine NCH_2), 2.75–2.83 (m, 1H, piperidine-CHPh), 3.09 (app t, *J* = 12.0 Hz, minor) and 3.14 (app t, *J* = 12.0 Hz, major, 1H, H_{ax} of *s*-anti amide piperidine NCH_2), 4.19 (d, 1H, *J* = 13.2 Hz, H_{eq} of *s*-anti amide piperidine NCH_2), 4.53–4.59 (m, 2H, Cha-α-CH and H_{eq} of *s*-syn amide piperidine NCH_2), 4.60–4.65 (m, 1H, Ile-α-CH), 7.14–7.41 (m, 6H), 8.14 (d, *J* = 8.4 Hz, minor) and 8.28 (d, *J* = 9.0 Hz,

major, 1H, Ile-NH), 8.74 (d, *J* = 1.8 Hz, major) and 8.75 (d, *J* = 1.8 Hz, minor, 1H, isoxazole-CH), 8.94 (d, *J* = 7.2 Hz, minor) and 8.95 (d, *J* = 7.8 Hz, major, 1H, Cha-NH); ^{13}C NMR (150 MHz, $CDCl_3$): 11.5/11.7 (CH_3, Ile-δ-CH_3), 16.0/16.3 (CH_3, Ile-γ-CH_3), 24.3/24.4 (CH_2, Ile-γ-CH_2), 26.0(8)/26.1(7) (CH_2), 26.2(5)/26.3(0) (CH_2), 26.5 (CH_2), 32.1(8)/32.2(0) (CH_2), 33.3(8)/33.4(4) (CH_2), 33.6(7)/33.7(0) (CH_2), 34.1 (CH, Cha-γ-CH), 34.2 (CH_2), 36.6/36.9 (CH, Ile-β-CH), 39.3/39.5 (CH_2, Cha-β-CH_2), 42.0/42.2 (CH, piperidine CHPh), 42.6/42.7 (CH_2, *s*-syn amide piperidine NCH_2), 46.1/46.3 (CH_2, *s*-anti amide piperidine NCH_2), 51.3/51.4 (CH, Cha-α-CH), 52.5/52.6 (CH, Ile-α-CH), 106.6/106.7 (CH, isoxazole-4-CH), 126.7 (CH), 127.0(9)/127.1(3) (CH), 128.9/129.0 (CH), 145.9/146.0 (C), 152.2 (CH, isoxazole-3-CH), 155.8 (C), 162.8 (C), 169.6/169.7 (C, Ile-CO), 171.6/171.7 (C, Cha-CO). HRMS: calc. for $C_{30}H_{43}N_4O_4^+$ $[MH]^+$ 523.3279, found 523.3280. HPLC t_R = 10.0 min (Method II).

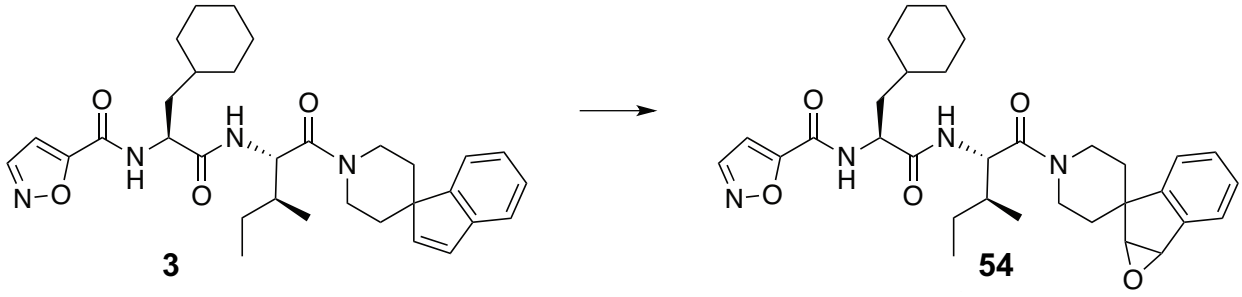


44

Scheme S1. Synthesis of **44**. (a) $O(CH_2CH_2Cl)_2$ (1 eq), 2M NaOH (3 eq), 1,4-dioxane, microwave (Biotage Initiator), 100 ºC, 2 h; (b) HBTU (1.1 eq), DIPEA (1.1 eq), DMF, rt, 18 h; (c) 20% TFA in DCM, rt, 2 h; (d) isoxazole-5-carboxylic acid (1.2 eq), HBTU (1.2 eq), DIPEA (1.2 eq), DMF, rt, 18 h.



Scheme S2. Synthesis of **50**. (a) carboxylic acid (i.e., Boc-Thr-OH, Boc-Cha-OH or isoxazole-5-carboxylic acid, 1.2 eq), HBTU (1.2 eq), DIPEA (1.2 eq), DMF, rt, 18 h; (b) i) LiO^{t}Bu (1.05 eq), DMF, rt, 1 h; ii) MeI (1.1 eq), rt, 18 h; (c) 20% TFA in DCM, rt, 2 h.



Scheme S3. Synthesis of **54**. mCPBA (2 eq), DCM–sat. $NaHCO_3$ (1:1, v/v). rt, 3 days.

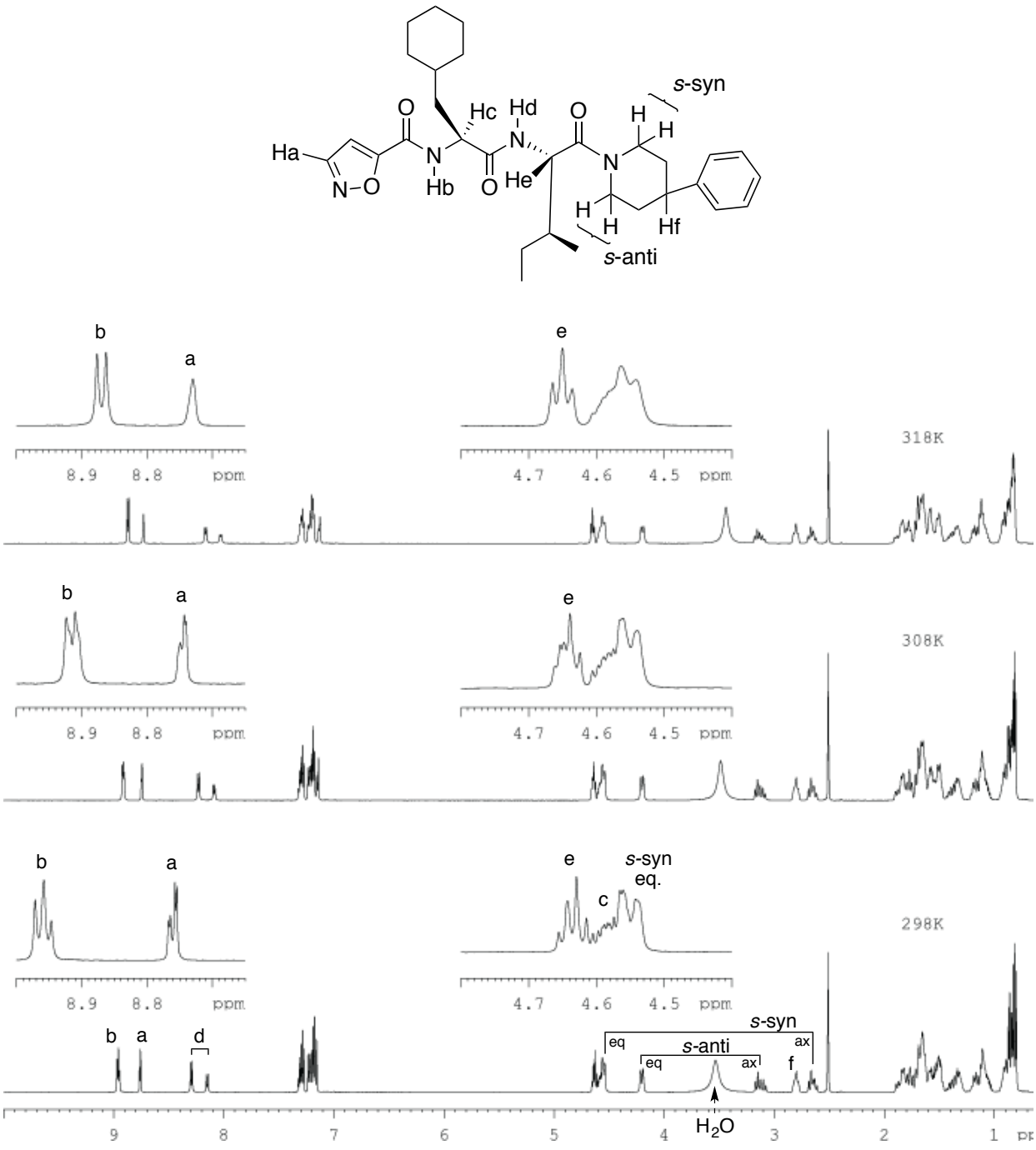
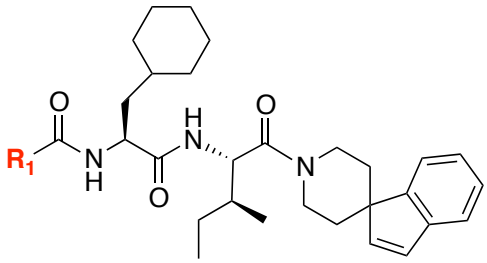
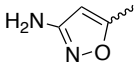
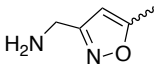
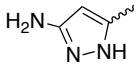
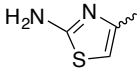
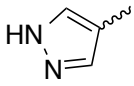
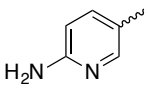


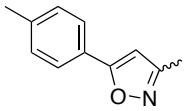
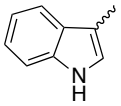
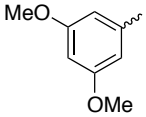
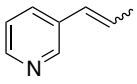
Figure S5. ^{1}H NMR of representative compound **65** at elevated temperature in DMSO-d_6. At 298K, isoxazole proton Ha split into 2 sets of doublets (8.75 ppm), amide NH_b into 2 sets of doublets (8.94 ppm, appeared as triplet due to overlapping signals) and Ile-α-CH (He) was multiplet at 4.64 ppm. They all simplified at 318K with Ha appeared as one broad singlet, Hb as a sharp doublet (coupling with Cha-α-CH, Hc) and He as a triplet (coupling with Ile-NH, Hd and Ile-β-CH). However, at this temperature, the asymmetry caused by amide group derived from piperidine ring was still present with two distinguished methylene pairs (H_{axial} and $H_{equatorial}$) of *s*-syn and *s*-anti NCH_2 of piperidine ring clearly observable.

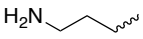
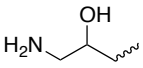


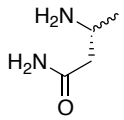
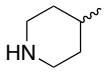
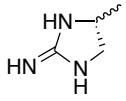
ID	R_1	Analytical data
12		t_R = 13.0 min (Method II); HRMS: calc. for $C_{32}H_{43}N_4O_4^+$ $[MH]^+$ 547.3279, found 547.3279; ^{1}H NMR (600 MHz, DMSO-d_6) (two rotamers in a ratio of 62:38) δ 0.82-0.97 (m, 8H), 1.06–1.36 (m, 7H), 1.47–1.97 (m, 11H), 3.00–3.09 (m, 1H), 3.44–3.48 (m, 1H), 4.15–4.19 (m, 1H), 4.41 (br d, J = 13.5 Hz, 0.38H) and 4.50 (br d, J = 13.4 Hz, 0.62H), 4.56–4.63 (m, 1H), 4.68–4.72 (m, 1H), 6.82 (d, J = 5.6 Hz, 1H), 6.93 (d, J = 1.60 Hz, 1H), 7.05–7.35 (m, 5H), 8.11 (d, J = 8.7 Hz, 0.38H) and 8.24 (d, J = 9.04 Hz, 0.62H), 8.74 (d, J = 8.5 Hz, 0.38H) and 8.78 (d, J = 8.4 Hz, 0.62H), 9.10–9.11 (m, 1H).
13		t_R = 10.9 min (Method II); HRMS: calc. for $C_{33}H_{44}N_3O_4^+$ $[MH]^+$ 546.3326, found 546.3330; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.85–0.94 (m, 4H), 0.97–1.0 (m, 2H), 1.05–1.28 (m, 4H), 1.37–1.58 (m, 4H), 1.62–1.74 (m, 4H), 1.75–1.85 (m, 6H), 1.91–2.14 (m, 2H), 3.00–3.02 (m, 1H), 3.41–3.52 (m, 1H), 4.12–4.18 (m, 1H), 4.61–4.75 (m, 2H), 4.88–4.95 (m, 1H), 6.51 (dd, 1H, J = 2.0, 3.4 Hz), 6.77–6.87 (m, 4H), 7.14 (d, 1H, J = 3.2 Hz), 7.19–7.36 (m, 4H), 7.46 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): 11.6/11.8 (CH_3, Ile-δ-CH_3), 16.1/16.4 (CH_3, Ile-γ-CH_3), 24.3, 26.2(7)/26.3(0), 26.4(0)/26.4(5), 26.6, 32.8(6)/32.9(1), 33.5/33.6, 34.0, 34.2(8)/34.3(5), 34.3(9)/34.5, 38.4, 40.4/40.5, 41.3 (br, *s*-syn amide piperidine NCH_2), 45.0/45.4, 51.1 (br, *s*-anti amide piperidine NCH_2), 52.1/52.3 (spiro-C), 53.1(6)/53.2(2)/53.2(9) (2 × α-CH), 112.3(8)/112.4(4), 115.0, 121.7/121.8, 121.9/122.0, 125.7/125.8, 127.4/127.5, 131.0(7)/131.1(1), 139.8/140.0, 142.8/143.0, 144.3(8)/144.4(2), 147.7, 151.1/151.2, 158.3, 170.3, 172.1.
14		t_R = 6.6 min (Method II); HRMS: calc. for $C_{32}H_{44}N_5O_3^+$ $[MH]^+$ 546.3439, found 546.3437; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.78–0.99 (m, 8H), 1.10–1.25 (m, 4H), 1.38–1.50 (m, 4H), 1.60–1.62 (m, 2H), 1.70–1.89 (m, 6H), 1.93–2.11 (m, 2H), 3.00–3.10 (m, 1H), 3.46 (t, 1H, J = 12.8 Hz), 4.25 (d, 1H, J = 12.4 Hz), 4.57–4.66 (m, 1H), 4.70–4.90 (m, 1H), 5.02 (q, 1H, J = 8.0 Hz), 6.77–6.82 (m, 2H), 7.19–7.26 (m, 5H), 7.31–7.35 (m, 1H), 7.90–8.00 (br s, 1H), 8.40–8.48 (br s, 1H), 8.49–8.62 (br s, 1H).
15		t_R = 9.7 min (Method II); HRMS: calc. for $C_{32}H_{43}N_4O_4^+$ $[MH]^+$ 547.3279, found 547.3278; ^{1}H NMR (600MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.86–0.97 (m, 5H), 1.01 (d, 2H, J = 6.8 Hz), 1.1–1.28 (m, 4H), 1.28–1.6 (m, 4H), 1.61–1.78 (m, 5H), 1.78–1.90 (m, 2H), 1.91–2.07 (m, 2H), 2.09–2.16 (m, 1H), 3.05–3.16 (m, 1H), 3.44–3.56 (m, 2H), 4.14–4.22 (m, 1H), 4.61–4.76 (m, 2H), 4.93 (q, 1H, J = 7.2

		Hz), 6.83–6.92 (m, 2H), 7.09 (dd, 1H, *J* = 8.8, 18 Hz), 7.18–7.36 (m, 5H), 7.76 (s, 1H), 7.95 (s, 1H).
16		t_R = 8.9 min (Method II); HRMS: calc. for $C_{31}H_{43}N_6O_3^+$ $[MH]^+$ 547.3391, found 547.3391; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.80–1.01 (m, 9H), 1.10–1.26 (m, 5H), 1.40–1.58 (m, 5H), 1.66–1.88 (m, 5H), 1.98–2.18 (m, 2H), 3.16 (t, 1H, *J* = 12.4 Hz), 3.56 (q, 1H, *J* = 13.6 Hz), 4.78–4.85 (m, 1H), 5.10–5.20 (m, 1H), 5.21–5.40 (m, 1H), 6.84–6.88 (m, 2H), 7.21–7.37 (m, 4H), 8.02–8.21 (m, 2H), 8.80–8.85 (m, 1H).
17		t_R = 15.0 min (Method IV); HRMS: calc. for $C_{32}H_{44}N_5O_4^+$ $[MH]^+$ 562.3388, found 562.3388; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.84–1.04 (m, 8H), 1.08–2.24 (m, 18H), 3.05–3.16 (m, 1H), 3.44–3.59 (m, 1H), 4.19–4.26 (m, 1H), 4.60–4.77 (m, 2H), 4.93–5.01 (m, 1H), 6.47 (s, 1H), 6.82–6.87 (m, 2H), 7.18–7.49 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 11.2/11.4 (Ile-δ-CH_3), 15.6/16.0 (Ile-γ-CH_3), 24.2 (Ile-γ-CH_2), 26.0, 26.1(0)/26.1(3), 26.2, 32.4(0)/32.4(2), 33.2/33.4, 33.5(9)/33.6(2), 34.1, 37.8(8)/37.9(4), 40.1/40.2, 41.4/41.5, 45.1/45.6, 51.4/51.5, 51.8/51.9, 53.1, 99.9 (isoxazole-4-CH), 121.4/121.6, 121.8, 125.5/125.7, 127.3/127.4, 131.0/131.1 (indene-3-CH), 139.3/139.7 (indene-2-CH), 142.5/142.8, 150.7/150.8, 155.8, 162.0/163.6, 170.6(5)/170.6(8), 171.8/171.9.
18		t_R = 6.2 min (Method II); HRMS: calc. for $C_{33}H_{46}N_5O_4^+$ $[MH]^+$ 576.3544, found 576.3544; ^{1}H NMR (600 MHz, DMSO-d_6) (two rotamers in a ratio of 2:1) δ 0.80–0.97 (m, 8H), 1.04–1.21 (m, 5H), 1.22–1.38 (m, 2H), 1.47–1.62 (m, 3H), 1.62–1.77 (m, 5H), 1.77–1.99 (m, 3H), 2.99–3.10 (m, 1H), 3.46 (t, 1H, *J* = 13 Hz), 4.17 (t, 1H, *J* = 14 Hz), 4.24–4.30 (m, 2H), 4.43 (d, *J* = 13 Hz, minor) and 4.50 (d, *J* = 13 Hz, major, 1H), 4.54–4.62 (m, 1H), 4.64–4.70 (m, 1H), 6.84 (d, *J* = 5.6 Hz, major) and 7.06 (d, *J* = 5.6 Hz, minor, 1H), 7.13–7.20 (m, 1H), 7.21–7.28 (m, 2H), 7.32–7.36 (m, 1H), 8.16 (d, *J* = 8.8 Hz, minor) and 8.31 (d, *J* = 8.8 Hz, major, 1H), 8.50 (br s, 2H), 9.12 (d, *J* = 8.0 Hz, minor) and 9.14 (d, *J* = 8.0 Hz, major, 1H)
19		t_R = 9.6 min (Method I) or 13.7 (Method IV); HRMS: calc. for $C_{32}H_{45}N_6O_3^+$ $[MH]^+$ 561.3548, found 561.3548; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.80–1.04 (m, 8H), 1.06–2.20 (m, 18H), 3.04–3.16 (m, 1H), 3.46–3.56 (m, 1H), 4.17–4.24 (m, 1H), 4.76–4.81 (m, 2H), 5.01–5.08 (m, 1H), 6.04 (br s, 1H), 6.80–6.85 (m, 2H), 7.18–7.35 (m, 5H), 7.78 (br s, 1H), 8.40 (br s, 1H).
20		t_R = 12.8 min (Method II); HRMS: calc. for $C_{32}H_{43}N_4O_3S^+$ $[MH]^+$ 563.3051 found 563.3049; ^{1}H NMR (600 MHz, DMSO-d_6) (two rotamers in a ratio of 2:1) δ 0.78–0.97 (m, 8H), 1.04–1.36 (m, 7H), 1.44–1.73 (m, 7H), 1.73–1.98 (m, 4H), 2.97–3.11 (m, 1H), 3.46 (t, J = 13.2 Hz, 1H), 4.14–4.23 (m, 1H), 4.41 (d, *J* = 13 Hz, minor) and 4.49 (d, *J* = 13 Hz, major, 1H), 4.60–4.74 (m, 2H), 6.83 (d, *J* = 5.7 Hz, major) and 7.04 (d, *J* = 5.7 Hz, minor, 1H), 7.08–7.27 (m, 3H), 7.31–7.38 (m, 1H), 8.25 (d, *J* = 8.8 Hz, 0.4H), 8.33–8.45 (m, 2.6H), 9.22 (d, *J* = 2.0 Hz, minor) and 9.23 (d, *J* = 2.0 Hz, major, 1H).
21		t_R = 13.2 min (Method II); HRMS: calc. for $C_{32}H_{43}N_4O_3S^+$ $[MH]^+$ 563.3051, found 563.3049; ^{1}H NMR (600 MHz, DMSO-d_6) (two

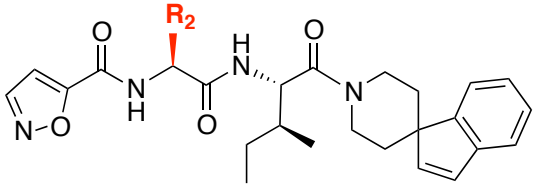
		rotamers in a ratio of 2:1) δ 0.79–1.00 (m, 8H), 1.03–1.45 (m, 7H), 1.46–1.77 (m, 8H), 1.77–2.03 (m, 3H), 2.97–3.12 (m, 1H), 3.46 (t, *J* = 13.3 Hz, 1H), 4.12–4.23 (m, 1H), 4.41 (d, *J* = 13.3 Hz, minor) and 4.51 (d, *J* = 13.3 Hz, major, 1H), 4.53–4.61 (m, 1H), 4.64–4.70 (m, 1H), 6.84 (d, *J* = 5.6 Hz, major) and 7.05 (d, *J* = 5.6 Hz, minor, 1H), 7.12–7.31 (m, 3H), 7.31–7.37 (m, 1H), 8.07 (d, *J* = 9.0 Hz, minor) and 8.22 (d, *J* = 9.0 Hz, major, 1H), 8.56–8.62 (m, 1H), 8.68–8.76 (m, 1H), 9.23 (br s, 1H).
22		t_R = 15.0 min (Method IV); HRMS: calc. for $C_{32}H_{44}N_5O_3S^+$ $[MH]^+$ 578.3159, found 578.3161; ^{1}H NMR (600 MHz, DMSO-d_6) (two rotamers in a ratio of 2:1) δ 0.79–0.97 (m, 8H), 1.03–1.35 (m, 7H), 1.44–1.72 (m, 7H), 1.73–2.00 (m, 4H), 2.97–3.11 (m, 1H), 3.41–3.52 (m, 1H), 4.13–4.24 (m, 1H), 4.43 (d, *J* = 13 Hz, minor) and 4.51 (d, *J* = 13 Hz, major, 1H), 4.55–4.64 (m, 1H), 4.64–4.71 (m, 1H), 6.84 (d, *J* = 5.6 Hz, major) and 7.06 (d, *J* = 5.6 Hz, minor, 1H), 7.12–7.26 (m, 3H), 7.29–7.37 (m, 2H), 7.86–8.00 (m, 1H), 8.32 (d, *J* = 9.2 Hz, minor) and 8.43 (d, *J* = 9.2 Hz, major, 1H).
23		t_R = 13.1 min (Method II); HRMS: calc. for $C_{33}H_{44}N_3O_3S^+$ $[MH]^+$ 562.3098, found 562.3096; ^{1}H NMR (600 MHz, DMSO-d_6) (two rotamers in a ratio of 2:1) δ 0.77–1.01 (m, 8H), 1.02–1.42 (m, 7H), 1.45–1.76 (m, 8H), 1.77–2.02 (m, 3H), 2.97–3.11 (m, 1H), 4.12–4.24 (m, 1H), 4.40 (d, *J* = 13.6 Hz, minor) and 4.50 (d, *J* = 13.6 Hz, major, 1H), 4.52–4.59 (m, 1H), 4.65–4.72 (m, 1H), 6.84 (d, *J* = 5.6 Hz, major) and 7.05 (d, *J* = 5.6 Hz, minor, 1H), 7.11–7.26 (m, 4H), 7.29–7.36 (m, 2H), 7.77 (d, *J* = 5.1 Hz, 1H), 7.86–7.92 (m, 1H), 7.97 (d, *J* = 9.0 Hz, minor) and 8.11 (d, *J* = 9.0 Hz, major, 1H), 8.45–8.53 (m, 1H).
24		t_R = 9.9 min (Method II); HRMS: calc. for $C_{32}H_{44}N_5O_3^+$ $[MH]^+$ 546.3439, found 546.3438; ^{1}H NMR (600 MHz, DMSO-d_6) (two rotamers in a ratio of 2:1) δ
25		t_R = 7.3 min (Method II); HRMS: calc. for $C_{34}H_{45}N_4O_3^+$ $[MH]^+$ 557.3486, found 557.3494; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.85–0.95 (m, 4H), 0.98–1.04 (m, 2H), 1.05–1.08 (m, 4H), 1.38–1.58 (m, 4H), 1.64–1.85 (m, 10H), 1.91–2.14 (m, 2H), 3.03–3.12 (m, 1H), 3.41–3.51 (m, 1H), 4.12–4.18 (m, 1H), 4.63–4.71 (m, 1H), 4.75–4.83 (m, 1H), 4.91–4.97 (m, 1H), 6.82–6.93 (m, 4H), 7.19–7.29 (m, 3H), 7.33–7.40 (m, 2H), 8.11–8.15 (m, 1H), 8.73 (dt, 1H, *J* = 1.6, 4.8 Hz), 9.03 (t, 1H, *J* = 2.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.6/11.9 (Ile-δ-CH_3), 16.1/16.4 (Ile-γ-CH_3), 24.2 (Ile-γ-CH_2), 26.3(1)/26.3(5), 26.4(2)/26.4(7), 26.5(4), 33.0/33.1, 33.5/33.6, 33.9, 34.4(2)/34.4(5), 34.5(3), 38.4(7)/38.5(1), 40.5(7)/40.6(3), 41.3 (br), 44.9/45.3, 51.9 (br), 52.1/52.3, 53.2/53.3/53.4 (2 × α-CH), 121.7(0)/121.8(7)/121.9(4), 123.7, 125.8, 127.4(8)/127.5(5), 130.0, 131.1, 135.3, 139.8/139.9, 142.8/143.0, 148.4, 151.0(9)/151.1(3), 152.6, 165.5, 170.1(6)/170.2(0), 172.0/172.1.
26		t_R = 12.9 min (Method IV); HRMS: calc. for $C_{34}H_{46}N_5O_3^+$ $[MH]^+$ 572.3595, found 572.3599; ^{1}H NMR (600 MHz, DMSO-d_6) (two rotamers in a ratio of 1:1.3) δ 0.77–1.00 (m, 8H), 1.01–1.42 (m, 8H), 1.45–1.56 (m, 1H), 1.56–1.77 (m, 7H), 1.77–2.00 (m, 3H), 2.98–3.12 (m, 1H), 3.41–3.49 (m, 1H), 4.09–4.23 (m, 2H), 4.41 (d, *J* = 13 Hz,

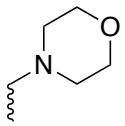
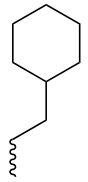
		minor) and 4.51 (d, *J* = 13 Hz, minor, 1H), 4.53–4.62 (m, 1H), 4.62–4.70 (m, 1H), 6.84 (d, *J* = 5.7 Hz, major) and 7.05 (d, *J* = 5.7 Hz, minor, 1H), 6.95 (d, *J* = 9.3 Hz, 1H), 7.11–7.28 (m, 3H), 7.35 (d, *J* = 7.2 Hz, 1H), 8.07 (d, *J* = 9.1 Hz, 0.4H), 8.19–8.29 (m, 2H), 8.48–8.57 (m, 2H).
27		t_R = 11.6 min (Method II); HRMS: calc. for $C_{33}H_{44}N_5O_3^+$ $[MH]^+$ 558.3439, found 558.3440; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.86–1.05 (m, 7H), 1.12–1.28 (m, 4H), 1.40–1.57 (m, 4H), 1.66–1.89 (m, 9H), 1.91–2.21 (m, 2H), 3.04–3.17 (m, 1H), 3.44–3.58 (m, 1H), 4.18–4.26 (m, 1H), 4.62–4.78 (m, 2H), 4.91–4.97 (m, 1H), 6.83–6.87 (m, 2H), 7.13–7.30 (m, 4H), 7.34–7.37 (m, 1H), 8.17–8.20 (m, 1H), 8.60 (br s, 1H), 8.78 (br s, 1H), 9.41 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.4/11.6 (Ile-δ-CH_3), 15.9/16.3 (Ile-γ-CH_3), 24.4 (Ile-γ-CH_2), 26.2(2)/26.2(5), 26.3(6)/26.3(9), 26.5, 32.6(9)/32.7(4), 33.5/33.6, 33.9, 34.4/34.5, 38.0/38.1, 40.1(5)/40.2(0), 41.7/41.8, 45.3/45.7, 51.7(1)/51.7(5), 51.8(2)/51.8(7), 52.0/52.2, 53.2/53.3/53.4 (2 × α-CH), 121.7/121.8, 121.9(7)/122.0(0), 125.8/125.9, 127.5/127.6, 131.1(9)/131.2(5), 139.6/139.9, 142.8/143.0, 143.1, 144.4, 147.4,, 150.9/151.0, 163.2, 170.7, 172.4.
28		t_R = 15.0 min (Method I); HRMS: calc. for $C_{39}H_{49}N_4O_4^+$ $[MH]^+$ 637.3748, found 637.3752; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.86–1.06 (m, 7H), 1.09–1.30 (m, 4H), 1.36–1.55 (m, 4H), 1.61–1.76 (m, 5H), 1.80–1.89 (m, 3H), 1.94–2.20 (m, 3H), 2.41 (s, 3H), 3.02–3.16 (m, 1H), 3.42–3.55 (m, 1H), 4.13–4.23 (m, 1H), 4.60–4.76 (m, 2H), 4.90–4.98 (m, 1H), 6.83–6.86 (m, 2H), 6.92–7.04 (m, 4H), 7.19–7.36 (m, 6H), 7.66–7.70 (m, 1H).
29		t_R = 12.1 min (Method I); HRMS: calc. for $C_{37}H_{47}N_4O_3^+$ $[MH]^+$ 595.3643, found 595.3644; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.86 (t, 2H, *J* = 7.2 Hz), 0.89–0.95 (m, 4H), 0.96–1.08 (m, 3H), 1.10–1.30 (m, 4H), 1.36–1.67 (m, 5H), 1.68–2.07 (m, 9H), 3.04–3.14 (m, 1H), 3.43–3.54 (m, 1H), 4.14–4.24 (m, 1H), 4.61–4.74 (m, 1H), 4.80–4.89 (m, 1H), 4.91–4.98 (m, 1H), 6.66–6.74 (m, 1H), 6.81–6.88 (m, 2H), 7.17–7.24 (m, 2H), 7.26–7.30 (m, 3H), 7.30–7.36 (m, 2H), 7.41–7.46 (m, 1H), 7.86 (t, 1 H, *J* = 3.2Hz), 7.98–8.03 (m, 1H), 8.65 (br s, 1H).
30		t_R = 12.8 min (Method I); HRMS: calc. for $C_{37}H_{49}NaN_3O_5^+$ $[M+Na]^+$ 638.3564, found 38.3573; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.85–1.03 (m, 9H), 1.08–1.28 (m, 5H), 1.37–1.52 (m, 4H), 1.61–1.89 (m, 5H), 1.92–2.13 (m, 2H), 3.30–3.13 (m, 1H), 3.41–3.52 (m, 1H), 3.83 (s, 6H), 4.10–4.19 (m, 1H), 4.62–4.78 (m, 2H), 4.89–4.96 (m, 1H), 6.58–6.63 (m, 2H), 6.82–6.92 (m, 3H), 7.19–7.36 (m, 6H).
31		t_R = 10.3 min (Method I); HRMS: calc. for $C_{36}H_{47}N_4O_3^+$ $[MH]^+$ 583.3643, found 583.3645; ^{1}H NMR (400 MHz, DMSO–d_6) (two rotamers in a ratio of 1:1) δ 0.80–0.96 (m, 8H), 1.03–1.32 (m, 7H), 1.47–1.70 (m, 7H), 1.74–2.00 (m, 4H), 2.97–3.10 (m, 1H), 3.45 (t, 1H, *J* = 12.8 Hz), 4.13–4.20 (m, 1H), 4.40 (d, 1H, *J* = 14 Hz), 4.48–4.60 (m, 1H), 4.63–4.67 (m, 1H), 6.82 (d, 1H, *J* = 5.6 Hz), 6.89 (d, 1H, *J* = 16 Hz), 7.11–7.34 (m, 5H), 7.49 (d, 1H, *J* = 15.6 Hz), 7.59 (dd, 1H, *J* =

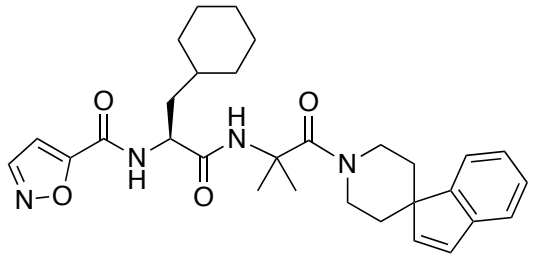
		5.2, 7.6 Hz), 8.15 (d, 1H, *J*= 8 Hz), 8.22 (d, 1H, *J* = 9.2 Hz), 8.29–8.36 (m, 1H), 8.61 (s, 1H), 8.84 (s, 1H).
32		t_R = 12.2 min (Method I); HRMS: calc. for $C_{32}H_{48}N_3O_3^+$ $[MH]^+$ 522.3690, found 522.3698; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.87–1.01 (m, 8H), 1.07–1.33 (m, 4H), 1.37–1.58 (m, 3H), 1.64–1.72 (m, 5H), 1.78–2.11 (m, 11H), 2.18–2.23 (m, 2H), 3.02–3.12 (m, 1H), 3.41–3.51 (m, 1H), 4.11–4.17 (m, 1H), 4.50–4.71 (m, 2H), 4.86–4.92 (m, 1H), 5.95 (t, 1H, *J* = 6.4 Hz), 6.78–6.87 (m, 2H), 7.20–7.35 (m, 6H).
33		t_R = 5.4 min (Method II); HRMS: calc. for $C_{30}H_{45}N_4O_3^+$ $[MH]^+$ 509.3486, found 509.3492; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.81–0.95 (m, 7H), 1.06–1.19 (m, 4H), 1.25–1.46 (m, 4H), 1.56–1.71 (m, 7H), 1.75–2.10 (m, 4H), 2.97–3.04 (m, 1H), 3.37–3.47 (m, 1H), 3.72–3.76 (m, 1H), 3.97–4.02 (m, 1H), 4.15–4.20 (m, 1H), 4.51–4.60 (m, 2H), 4.83–4.92 (m, 1H), 6.78 (d, 2H, *J* = 2 Hz), 7.13–7.34 (m, 6H), 7.55 (br s, 1 H), 8.28–8.46 (br d, 1H).
34		t_R = 8.1 min (Method IV). HRMS: calc. for $C_{32}H_{49}N_4O_3^+$ $[MH]^+$ 537.3799, found 537.3790; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1), δ 0.83–0.98 (m, 8H), 1.06–2.15 (m, 20H), 2.41–2.57 (m, 2H, $COCH_2$), 2.99–3.10 (m, 3H, $\underline{CH_2}NH_3^+$ and 1 × H of piperidine-CH_2), 3.41–3.51 (m, 1H, piperidine-CH_2), 4.16–4.23 (m, 1H, piperidine-CH_2), 4.47–4.61 (m, 2H, α-CH and piperidine-CH_2), 4.84–4.91 (m, 1H, α-CH), 6.82 (s, 2H, indene 2 × CH), 7.18–7.34 (m, 4H), 7.41–7.53 (m, 2H, exchangeable with D_2O), 8.06 (br s, 3H, exchangeable with D_2O, NH_3^+); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.9/11.1 (Ile-δ-CH_3), 15.5/15.8 (Ile-γ-CH_3), 22.8, 24.1, 25.9, 26.1, 26.3, 32.2(8)/32.3(2), 33.2/33.4, 33.4(8)/33.5(3), 34.0(5)/34.1(1)/34.1(6), 37.5/37.6, 39.3, 39.6(6)/39.7(4), 41.3 (*s*-syn amide piperidine NCH_2), 45.0/45.4 (*s*-anti amide piperidine NCH_2), 51.7/51.9 (spiro-C), 52.3/52.5 (α-CH), 52.8(6)/52.9(0) (α-CH), 121.4/121.6 (CH), 121.7/121.8 (CH), 125.5/125.7 (CH), 127.3/127.4 (CH), 130.9(8)/131.0(2) (indene-CH), 139.3/139.5 (indene-CH), 142.5/142.7 (C), 150.6(6)/150.7(3) (C), 170.7 (C), 172.7/172.9 (C), 173.2/173.3 (C).
35		t_R = 5.2 min (Method II); HRMS: calc. for $C_{32}H_{49}N_4O_4^+$ $[MH]^+$ 553.3748, found 553.3748; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.83–0.99 (m, 7H), 1.05–1.29 (m, 4H), 1.31–1.47 (m, 3H), 1.48–1.58 (m, 2H), 1.61–1.76 (m, 5H), 1.77–1.91 (m, 3H), 1.93–2.04 (m, 2H), 2.35–2.46 (m, 2H), 2.81–2.90 (m, 1H), 2.98–3.11 (m, 1H), 3.20–3.34 (m, 2H), 3.39–3.58 (m, 1H), 4.28–4.41 (m, 1H), 4.44–4.57 (m, 2H), 4.77–4.88 (m, 1H), 6.81 (br s, 2H), 7.18–7.43 (m, 6H), 7.89–7.94 (m, 1H), 8.07 (br s, 2H).
36		t_R = 9.7 min (Method I); HRMS: calc. for $C_{31}H_{47}N_4O_4^+$ $[MH]^+$ 539.3592, found 539.3589; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.81–0.96 (m, 8H), 1.06–2.15 (m, 18H), 2.93–3.07 (m, 1H), 3.36–3.54 (m, 1H), 3.92–4.17 (m, 3H), 4.28–4.55 (m, 3H), 4.80–4.89 (m, 1H), 6.71–6.86 (m, 2H), 7.12–7.29 (m, 6H), 7.34–7.47 (m, 2H).
37		t_R = 8.7 min (5 to 100%B in 15 min); HRMS: calc. for $C_{31}H_{48}N_5O_3^+$ $[MH]^+$ 538.3752, found 538.3752; ^{1}H NMR (400 MHz, $CDCl_3$) (two

		rotamers in a ratio of 1:1), δ 0.80–0.96 (m, 8H), 1.02–1.22 (m, 4H), 1.26–2.08 (m, 14H), 2.95–3.08 (m, 1H, 1 × H of piperidine-CH_2), 3.41 (br m, 1H, 1 × of piperidine-CH_2), 3.55 (br m, 1H, 1 × H of Dap-β-CH_2), 3.75 (br m, 1H, 1 × H of Dap-β-CH_2), 4.04 (br s, 1H, 1 × H of piperidine-CH_2), 4.61–4.36 (m, 3H, 2 × α-CH and 1 × H of piperidine-CH_2), 4.80–4.88 (m, 1H, α-CH), 6.75–6.83 (m, 2H), 7.12–7.30 (m, 4H), 7.31–9.60 (6H, incl. 1 × very br s at δ 8.50 and 3 × br s at δ 7.36, 8.89 and 8.96).
38		t_R = 9.6 min (5 to 100%B in 15 min); HRMS: calc. for $C_{32}H_{48}N_5O_4^+$ $[MH]^+$ 566.3701, found 566.3701; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.81–0.98 (m, 8H), 1.02–1.23 (m, 4H), 1.53–1.24 (m, 4H), 1.54–2.12 (m, 10H), 2.88–3.08 (m, 3H, 1 × H of piperidine-CH_2 and Asn-β-CH_2), 3.38–3.48 (m, 1H), 4.13–4.19 (m, 1H), 4.46–4.62 (m, 3H, 2 × α-CH and 1 × H of piperidine-CH_2), 4.82–4.88 (m, 1H, α-CH), 6.75–6.82 (m, 2H), 7.00–7.32 (m, 4H), 7.52–8.72 (m, 6H, incl. 1 × very br s at δ 8.32, 1 × br s at δ 7.58, and 3 × doublets at δ 7.65, J = 7.6 Hz, δ 8.59, J = 6.4 Hz and δ 8.70, J = 6.4 Hz).
39		t_R = 5.6 min (Method II); HRMS: calc. for $C_{33}H_{49}N_4O_3^+$ $[MH]^+$ 549.3799, found 549.3792; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.83–0.98 (m, 9H), 1.07–1.23 (m, 4H), 1.37–1.50 (m, 3H), 1.60–1.75 (m, 7H), 1.88–2.14 (m, 5H), 2.40–2.53 (m, 2H), 3.00–3.09 (m, 1H), 3.40–3.50 (m, 3H), 4.11–4.21 (m, 1H), 4.52–4.69 (m, 2H), 4.71–4.80 (m, 1H), 4.87–4.99 (m, 1H), 6.80–6.85 (m, 2H), 7.17–7.29 (m, 4H), 7.29–7.36 (m, 1H), 7.37–7.47 (m, 1H), 7.90–8.0 (m, 1H).
40		t_R = 8.3 min (Method II); HRMS: calc. for $C_{33}H_{47}N_4O_4^+$ $[MH]^+$ 563.3592, found 563.3598; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.87–1.03 (m, 7H), 1.09–1.31 (m, 5H), 1.38–1.57 (m, 4H), 1.58–1.84 (m, 7H), 1.90–2.04 (m, 2H), 2.06–2.30 (m, 2H), 2.39–2.59 (m, 3H), 3.04–3.14 (m, 1H), 3.44–3.55 (m, 1H), 4.15–4.28 (m, 2H), 4.53–4.68 (m, 2H), 4.90–4.95 (m, 1H), 6.81–6.86 (m, 2H), 6.99–7.01 (m, 1H), 7.20–7.39 (m, 6H).
41		Rt = 9.8 min (Method I); HRMS: calc. for $C_{34}H_{51}N_4O_3^+$ $[MH]^+$ 563.3956, found 563.3956; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.85–0.99 (m, 7H), 1.15–1.27 (m, 7H), 1.33–1.55 (m, 4H), 1.88–2.17 (m, 12H), 2.50–2.63 (m, 1H), 2.98–3.04 (m, 3H), 3.40–3.57 (m, 3H), 4.13–4.19 (m, 1H), 4.53–4.65 (m, 2H), 4.89 (q, 1H, J = 8.4 Hz), 6.73–6.77 (m, 1H), 6.80–6.85 (m, 2H), 7.17–7.35 (m, 6H).
42		V= 8.0 min (Method IV); HRMS: calc. for $C_{32}H_{47}N_6O_3^+$ $[MH]^+$ 563.3704, found 563.3704; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 64:36) δ 0.84–0.99 (m, 8H), 1.06–2.12 (m, 18H), 3.00–3.07 (m, 1H), 3.39–3.50 (m, 1H), 3.84 (br s, 1H), 3.92–3.99 (m, 1H), 4.11–4.20 (m, 1H), 4.38–4.48 (m, 1H), 4.52–4.59 (m, 2H), 4.79–4.87 (m, 1H), 6.79–6.83 (m, 2H), 7.16–7.34 (m, 4H), 7.40 (br m, 1H), 7.83 (br s, 2H), 8.00 (d, 0.36H, J = 7.2 Hz), 8.09 (d, 0.36H, J = 6.0 Hz), 8.35 (br s, 0.64H), 8.69 (br s, 0.64H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.2/11.5 (Ile-δ-CH_3), 15.8/16.1 (Ile-γ-CH_3), 24.2(8)/24.3(3), 26.2, 26.4, 32.6(7)/32.6(9), 33.3(8)/33.4(4), 33.4(8)/33.5(2), 34.3/34.4, 37.7, 39.4, 41.4/41.5, 45.1/45.5, 47.2/47.3, 51.9/52.1, 53.0/53.2, 53.4/53.5,

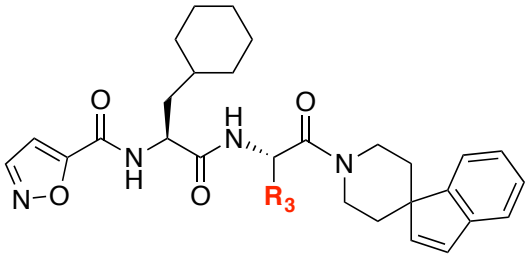
		57.2(6)/57.2(9), 121.5/121.8, 121.9, 125.7/125.8, 127.4/127.5, 131.1/131.2, 139.6/139.7, 142.7/142.9, 150.9/151.0, 160.5, 170.6/170.7, 172.7/172.9.

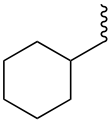
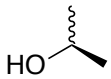
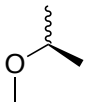


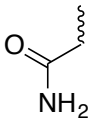
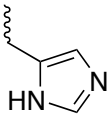
ID	R_2	Analytical data
43		t_R 9.2 min (Method II); HRMS: calc. for $C_{29}H_{39}N_4O_4^+$ $[MH]^+$ 507.2966, found 507.2965; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 57:43) δ 0.86–0.95 (m, 5H), 1.00 (d, 1H, *J* = 6.8 Hz), 1.07 (s, 4H), 1.13 (s, 5H), 1.39–1.60 (m, 2H), 1.79–1.88 (m, 1H), 1.90–2.04 (m, 2H), 2.10–2.20 (m, 2H), 3.02–3.17 (m, 1H), 3.43–3.57 (m, 1H), 4.12–4.27 (m, 1H), 4.50–4.75 (m, 2H), 4.92–4.98 (m, 1H), 6.83–6.88 (m, 2H), 6.97–6.98 (m, 1H), 7.20–7.35 (m, 6H), 8.34 (d, 1H, *J* = 2.0 Hz).
44		t_R 10.0 min (0% to100% B in 10 min, then 100%B for extra 5min); HRMS: calc. for $C_{30}H_{40}N_5O_5^+$ $[MH]^+$ 550.3024, found 550.3022; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.85–1.54 (m, 10H), 1.80–2.22 (m, 3H), 3.06–3.13 (m, 1H), 3.37 (br s, 4H), 3.45–3.55 (m, 1H), 3.65–3.71 (m, 1H), 3.83–3.92 (m, 1H), 3.99 (s, 4H), 4.14 (d, 1H, *J* = 12Hz), 4.58–4.66 (m, 1H), 4.80–4.85 (m, 1H), 5.26–5.34 (m, 1H), 6.81–6.86 (m, 2H), 6.99 (d)/7.00 (d, *J* = 1.6 Hz, 1H), 7.19–7.37 (m, 4H), 7.96 (d, 1H, *J* = 6.8 Hz), 8.34 (d)/8.35 (d, *J* = 1.6 Hz, 1H), 8.77–8.82 (m, 1H); ^{13}C NMR (100 MHz,$CDCl_3$) δ 11.4/11.6 (Ile-δ-CH_3), 15.8/16.2 (Ile-γ-CH_3), 24.3 (Ile-γ-CH_2), 33.5/33.6, 34.2/34.3, 37.4, 41.8/42.0, 45.2/45.7, 48.6, 51.9/52.1, 53.3, 54.6/54.7, 58.3/58.4, 63.9, 107.5, 121.6/121.9, 122.0, 125.8/125.9, 127.6/127.7, 131.4, 139.5/139.6, 142.8/142.9, 150.8/150.9, 151.1, 157.0, 161.6, 167.8, 170.7.
45		t_R = 11.4 min (Method II); HRMS: calc. for $C_{33}H_{45}N_4O_4^+$ $[MH]^+$ 561.3435, found 561.3438; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.84–0.96 (m, 6H), 1.02 (d, 1H, *J* = 6.4 Hz), 1.09–1.32 (m, 7H), 1.40–1.57 (m, 3H), 1.60–1.76 (m, 5H), 1.78–1.87 (m, 2H), 1.90–2.05 (m, 3H), 2.10–2.18 (m, 1H), 3.04–3.16 (m, 1H), 3.44–3.57 (m, 1H), 4.15–4.23 (m, 1H), 4.58–4.73 (m, 2H), 4.92–4.98 (m, 1H), 6.83–6.87 (m, 2H), 6.94 (t, 1H, *J* = 2.0 Hz), 7.03 (dd, 1H, *J* = 8.8 Hz, 16.6 Hz), 7.20–7.36 (m, 5H), 8.34–8.35 (m, 1H).

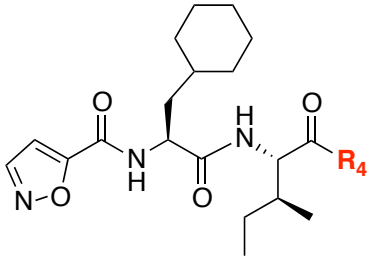


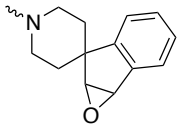
46: t_R 8.7 min (Method II); HRMS: calc. for $C_{30}H_{39}N_4O_4^+$ $[MH]^+$ 519.2966, found 519.2968; ^1H NMR ($CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.90–1.03 (m, 2H), 1.10–1.29 (m, 3H), 1.35–1.42 (m, 3H), 1.65–1.72 (m, 12H), 1.75–1.86 (m, 3H), 1.96–2.17 (m, 2H), 3.23 (br s, 1H), 4.48 (br s, 1H), 4.60–4.64 (m, 1H), 6.79–6.81 (m, 2H), 6.87 (d, 1H, *J* = 1.2 Hz), 7.08–7.12 (m, 1H), 7.16–7.18 (m, 1 H), 7.23–7.26 (m, 2H), 7.32 (d, 1H, *J* = 6.6 Hz), 7.44 (br s, 1H), 8.31 (d, 1H, *J* = 1.8 Hz).

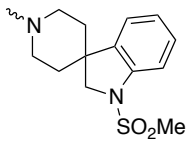
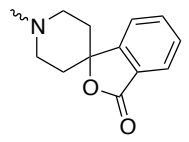
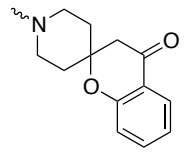
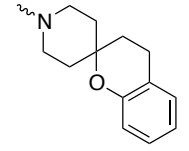


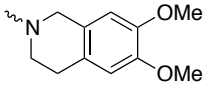
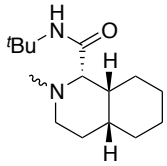
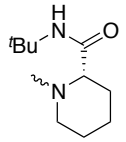
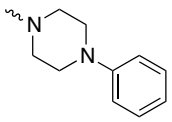
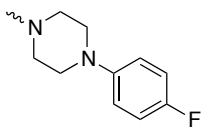
ID	R_3	Analytical data
47		t_R = 11.1 min (Method II); HRMS: calc. for $C_{32}H_{43}N_4O_4^+$ $[MH]^+$ 547.3279, found 547.3278; ^1H NMR (600MHz, $CDCl_3$, two rotamers in a ratio of 3:2) δ 0.92–1.08 (m, 12H), 1.11–1.36 (m, 4H), 1.36–1.46 (m, 2H), 1.46–1.52 (m, 1H), 1.63–1.77 (m, 6H), 1.77–1.87 (m, 2H), 1.91–2.09 (m, 2H), 2.99–3.04 (m, 0.6H)/3.09–3.14 (m, 0.4H), 3.41–3.46 (m, 0.6H)/3.49–3.54 (m, 0.4H), 4.20–4.25 (m, 1H), 4.61–4.75 (m, 2H), 4.96 (d, 0.4H, *J* = 9.6 Hz)/ 4.99 (d, 0.6H, *J* = 9.6 Hz), 6.71–6.88 (m, 3H), 6.94–6.95 (m, 1H), 7.05 (dd, 1H, *J* = 7.8, 17.4 Hz), 7.19–7.23 (m, 1H), 7.27–7.32 (m, 1H), 7.33–7.37 (m, 1H), 8.34 (d, 1H, *J* = 1.8 Hz).
48		t_R = 11.9 min (Method II); HRMS: calc. for $C_{35}H_{47}N_4O_4^+$ $[MH]^+$ 587.3592, found 587.3588; ^1H NMR (400MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.84–1.07 (m, 4H), 1.08–1.28 (m, 6H), 1.30–1.56 (m, 5H), 1.57–1.91 (m, 12H), 1.95–2.20 (m, 3H), 3.01–3.15 (m, 1H), 3.40–3.56 (m, 1H), 4.04–4.12 (m, 1H), 4.58–4.75 (m, 1H), 5.07–5.15 (m, 1H), 6.82–6.86 (m, 1H), 6.94–6.95 (m, 1H), 6.99–7.04 (m, 1H), 7.10–7.15 (m, 1H), 7.20–7.36 (m, 4H), 8.34 (dd, 1H, *J* = 0.8, 1.6 Hz).
49		t_R = 11.1 min (Method II); HRMS: calc. for $C_{30}H_{39}N_4O_5^+$ $[MH]^+$ 535.2915, found 535.2915; ^1H NMR (400MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.86–1.28 (m, 8H), 1.34–2.14 (m, 12H), 2.84–3.33 (m, 2H), 3.42–3.64 (m, 1H), 4.06–4.13 (m, 1H), 4.52–4.62 (m, 1H), 4.91—4.97 (m, 1H), 5.16–5.22 (m, 1H), 6.80–6.94 (m, 2H), 6.96 (d, 1H, *J* = 2.0 Hz), 7.11–7.67 (m, 6H), 8.35 (d, 1H, *J* = 2.0 Hz).
50		t_R = 9.2 min (Method II); HRMS: calc. for $C_{31}H_{41}N_4O_5^+$ $[MH]^+$ 549.3071, found 549.3072; ^1H NMR (400 MHz, $CDCl_3$, two rotamers in a ratio of 1:1) δ 0.88–1.32 (m, 8H), 1.36–1.50 (m, 3H), 1.63–2.16 (m, 9H), 3.07–3.17 (m, 1H, *s*-syn amide piperidine NCH_2), 3.35 (s)/3.40 (s, 3H, OCH_3), 3.44–3.53 (m, 1H, *s*-anti amide piperidine NCH_2), 3.66 (m, 1H, Thr-β-CH), 4.17 (br s, 1H, *s*-anti amide piperidine NCH_2), 4.65–4.72 (m, 1H, *s*-syn amide piperidine NCH_2), 4.72–4.79 (m, 1H, Cha-α-CH), 5.12 (dd, 1H, *J* = 8.0, 4.0 Hz, Thr-α-CH), 6.83 (d, 1H, *J* = 6.0 Hz, indene-CH), 6.85 (d, 1H, *J* = 6.0 Hz, indene-CH), 6.96 (d, 1H, *J* = 1.6 Hz, isoxazole-CH), 7.40–7.19 (m, 6H), 8.35 (d, 1H, *J* = 2.0 Hz, isoxazole-CH); ^{13}C NMR (100 MHz,$CDCl_3$) δ 15.1/15.2 (Thr-γ-CH_3), 25.9, 26.1(0)/26.1(4), 26.3, 32.3, 33.2/33.4, 33.7, 34.0, 40.1, 41.6/41.8

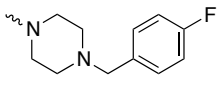
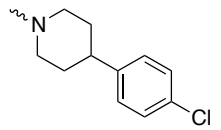
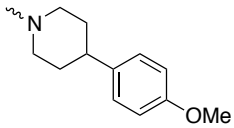
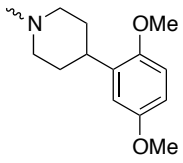
		(*s*-syn amide piperidine NCH_2), 45.2/45.6 (*s*-anti amide piperidine NCH_2), 51.4 (Cha-α-CH), 51.8/52.0 (spiro-C), 52.7/52.9 (Thr-α-CH), 56.9 (OCH_3), 106.9 (isoxazole-CH), 121.5/121.6 (CH), 121.7 (CH), 125.5/125.6 (CH), 127.3 (CH), 130.9(6)/131.0(0) (indene-CH), 139.5/139.6 (indene-CH), 142.6/142.7, 150.7(6)/150.8(4), 151.0(4) (isoxazole-CH), 155.7, 162.2, 168.1/168.2, 171.6/171.8.
51		t_R = 10.1 min (Method I); HRMS: calc. for $C_{30}H_{40}N_5O_4^+$ $[MH]^+$ 534.3075, found 534.3070; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.82–1.22 (m, 5H), 1.32–1.48 (m, 2H), 1.54–1.82 (m, 8H), 1.92–2.08 (m, 3H), 2.20–2.38 (m, 1H), 2.82–3.20 (m, 3H), 3.38–3.46 (m, 1H), 3.90–4.08 (m, 1H), 4.46–4.74 (m, 2H), 5.04–5.14 (m, 1H), 6.74–6.83 (m, 2H), 6.91 (br s, 1H), 7.14–7.33 (m, 4H), 8.0–8.17 (br m, 5H), 8.27 (s, 1H); ^{13}C NMR (100 MHz,$CDCl_3$) δ 25.9, 26.1, 26.2, 30.0/30.4, 32.1(5)/32.2(2), 33.1/33.2, 33.5/33.6/33.8, 34.1, 36.1, 39.1/39.2, 41.5/41.6 (*s*-syn amide piperidine NCH_2), 44.5/44.7 (*s*-anti amide piperidine NCH_2), 47.0/47.1 (Dab-α-CH), 51.7/51.8 (spiro-C), 51.9/52.0 (Cha-α-CH), 107.0 (isoxazole-CH), 121.65(1)/121.6(0) (CH), 121.6(3)/121.6(9) (CH), 125.5/125.6 (CH), 127.3 (CH), 130.9(6)/131.0(0) (indene-CH), 139.4 (indene-CH), 142.6/142.7, 150.7/150.8, 151.2 (isoxazole-CH), 156.3(7)/156.4(1), 161.9(6)/162.0(4), 168.6/168.8, 173.1/173.2.
52		t_R = 10.1 min (Method I); HRMS: calc. for $C_{30}H_{38}N_5O_5^+$ $[MH]^+$ 548.2867, found 548.2869; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.82–2.30 (m, 17H), 2.56–2.90 (m, 2H), 3.05–3.12 (m, 1H), 3.43–3.54 (m, 1H), 4.06–4.12 (m, 1H), 4.48–4.64 (m, 2H), 5.26–5.34 (m, 1H), 6.06 (br s) and 6.17 (br s, 1H), 6.74–6.86(m, 3H), 6.93 (d, 1H, *J* = 1.2 Hz), 7.14–7.63 (m, 6H), 8.35 (d, 1H, *J* = 1.2 Hz).
53		t_R = 9.1 min (Method I); HRMS: calc. for $C_{32}H_{39}N_6O_4^+$ $[MH]^+$ 571.3027, found 571.3026; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 54:46) δ 0.80–1.20 (m, 5H), 1.30–1.80 (m, 10H), 1.95–2.12 (m, 2H), 2.92–3.30 (m, 3H, H_{ax} of *s*-syn amide piperidine NCH_2 and His-β-CH_2), 3.44–3.58 (m, 1H, H_{ax} of *s*-anti amide piperidine NCH_2), 3.96–4.12 (m, 1H, H_{eq} of *s*-anti amide piperidine NCH_2), 4.48–4.71 (m, 2H, H_{eq} of *s*-syn amide piperidine NCH_2 and Cha-α-CH), 5.24–5.33 (m, 1H, His-α-CH), 6.77–7.00 (m, 3H), 7.14–7.35 (m, 6H), 7.85 (d, 1H, *J* = 7.6 Hz, Cha-NH), 8.02 (d, *J* = 8.0 Hz, major) and 8.04 (d, *J* = 8.4 Hz, minor, 1H, His-NH), 8.32 (d, 1H, *J* = 1.6 Hz, His-CH), 8.51 (br s, 1H, isoxazole-CH).

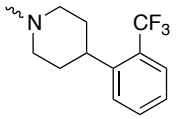
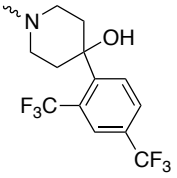
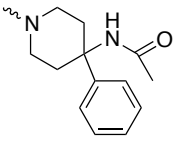


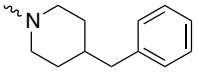
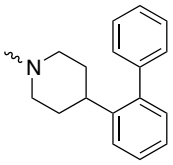
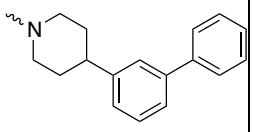
ID	R_4	Analytical data
54		t_R = 7.2 and 7.8 min (Method II, two diastereomers in a ratio of 2:1); HRMS: calc. for $C_{32}H_{43}N_4O_5^+$ $[MH]^+$ 563.3228, found 563.3228; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 0.83-1.01 (m, 8H), 1.04–2.10 (m, 18H), 3.03–3.12 (m, 1H, H_{ax} of *s*-syn amide piperidine NCH_2), 3.48–3.60 (m, 1H, H_{ax} of *s*-anti amide piperidine NCH_2), 4.08–4.22 (m, 2H,

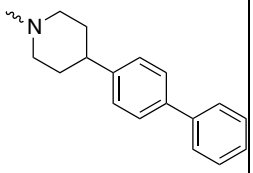
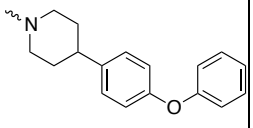
		incl. 1 × epoxide-CH and H_{eq} of *s*-anti amide piperidine NCH_2), 4.30 (d, 1H, *J* = 2.4 Hz, 1 × epoxide-CH), 4.60–4.78 (m, 1H, H_{eq} of *s*-syn amide piperidine NCH_2), 4.78–4.86 (m, 1H), 4.92–5.03 (m, 1H), 6.96 (m, 1H, isoxazole-CH), 7.05–7.53 (m, 6H, 4 × PhCH and 2 × NH), 8.32 (m, 1H, isoxazole-CH).
55		t_R = 9.9 min (Method II); HRMS: calc. for $C_{32}H_{45}N_5NaO_6S^+$ $[M+Na]^+$ 650.2983, found 650.2985; ^1H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 2:1) δ 0.85–1.03 (m, 8H), 1.09–2.10 (m, 18H), 2.80–2.93 (m, 1H, H_{ax} of *s*-syn amide piperidine NCH_2), 2.94(s)/2.93(s, 3H, SO_2Me), 3.24–3.41 (m, 1H, H_{ax} of *s*-anti amide piperidine NCH_2), 3.86–3.93 (m, 2H, indole-NCH_2), 4.14–4.24 (m, 1H, H_{eq} of *s*-anti amide piperidine NCH_2), 4.56–4.74 (m, 2H, Cha-α-CH and H_{eq} of *s*-syn amide piperidine NCH_2), 4.87–4.91 (m, 1H, Ile-α-CH), 6.95 (d, *J* = 2.0 Hz, major) and 6.96 (d, *J* = 2.0 Hz, minor, 1H, isoxazole-CH), 7.05–7.31 (m, 4H), 7.37–7.42 (m, 1H, NH), 7.45 (d, *J* =8.8 Hz, minor) and 7.55 (d, *J* = 8.8 Hz, major, 1H, NH), 8.35 (d, 1H, *J* = 2.0 Hz, isoxazole-CH).
56		t_R = 9.6 min (Method II); HRMS: calc. for $C_{31}H_{40}N_4NaO_6^+$ $[M+Na]^+$ 587.2840, found 587.2843; ^1H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 72:28) δ 0.88–1.90 (m, 24H), 2.00–2.16 (m, 1H, 1 × H of *s*-syn amide piperidine $NCH_2\underline{CH_2}$), 2.29–2.37 (m, 1H, 1 × H of *s*-anti amide piperidine $NCH_2\underline{CH_2}$), 3.14–3.24 (m, 1H, H_{ax} of *s*-syn amide piperidine NCH_2), 3.63–3.71 (m, 1H, H_{ax} of *s*-anti amide piperidine NCH_2), 4.17–4.21 (m, 1 H, H_{eq} of *s*-anti amide piperidine NCH_2), 4.66–4.76 (m, 2H, Cha-α-CH and H_{eq} of *s*-syn amide piperidine NCH_2), 4.89–4.94 (m, 1H, Ile-α-CH), 6.95 (d, 1H, *J* = 2.0Hz), 6.88 (d, 0.28H, *J* = 8.8Hz) and 7.03 (d, 0.72H, *J* = 8.8 Hz, Ile-NH), 7.16–7.21 (m, 1H, Cha-NH), 7.35 (d, 0.28H, *J* = 8.0Hz) and 7.45 (d, 0.72H, *J* = 8.0 Hz), 7.54–7.60 (m, 1H), 7.68–7.73 (m, 1H), 7.90–7.95 (m, 1H), 8.35 (d, 0.28H, *J* = 2.0 Hz) and 8.36 (d, 1H, 0.72H, *J* = 2.0 Hz, isoxazole-CH).
57		t_R = 10.4 min (Method II); HRMS: calc. for $C_{32}H_{43}N_4O_6^+$ $[MH]^+$ 579.3177, found 579.3178; ^1H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 55:45) δ 0.85–1.08 (m, 8H), 1.03–1.40 (m, 5H), 1.43–1.84 (m, 11H), 2.11–2.24 (m, 2H), 2.71-2.82 (m, 2H, benzopyranone-CH_2), 3.10–3.26 (m, 1H, H_{ax} of *s*-syn amide piperidine NCH_2), 3.56–3.69 (m, 1H, H_{ax} of *s*-anti amide piperidine NCH_2), 3.93–4.06 (m, 1H, H_{eq} of *s*-anti amide piperidine NCH_2), 4.37–4.47 (m, 1H, H_{eq} of *s*-syn amide piperidine NCH_2), 4.62–4.72 (m, 1H, Cha-α-CH), 4.85 (app t, 1H, *J* = 8.6 Hz, Ile-α-CH), 6.94 (d, *J* = 2.0 Hz) and 6.96 (d, *J* = 2.0 Hz, 1H, isoxazole-CH), 6.99–7.08 (m, 2H), 7.18 (d, *J* = 7.6 Hz, major) and 7.23 (d, *J* = 8.0 Hz, minor, 1H, Cha-NH), 7.36 (d, *J* = 9.2 Hz, minor) and 7.44 (d, *J* = 8.8 Hz, major, 1H, Ile-NH), 7.50–7.56 (m, 1H), 7.87–7.91 (m, 1H), 8.35 (d, 1H, *J* = 2.0 Hz, isoxazole-CH).
58		t_R = 10.6 min (Method II); HRMS: calc. for $C_{32}H_{45}N_4O_5^+$ $[MH]^+$ 565.3384, found 565.3385; ^1H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.85–1.00 (m, 8H), 1.11–1.24 (m, 6H), 1.49–2.22 (m, 14H), 2.77–2.82 (m, 2H), 3.15–3.28 (m, 1H, H_{ax} of *s*-

		syn amide piperidine NCH_2), 3.57–3.69 (m, 1H, H_{ax} of *s*-anti amide piperidine NCH_2), 3.92–4.00 (m, 1H, H_{eq} of *s*-anti amide piperidine NCH_2), 4.40–4.47 (m, 1H, H_{eq} of *s*-syn amide piperidine NCH_2), 4.68–4.76 (m, 1H, Cha-α-CH), 4.90 (t, 1H, *J* = 8.4 Hz, Ile-α-CH), 6.82–7.14 (m, 5H), 7.31–7.58 (m, 2H, 2 × NH), 8.34 (d, 1H, *J* = 1.2 Hz, isoxazole-CH).
59		t_R = 9.1 min (Method II); HRMS: calc. for $C_{30}H_{43}N_4O_6^+$ $[MH]^+$ 555.3177, found 555.3179; ^{1}H NMR (400 MHz, DMSO-d_6) (two rotamers in a ratio of 58:42) δ 0.89–1.98 (m, 22H), 2.75–2.95 (m, 2H), 3.64–3.97 (m, 8H, including MeO singlets at δ 3.82 and 3.84), 4.54–4.87 (m, 4H), 6.83 (s)/6.87 (s)/6.89 (s, 2H), 7.26 (d, *J* = 2.0 Hz, minor) and 7.27 (d, *J* = 2.0 Hz, major, 1H, isoxazole-CH), 8.29 (d, *J* = 8.8 Hz, minor) and 8.32 (d, *J* = 8.8 Hz, major, 1H, NH), 8.86 (d, 1H, *J* = 2.0 Hz, isoxazole-CH), 9.00 (d, *J* = 8.0 Hz, minor) and 9.01 (d, *J* = 8.0 Hz, major, 1H, NH).
60		t_R = 13.6 min (Method II); HRMS: calc. for $C_{33}H_{54}N_5O_5^+$ $[MH]^+$ 600.4119, found 600.4118; ^{1}H NMR (400 MHz, DMSO-d_6) (two rotamers in a ratio of 1:1) δ 0.76–0.91 (m, 8H), 0.97–1.36 (m, 18H), 1.40–1.70 (m, 15H), 1.77–1.84 (m, 1H), 1.91–1.95 (m, 1H), 3.50 (t, 1H, *J* = 12.4 Hz), 3.64 (dd, 1H, *J* = 4.4, 13.2 Hz), 4.41–4.54 (m, 2H), 4.65 (t, 1H, *J* = 8.8 Hz), 4.78–4.81 (m, 1H), 7.12 (s, 1H), 7.13 (d, 1H, J = 1.6 Hz), 8.11 (d, 1H, *J* = 8.8 Hz), 8.72 (d, 1H, *J* = 1.6 Hz), 8.91 (d, 1H, *J* = 8.4 Hz).
61		t_R = 9.3 min (Method II); HRMS: calc. for $C_{29}H_{48}N_5O_5^+$ $[MH]^+$ 546.3650, found 546.3653; ^{1}H NMR (400 MHz, DMSO-d_6) (two rotamers in a ratio of 2:1) δ 0.76–0.92 (m, 10H), 0.97–1.23 (m, 6H), 1.16–1.26 (m, 11H), 1.27–1.44 (m, 5H), 1.47–1.67 (m, 12H), 1.76–1.81 (m, 1H), 1.95–1.98 (m, 1H), 3.92 (br d, 1H, *J* = 13.2 Hz), 4.49–4.55 (m, 2H), 4.64 (t, 1H, *J* = 8.4 Hz), 4.92–4.94 (m, 1H), 7.04 (s, 1H), 7.13 (d, 1H, *J* = 2.0 Hz), 8.06 (d, 1H, *J* = 2.0 Hz), 8.72 (d, 1H, *J* = 2.0 Hz), 8.91 (d, 1H, *J* = 8.4 Hz).
62		t_R = 28.6 min (0% to 100% B linear gradient over 30 min followed by a further 10 min at 100% B); HRMS: calc. for $C_{29}H_{42}N_5O_4^+$ $[MH]^+$524.3231, found 524.3243; ^{1}H NMR (400 MHz, DMSO-d_6) (two rotamers in a ratio of 1:1) 0.78-0.72 (m, 6H); 0.84–0.89 (m, 2H), 1.02–1.11 (m, 4H), 1.22–1.30 (m, 1H), 1.45–1.64 (m, 8H), 1.77–1.83 (m, 1H), 2.94–3.13 (m, 5H), 3.49–3.77 (m, 8H), 4.49–4.55 (m, 1H), 4.59 (t, 1H, *J* = 8.6 Hz), 6.79 (t, 1H, *J* = 7.3 Hz), 6.92 (d, 2H, *J* = 8.8 Hz), 7.10 (d, 1H, *J* = 4.0 Hz), 7.20 (dd, 1H, *J* = 7.3, 8.7 Hz), 8.19 (d, 1H, *J* = 8.7 Hz), 8.88 (d, 1H, *J* = 8.3 Hz).
63		t_R = 8.2 min (Method II); HRMS: calc. for $C_{29}H_{41}FN_5O_4^+$ $[MH]^+$ 542.3137, found 542.3137; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.85–1.02 (m, 8H), 1.07–1.29 (m, 4H), 1.30–1.40 (m, 1H), 1.46–1.55 (m, 1H), 1.62–1.85 (m, 10H), 3.16–3.36 (m, 4H), 3.68–3.73 (m, 1H), 3.80–3.87 (m, 1H), 4.01–4.07 (m, 1H), 4.11–4.17 (m, 1H), 4.59–4.65 (m, 1H, Cha-α-CH), 4.80 (d, *J* = 8.0 Hz) and 4.82 (d, *J* = 8.0 Hz, 1H, Ile-α-CH), 6.91 (d, 1H, *J* = 2.0 Hz), 6.93–7.17 (m, 6H), 8.34 (d, 1H, *J* = 2.0 Hz).

64		t_R = 5.7 min (Method II); HRMS: calc. for $C_{30}H_{43}FN_5O_4^+$ $[MH]^+$ 556.3294, found 556.3291; ^{1}H NMR (400 MHz, DMSO-d_6) (two rotamers in a ratio of 1:1) δ 0.90–1.09 (m, 8H) 1.13–1.49 (m, 6H), 1.52–1.94 (m, 10H), 2.82–3.18 (m, 4H), 3.58 (CH_2Ar, overlapped with water peak), 4.38–4.58 (m, 4H), 4.59–4.64 (m, 1H, Cha-α-CH), 4.65–4.70 (app t, 1H, *J* = 8.4 Hz, Ile-α-CH), 7.27 (s, 1H, isoxazole-CH), 7.43–7.45 (m, 2H), 7.65–7.67 (m, 2H), 8.28 (d, 1H, *J* = 8.0 Hz), 8.88 (s, 1H, isoxazole-CH), 9.05 (br s, 1H).
66		t_R = 12.7 min (Method II); HRMS: calc. for $C_{30}H_{42}ClN_4O_4^+$ $[MH]^+$ 577.2889, found 577.2894; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.84–1.03 (m, 8H), 1.07–1.38 (m, 6H), 1.44–2.00 (m, 12H), 2.66–2.79 (m, 2H, piperidine-CHAr and H_{ax} of *s*-syn amide piperidine NCH_2), 3.15–3.24 (m, 1H, H_{ax} of *s*-anti amide piperidine NCH_2), 4.12–4.19 (m, 1H, H_{eq} of *s*-anti amide piperidine NCH_2), 4.65–4.71 (m, 1H, α-CH), 4.74–4.79 (m, 1H, H_{eq} of *s*-syn amide piperidine NCH_2), 4.86–4.91 (m, 1H, α-CH), 6.74–6.79 (m, 1H), 6.93 (d, 1H, *J* = 1.6 Hz, isoxazole-CH), 7.04–7.08 (m, 1H), 7.10–7.14 (m, 2H), 7.27–7.30 (m, 2H), 8.33 (d, 1H, *J* = 2.0 Hz, isoxazole-CH).
67		t_R = 11.7 min (Method II); HRMS: calc. for $C_{31}H_{45}N_4O_5^+$ $[MH]^+$ 553.3384, found 553.3384; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.84–1.03 (m, 8H), 1.06–1.36 (m, 5H), 1.43–2.00 (m, 13H), 2.71–2.78 (m, 2H, piperidine-CHAr and H_{ax} of *s*-syn amide piperidine NCH_2), 3.17–3.22 (m, 1H, H_{ax} of *s*-anti amide piperidine NCH_2), 3.79 (s, 3H, OMe), 4.15–4.17 (m, 1H, H_{eq} of *s*-anti amide piperidine NCH_2), 4.65–4.70 (m, 1H, α-CH), 4.73–4.77 (m, 1H, H_{eq} of *s*-syn amide piperidine NCH_2), 4.87–4.93 (m, 1H, α-CH), 6.84–6.94 (m, 4H), 7.08–7.12 (m, 3H), 8.33 (d, 1H, *J* = 2.0 Hz, isoxazole-CH); ^{13}C NMR (100 MHz, $CDCl_3$): 11.3/11.5 (Ile-δ-CH_3), 15.7/16.1 (Ile-γ-CH_3), 24.0/24.1 (Ile-γ-CH_2), 26.0, 26.1, 26.3 32.6/32.7, 33.1/33.3, 33.6, 34.0, 38.0(6)/38.1(1), 40.2, 41.5/41.8, 43.1 (br s), 46.6/47.1, 51.4 (br s), 53.0/53.1/53.2, 55.2/55.3, 106.8 (isoxazole-4-CH), 113.9(6)/140.0(1), 127.5/127.6, 136.8/136.9, 151.0, 155.4, 158.2/158.3, 162.4, 169.9/170.0, 171.1(5)/171.2(1).
68		t_R = 11.4 min (Method II); HRMS: calc. for $C_{32}H_{47}N_4O_6^+$ $[MH]^+$ 583.3490, found 583.3492; ^{1}H NMR (600 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1) δ 0.84–0.93 (m, 6H), 0.93–1.01 (m, 3H), 1.05–1.27 (m, 6H), 1.30–1.38 (m, 1H), 1.45–1.53 (m, 2H), 1.53–1.84 (m, 13H), 1.86–2.00 (m, 4H), 2.72–2.76 (m, 1H, H_{ax} of *s*-syn amide piperidine NCH_2), 3.14–3.25 (m, 2H, piperidine-CHAr and H_{ax} of *s*-anti amide piperidine NCH_2), 3.76 (s) and 3.75 (s, 3H, OMe), 3.79 (s, 3H, OMe), 4.09–4.16 (m, 1H, H_{eq} of *s*-anti amide piperidine NCH_2), 4.65–4.70 (m, 1H, α-CH), 4.74–4.76 (m, 1H, H_{eq} of *s*-syn amide piperidine NCH_2), 4.87–4.90 (m, 1H, α-CH), 6.71–6.73 (m, 2H), 6.78–6.80 (m, 1H), 6.87 (d, *J* = 9.0 Hz) and 6.89 (d, *J* = 9.0 Hz, 1H, NH), 6.93 (d, 1H, *J* = 1.8 Hz, isoxazole-CH), 7.08–7.11 (m, 1H, NH), 8.34 (s, 1H, isoxazole-CH); ^{13}C NMR (100 MHz, $CDCl_3$): 11.3/11.5 (Ile-δ-CH_3), 15.8/16.1 (Ile-γ-CH_3),

		23.9(9)/24.0(4) (Ile-γ-CH_2), 26.0, 26.1, 26.3 31.4(5)/31.5(3), 32.2, 32.5/32.6, 33.6, 34.0/34.1, 35.5/35.9, 38.1, 40.2, 43.3/43.4, 46.7/47.1, 51.3(5)/51.4(2), 53.0/53.1, 55.7, 55.8/55.9, 106.7, 110.6/111.0, 111.3, 113.4/113.8, 127.3, 134.1, 151.0, 153.7, 155.4, 162.4, 169.8/169.9, 171.1/171.2.
69		t_R = 12.8 min (Method II); HRMS: calc. for $C_{31}H_{42}F_3N_4O_4^+$ $[MH]^+$ 591.3153, found 591.3156; ^{1}H NMR (600 MHz, $CDCl_3$) (two rotamers in a ratio of 59:41) δ 0.86–1.07 (m, 8H), 1.08–1.59 (m, 7H), 1.61–2.01 (m, 9H), 2.70–2.78 (m, 1H, H_{ax} of *s*-syn amide piperidine NCH_2), 3.19–3.28 (m, 2H, piperidine-CHAr and H_{ax} of *s*-anti amide piperidine NCH_2), 4.16–4.23 (m, 1H, H_{eq} of *s*-anti amide piperidine NCH_2), 4.66–4.74 (m, 1H, Cha-α-CH), 4.81 (d, *J* = 13.0 Hz, 1H, H_{eq} of *s*-syn amide piperidine NCH_2), 4.89–4.94 (m, 1H, Ile-α-CH), 6.83 (d, *J* = 8.4 Hz, minor) and 6.92 (d, *J* = 9.0 Hz, major, 1H), 6.95 (d, 1H, *J* = 1.8 Hz, isoxazole-CH), 7.08–7.13 (m, 1H), 7.31–7.42 (m, 2H), 7.51–7.56 (m, 1H), 7.64–7.67 (m, 1H), 8.36 (d, 1H, *J* = 1.8 Hz, isoxazole-CH).
70		t_R = 12.0 min (Method II); HRMS: calc. for $C_{32}H_{41}F_6N_4O_5^+$ $[MH]^+$ 675.2976, found 675.2976; ^{1}H NMR (600 MHz, $CDCl_3$) (two rotamers in a ratio of 2:1), δ 0.86–1.03 (m, 8H), 1.08–2.30 (m, 19H), 3.17–3.22 (m, 1H, H_{ax} of *s*-syn amide piperidine NCH_2), 3.66–3.71 (m, 1H, H_{ax} of *s*-anti amide piperidine NCH_2), 3.98 (d, *J* = 13.2 Hz, minor) and 4.04 (d, *J* = 13.2 Hz, major, 1H, H_{eq} of *s*-anti amide piperidine NCH_2), 4.61–4.68 (m, 2H, Cha-α-CH and H_{eq} of *s*-syn amide piperidine NCH_2), 4.87–4.91 (m, 1H, Ile-α-CH), 6.78 (d, *J* = 9.0 Hz, minor) and 6.88 (d, *J* = 8.4 Hz, major, 1H, NH), 6.92 (d, *J* = 1.8 Hz, major) and 6.94 (d, *J* = 1.8 Hz, minor, 1H, isoxazole-CH), 7.03 (d, J = 7.8 Hz, major) and 7.07 (d, *J* = 8.4 Hz, minor, 1H, NH), 7.71 (d, 1H, *J* = 9.0 Hz), 7.78–7.82 (m, 1H), 8.05 (br s, 1H), 8.35 (d, 1H, *J* = 1.8 Hz, isoxazole-CH).
71		t_R = 8.3 min (Method II); HRMS: calc. for $C_{32}H_{46}N_5O_5^+$ $[MH]^+$ 580.3493, found 580.3493; ^{1}H NMR (600 MHz, DMSO-d_6) (two rotamers in a ratio of 62:38), δ 0.79–0.93 (m, 8H), 1.01–1.33 (m, 5H), 1.46–1.85 (m, 11H), 1.88 (s, minor) and 1.91 (s, major, 3H, Ac), 2.38–2.45 (m, 2H), 2.83–2.90 (m, 1H, H_{ax} of *s*-syn amide piperidine NCH_2), 3.24–3.32 (m, 1H, H_{ax} of *s*-anti amide piperidine NCH_2), 3.97–4.03 (m, 1H, H_{eq} of *s*-anti amide piperidine NCH_2), 4.27 (d, *J* = 13.2 Hz, minor) and 4.34 (d, *J* = 12.6 H, major, 1H, H_{eq} of *s*-syn amide piperidine NCH_2), 4.52–4.58 (m, 2H, 2 × α-CH), 7.13 (d, *J* = 1.8 Hz, major), and 7.16 (d, *J* = 1.8 Hz, minor, 1H, isoxazole-CH), 7.18–7.22 (m, 1H), 7.26–7.34 (m, 4H), 8.06 (s, major) and 8.07 (s, minor, 1H, AcNH), 8.16 (d, J = 9.0 Hz, minor) and 8.28 (d, *J* = 9.0 Hz, major, 1H, NH), 8.73 (d, *J* = 1.8 Hz, major) and 8.75 (d, *J* = 1.8 Hz, minor, 1H, isoxazole-CH), 8.91 (d, *J* = 8.4 Hz, minor) and 8.92 (d, major, 1H, *J* = 8.4 Hz, NH); ^{13}C NMR (150 MHz, $CDCl_3$): 11.5/11.6 (Ile-δ-CH_3), 16.0/16.1 (Ile-γ-CH_3), 23.9(6)/24.0(3) (Ac), 24.3(9)/24.4(3) (Ile-γ-CH_2), 26.0, 26.1(6)/26.2(1)/26.2(8), 26.5, 32.2/32.3, 33.6/33.7, 34.1/34.2, 35.4, 36.1/36.3, 36.5/36.7, 38.2(9)/38.3(4), 39.3(3)/39.4(1), 42.0/42.2,

		51.3/51.4 (α-CH), 52.6/52.7 (α-CH), 56.5/56.7 (C), 106.6/106.7 (isoxazole-4-CH), 125.3/125.5 (CH), 126.7/126.8 (CH), 128.5/129.6 (CH), 147.1/147.2 (C), 152.1(8)/152.2(3) (isoxazole-3-CH), 155.8 (C), 162.8 (C), 163.2 (C), 169.6/169.8 (C), 171.6/171.7 (C).
72		t_R = 12.2 min (Method II); HRMS: calc. for $C_{31}H_{45}N_4O_4^+$ $[MH]^+$ 537.3435, found 537.3449; ^{1}H NMR (400 MHz, DMSO-d_6) (two rotamers in a ratio of 58:42), δ 0.76–1.40 (m, 15H), 1.40–1.82 (m, 12H), 2.32–2.50 (m, 1H, H_{ax} of *s*-syn amide piperidine NCH_2), 2.50 ($\underline{CH_2}$Ph, overlapped with the solvent peak), 2.89–2.98 (m, 1H, H_{ax} of *s*-anti amide piperidine NCH_2), 4.02 (d, 1H, *J* = 12.8 Hz, H_{eq} of *s*-anti amide piperidine NCH_2), 4.31–4.38 (m, 1H, H_{eq} of *s*-syn amide piperidine NCH_2), 4.50–4.60 (m, 2H, 2 × α-CH), 7.12–7.30 (m, 6H), 8.04 (d, *J* = 8.8 Hz, minor) and 8.16 (d, *J* = 9.2 Hz, major, 1H, NH) 8.74 (s, minor) and 8.76 (s, major, 1H, isoxazole-CH), 8.89–8.95 (m, 1H, NH).
73		t_R = 14.3 min (Method II); HRMS: calc. for $C_{36}H_{47}N_4O_4^+$ $[MH]^+$ 599.3592, found 599.3597; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 56:44), δ 0.79–1.54 (m, 14H), 1.54–1.86 (m, 12H), 2.45–2.51 (m, 1H, H_{ax} of *s*-syn amide piperidine NCH_2), 2.91–2.99 (m, 2H, piperidine-CHAr and H_{ax} of *s*-anti amide piperidine NCH_2), 4.05 (d, 1H, *J* = 13.2 Hz, H_{eq} of *s*-anti amide piperidine NCH_2), 4.65–4.72 (m, 2H, α-CH and H_{eq} of *s*-syn amide piperidine NCH_2), 4.82–4.90 (m, 1H, α-CH), 6.86 (d, *J* = 9.2 Hz) and 6.92 (d, *J* = 9.2 Hz, 1H, NH), 6.93 (d, *J* = 1.8 Hz, minor) and 6.94 (d, *J* = 1.8 Hz, major, 1H, isoxazole-CH), 7.11 (d, *J* = 8.8 Hz) and 7.16 (d, *J* = 8.4 Hz, 1H, NH), 7.19–7.45 (m, 9H), 8.33 (d, *J* = 1.8 Hz, major) and 8.34 (d, *J* = 1.8 Hz, major, 1H, isoxazole-CH); ^{13}C NMR (100 MHz, $CDCl_3$): 11.4/11.7 (Ile-δ-CH_3), 15.8/16.3 (Ile-γ-CH_3), 24.2 (Ile-γ-CH_2), 26.1(3)/26.2(0)/26.2(5)/26.3(3), 26.4(3)/26.4(4), 32.7/32.8, 33.2, 33.8/34.1/34.3, 38.2(6), 38.3(4)/38.4(8), 40.4/40.5, 43.1(8)/43.2(3), 46.7/47.1, 51.4/51.5/51.6, 53.0(6)/53.1(1)/53.1(6), 106.9, 126.2(6)/126.2(8), 127.1(6)/127.2(4), 128.0, 128.4, 129.3, 130.4/139.6, 141.6, 141.7/141.8, 142.1(9)/141.2(3), 151.2, 155.5, 162.6, 170.0, 171.3.
74		t_R = 14.3 min (Method II); HRMS: calc. for $C_{36}H_{47}N_4O_4^+$ $[MH]^+$ 599.3592, found 599.3590; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1), δ 0.84–2.09 (m, 26H), 2.72–2.89 (m, 2H, piperidine-CHAr and H_{ax} of *s*-syn amide piperidine NCH_2), 3.20–3.26 (m, 1H, H_{ax} of *s*-anti amide piperidine NCH_2), 4.18–4.25 (m, 1H, H_{eq} of *s*-anti amide piperidine NCH_2), 4.66–4.72 (m, 1H, α-CH), 4.79 (d, 1H, J = 12.8 Hz, H_{eq} of *s*-syn amide piperidine NCH_2), 4.88–4.96 (m, 1H, α-CH), 6.92 (d, *J* = 2.0 Hz) and 6.94 (d, *J* = 2.0 Hz, 1H, isoxazole-CH), 6.99–7.04 (m, 1H), 7.14–7.60 (m, 10H), 8.33 (d, *J* = 2.0 Hz) and 8.34 (d, *J* = 2.0 Hz, 1H, isoxazole-CH); ^{13}C NMR (100 MHz, $CDCl_3$): 11.3/11.5 (Ile-δ-CH_3), 15.7/16.1 (Ile-γ-CH_3), 24.0/24.1 (Ile-γ-CH_2), 26.0/26.1/26.2, 32.5/32.6, 32.9/33.0, 33.6, 33.8, 34.0(5)/34.1(0), 38.1, 40.2, 42.5/42.8, 43.2, 46.6/47.1, 51.3/51.4/51.5, 53.0/53.1/53.2, 106.8, 125.5/125.6/125.7, 127.2/127.3, 128.7/129.1, 141.1, 141.6(6)/141.7(0), 145.1/145.2,

		151.0, 155.4, 162.4, 170.1, 171.3/171.4.
75		t_R = 13.6 min (Method II); HRMS: calc. for $C_{36}H_{47}N_4O_4^+$ $[MH]^+$ 599.3592, found 599.3594; ^{1}H NMR (400 MHz, $CDCl_3$) (two rotamers in a ratio of 1:1), δ 0.85–2.07 (m, 26H), 2.70–2.88 (m, 2H, piperidine-CHAr and H_{ax} of *s*-syn amide piperidine NCH_2), 3.18–3.27 (m, 1H, H_{ax} of *s*-anti amide piperidine NCH_2), 4.14–4.20 (m, 1H, H_{eq} of *s*-anti amide piperidine NCH_2), 4.65–4.72 (m, 1H, α-CH), 4.79 (d, 1H, *J* = 11.6 Hz, H_{eq} of *s*-syn amide piperidine NCH_2), 4.88–4.94 (m, 1H, α-CH), 6.75 (app t, 1H, *J* = 9.6 Hz, NH), 6.93–6.94 (m, 1H, isoxazole-CH), 7.07 (app t, 1H, *J* = 9.6 Hz, NH), 7.26–7.58 (m, 9H), 8.33–8.34 (m, 1H, isoxazole-CH).
76		t_R = 13.2 min (Method II); HRMS: calc. for $C_{36}H_{47}N_4O_5^+$ $[MH]^+$ 615.3541, found 615.3546; ^{1}H NMR (400 MHz, DMSO-d_6) (two rotamers in a ratio of 64:36), δ 0.88–2.00 (m, 26H), 2.69–2.78 (m, 1H), 2.87–2.94 (m, 1H), 3.16–3.27 (m, 1H), 4.28 (d, 1H, *J* = 12.8 Hz, H_{eq} of *s*-anti amide piperidine NCH_2), 4.62–4.75 (m, 3H, 2 × α-CH and H_{eq} of *s*-syn amide piperidine NCH_2), 7.00–7.58 (m, 10H), 8.21 (d, *J* = 9.6 Hz, minor) and 8.34 (d, *J* = 9.6 Hz, major, NH), 8.83 (d, *J* = 1.8 Hz, major) and 8.85 (d, *J* = 1.8 Hz, minor, 1H, isoxazole-CH), 9.02 (d, 1H, *J* = 8.4 Hz, NH).

Calculation of Volume: Chemical structures of different R_1-R_4 substituents in ligands reported here were drawn in ChemDraw 13.0. "Ligand Preparation" module within Maestro, version 9.5 (Schrödinger, LLC, New York, NY, 2013) was used to convert 2D structures to 3D co-ordinates and saved as mol2 files. Gaussian 09 software was used for calculation of molar volume (cm^3/mole) of the R groups. Before quantum chemical calculations, a molecular mechanic minimization was performed with the UFF force field available in Gaussian 09. Further calculations were performed at the level of B3LYP/6-311+g(d,p) basis set in vacuum.

Intracellular calcium release assay. Adherent colorectal adenocarcinoma cells (HT29) were plated at ~2 x 10^4 cells/well in a 96-well cleared-bottomed black-walled assay plate (Corning) with equal amounts of medium added and incubated overnight at 37ºC. Before assay, the medium was removed and cells were incubated with dye-loading buffer (12 mL HBSS buffer, 2 mM Fluo-3 AM, 25 μL Pluronic acid F-127 and 1% v/v fetal calf serum) for an hour at 37ºC. After an hour, cells were washed once with assay buffer (HBSS supplemented with 2.5 mM probenecid and 20 mM HEPES, pH 7.4). All final compounds were dissolved in DMSO to make

a 10 mM stock solution for intracellular calcium release assays on HT29 cells. The stock sample was then diluted with HBSS buffer to give the desired concentrations for the calcium efflux assay. The concentration of DMSO in the assay was no more than 2%. For the antagonist assay, the cells were pre-incubated with desired concentrations of the synthesized compounds for 30 min before the addition of agonist (2f-LIGRLO-NH_2, 1 μM). Fluostar Optima (BMG Labtechnologies) or FLIPR (Molecular Devices) was used to monitor the intracellular release of Ca^{2+} via fluorescence measurement for at least 60 s (excitation 495 nm, emission 520 nm). The agonist assay was conducted in a similar manner, except that the intracellular Ca^{2+} release was monitored immediately after the injection of the desired concentration of the synthesized compounds. To assess potential antagonist in more detail, concentration dependent inhibition assay was conducted. A range of concentrations of the compound was pre-incubated with the cells. Duplicate measurements were made for each data point, mean ± SEM are reported from multiple experiments as indicated. Net changes in fluorescence were calculated as a percentage relative to the maximum response given by the test compound. Changes in fluorescence (% response) were plotted against logarithmic compound concentrations. The half maximal inhibitory concentration (IC_{50}) values were derived from the concentration response curve using a nonlinear regression curve in Graphpad Prism v5.

Antagonist surmountability. Cells were prepared as for the calcium mobilization assay. After 1 h incubation with dye loading buffer, cells were incubated with different concentrations of antagonist for 15 min before the addition of agonist (2f-LIGRLO-NH_2). The plate was read using FLIPR (Molecular Devices) and examined for concentration-dependent effects on the activity of agonist in the presence of different concentrations of antagonist.

PAR2 transfected CHO cells. Human PAR2 cDNA was cloned into pcDNA5/FRT vector (Invitrogen) at *BamHI* site and incorporated into Flp-In-CHO cells (Invitrogen) using

Lipofectamine 2000 (Invitrogen) according to manufacturer's instruction. Stable PAR2-CHO cells were then selected using 600 μg/mL of Hygromycin B.

cAMP accumulation. LANCE Ultra cAMP assay was performed in accordance with manufacturer's instructions (PerkinElmer). In brief, cells were dissociated from flasks by Versene (Invitrogen) on the day of experiment. Cells (5 μL, 4×10^5 cells/mL) were transferred to a 384-well proxiplate (PerkinElmer) and incubated with various concentrations of **65** for 20 min at room temperature. Forskolin (120 nM) was then added into each well and incubated for a further 10 min at room temperature. Finally, Eu-cAMP tracer (5 μL) and ULight™ -anti-cAMP (5 μL) were added to each well and incubated for 1 h at room temperature. The plate was read using a Pherastar FS fluorimeter (BMG, Germany).

ERK1/2 phosphorylation. SureFire phospho-ERK1/2 assay was performed in accordance with manufacturer's instructions (PerkinElmer). In brief, cells were seeded overnight in 96-well tissue culture plate (~5×10^4 cells per well). On the day of experiment, cells were treated with various concentrations of compounds dissolved in serum-free medium and incubated for 10 min at 37 °C. Supernatant was removed and cells were lysed with cell lysis buffer provided by the kit. Cell lysate (4 μL) was transferred to a 384-well proxiplate (PerkinElmer) and incubated with reaction mixture (7 μL) for 2 h at room temperature before plate reading.

Stability studies on PAR2 agonists and antagonists in rat plasma and liver homogenate. Blood and whole liver samples were collected from non-drug dosed male and female Wistar rats (aged 8–9 weeks, 200–250 g and 250–300 g respectively). Blood samples were centrifuged at 8k rpm for 5 min. Plasma samples were pooled and stored at –80 °C for later use. The rat livers were homogenized, diluted with three volumes of PBS and cloth filtered. The filtrate was used directly for stability studies. Each compound was dissolved in DMSO to make 5 mM stock solution. 10 μL of the stock was diluted with either rat plasma or liver homogenate (490 μL) to

make up a starting concentration of 10 μM (performed in triplicate). The mixtures were vortexed and incubated at 37 ºC. At each time point of 0, 30, 60 and 180 minutes, 100 μL of the mixture was taken and diluted with 300 μL of acetonitrile. The mixture was vortexed and centrifuged. 350 μL of the liquid was transferred into a microfuge tube and concentrated using a rotational vacuum concentrator (Christ Beta-RVC, supplied by Quantum Scientific). 100 μL of acetonitrile–water (9:1, v/v) was added to the residue, vortexed and immediately analyzed by LCMS/MS. Data from these experiments are expressed as percent of peak area recorded from the LCMS/MS trace at time zero (t_0).

In *vivo* efficacy using the PAR2-agonist induced paw oedema . The methods used are based on those previously described.[1-3] Male Wistar rats (n = 3–5 per group) were used. Briefly, rats were given 10 mg/kg of a compound orally (p.o. via gavage in olive oil, approx. 500 μL, weight adjusted). Control animals received only olive oil or saline (500 μL p.o.). After a relevant absorption period (2 h for oral), the PAR2 agonist 2-f-LIGRLO-NH_2 (350 μg/paw in saline, 100 μL) was injected into the plantar surface (i.pl.) of the right paw pad using a 30 G needle. The left paw acted as a control, receiving saline only. Paw thickness and width were measured at given time points (30 min, 60 min, 2 h, 3 h) thereafter using digital callipers (World Precision Instruments, USA) and swelling was calculated in area (mm^2; thickness multiplied by width). Data is expressed as a percentage change from baseline of each individual paw, mean ± SEM. Statistical tests performed were repeated measures ANOVA with Bonferroni planned comparisons, using GraphPad Prism software (v5.0c).

Human tubule epithelial cells (HTEC) isolation and culture. Segments of macroscopically and histologically normal renal cortex (5–10 g) were obtained aseptically from the non-cancerous pole of adult human kidneys removed surgically because of small renal cancers. Patients were otherwise healthy. Informed consent was obtained prior to each operative

procedure and the use of human renal tissue for primary culture was reviewed and approved by the Princess Alexandra Hospital Research Ethics Committee. The method for isolation and primary culture of human tubule epithelial cells (HTEC) is described in detail from literature.[4,5] Briefly the cortical tissue was minced finely, washed several times and agitated for 20 minutes at 37 °C in a Hank Balanced Salt Solution (HBSS) with Ca^{2+} & Mg^{2+} containing collagenase type II (1mg/ml). Cold HBSS was added and the solution passed through a 297 μm sieve (50 Mesh, Sigma). After washing three times the tubular fragments were resuspended in 45% percoll – KHB and centrifuged at 25000 xg. A high density band previously shown to be tubule fragments was removed and cultured in a serum free, hormonally defined media (DMEM/F12 containing 10 ng/ml epidermal growth factor, 5 μg/ml insulin, 5 μg/ml transferrin, 50nM hydrocortisone, 50 μM prostaglandin E1, 50 nM selenium and 5 pM triiodothyronine.

Treatment of human tubule epithelial cells (HTEC). All experiments were performed on confluent passage 2 HTEC, (in 48 well plates), made quiescent by two washes followed by incubation for 24 hours in serum and growth factor free DMEM/F12 media. Compounds diluted in DMEM/F12 were added to the cells at the concentrations shown. Antagonists were added 30 mins prior to agonist addition. Conditioned medium was collected from the cells at 24h and stored at –80°C until assayed.

Enzyme-linked immunosorbent assay. Cytokines (IL-6 & TNF-α) were examined either using BD Pharmingen ELISA set or R&D Duoset according to the manufacturer's protocols. Briefly, plate was coated with capturing antibody at 4°C overnight before being blocked with 10% serum or 1% BSA for 1 h at room temperature. Samples and standards were diluted in blocking buffer, added to each well and incubate for 2 h at room temperature. The HRP conjugated detection antibody was added and incubated for a further 1–2 h at room temperature. K-blue substrate (Elisa Systems, Brisbane Australia) was allowed to develop for 30 min in the

dark and stopped by 2 M sulfuric acid. A BMG Polarstar spectrofluorimeter was used to measure the absorbance at 450 nM.

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