sOptimization of Novel Antagonists to the Neurokinin-3 Receptor for the Treatment of Sex-Hormone Disorders (Part II)

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Supporting Information

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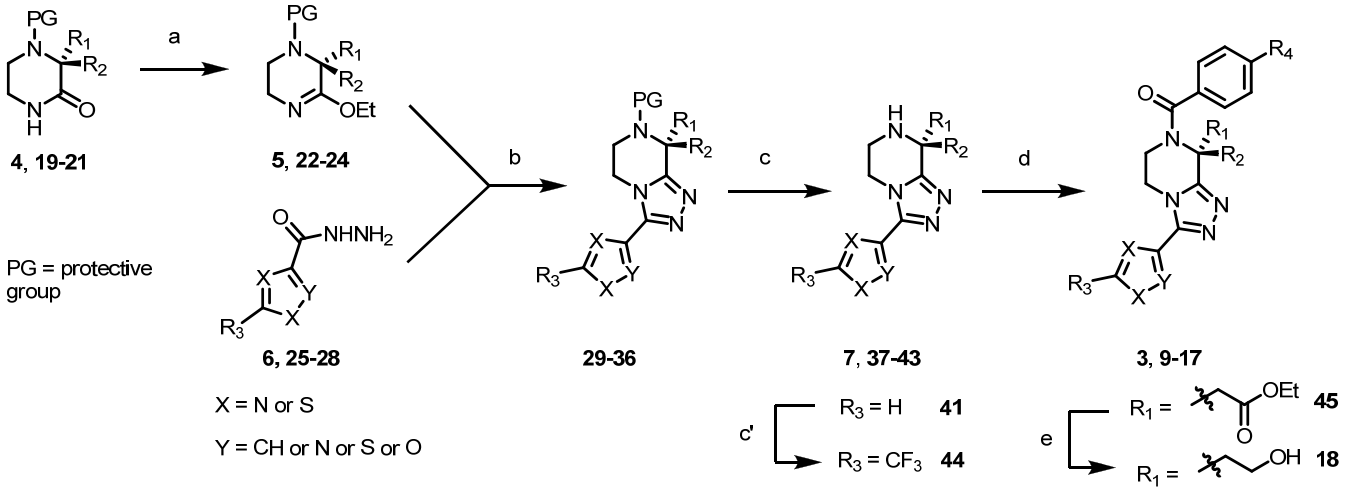
1. Synthesis and Characterization data for synthetic precursors and compounds 3 and 9-18.

General Notes. Solvents and reagents were of reagent grade and were used as supplied by the manufacturer. All reactions were performed under a N_2 atmosphere unless otherwise noted. Organic extracts were routinely dried over anhydrous $MgSO_4$. Piperazinone starting material, **4** (Scheme 1) was typically procured from a commercial vendor (J&W PharmLab, PA, USA). Chromatography refers to flash chromatography using silica gel. NMR spectra were recorded on Bruker ARX spectrometer (300 MHz) at room temperature. Chemical shifts are expressed in parts per million δ relative to residual solvent as an internal reference. Peak multiplicities are expressed as follows: singlet (s), doublet (d), triplet (t), quartet (q), heptuplet (h), multiplet (m), and broad singlet (bs). HPLC purity analysis of compounds was carried out using one of the following methods. Method A: LC/diode array detector coupled to single quadrupole mass spectrometry (LC/UV/MS); column: Sunfire C18 3µm 3.0 × 50 mm; mobile phase A: 0.1% TFA in water (v/v); mobile phase B: 0.1% TFA in acetonitrile (v/v); gradient begins at 5% B, hold 0.2 min at 5% B, increasing to 95% B over 6.0 min, hold at 95% B until 7.75 min; flow rate: 1.0 mL/min. Method B: LC/diode array detector coupled to single quadrupole mass spectrometry (LC/UV/MS); column: Sunfire C18 3µm 3.0 × 50 mm; mobile phase A: 0.1% TFA in water (v/v); mobile phase B: 0.1% TFA in acetonitrile (v/v); gradient begins at 5% B, hold 0.2 min at 5% B, increasing to 95% B over 2.0 min, hold at 95% B until 3.75 min; flow rate: 1.0 mL/min. Enantiomeric exces of chiral compounds and intermediates was carried out using one of the following methods. Method C: column: Chiralpak IA 5.0µm C18 4.6×250 mm; mobile phase: 0.1% DEA in TBME (v/v); flow rate: 1.0 mL/min; UV absorbance at 280 nm. Method D: column: Chiralpak IC 5.0µm C18 4.6×250 mm; mobile phase: 0.1% DEA in TBME/MeOH (92:2 v/v); flow rate: 1.0 mL/min; UV absorbance at 280 nm. Method E: column: Chiralpak IC 5.0µm C18 4.6×250 mm; mobile phase: 0.1% DEA in EtOAc/Hexane (1:1 v/v); flow rate: 1.5 mL/min; UV absorbance at 280 nm. Method F: column: Chiralpak IA 5.0µm C18 4.6×250 mm; mobile phase: 0.1% DEA in Hexane/EtOAc (1:1 v/v); flow rate: 1.0 mL/min; UV absorbance at 280 nm. Method G: column: Chiralpak IA 5.0µm C18 4.6×250 mm; mobile phase: 0.1% DEA in Hexane/EtOH (4:1 v/v); flow rate: 1.0 mL/min; UV absorbance at 280 nm. Method H: column: Chiralpak IA 5.0µm C18 4.6×250 mm; mobile phase: 0.1% DEA in EtOAc (v/v); flow rate: 1.0 mL/min; UV absorbance at 280 nm. Method I: column: Chiralpak IA 5.0µm C18 4.6×250 mm; mobile phase: 0.1% DEA in Hexane/EtOH (1:1 v/v); flow rate: 1.0 mL/min; UV absorbance at 280 nm. Nomenclature of compounds below was achieved using Beilstein AutoNom feature accessible within ChemBioDraw Ultra (ver 11).

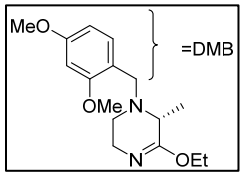
We are grateful to Dr. Lisa Haigh (Imperial College, London, UK) for obtaining HRMS characterization data provided herein.

Compounds **1**, **2** and **8** were prepared as described previously.[1]

Scheme S1. General synthetic scheme

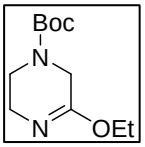


Step a$_1$. (*R*)-1-(2,4-Dimethoxybenzyl)-5-ethoxy-6-methyl-1,2,3,6-tetrahydropyrazine (5).



To a solution of (*R*)-4-(2,4-dimethoxybenzyl)-3-methylpiperazin-2-one, **4** (20 g, 76 mmol, 1 equiv.) in CH_2Cl_2 (250 mL) was added, at 15 °C, Na_2CO_3 (18 g, 170 mmol, 2.25 equiv.), followed by Et_3OBF_4[2] (18 g, 95 mmol, 1.25 equiv.) over 10 min, whereupon a slight exotherm was noted (15 °C to 21 °C). After stirring the reaction mixture 45 min further at room temperature, it was quenched with brine (340 mL), the organic layer separated, dried ($MgSO_4$), filtered and concentrated in vacuo (1-2 mbar, 30 °C) to afford the crude product as a yellow oil. Purification by silica gel flash chromatography (EtOAc/MeOH: 99/1) afforded pure **5** as a yellow oil (14.75 g, 67%). (NB: This product was stored at –20 °C under Ar and typically used for the subsequent reaction within a day of its preparation). LCMS (Method B) *m/z* 311 (M + H_2O + 1). HPLC purity 96% (280 nm). Chiral LC (Method C): 98.8 %ee ($t_{R(S)}$ = 4.4 min, $t_{R(R)}$ = 10.7 min). ^{1}H NMR ($CDCl_3$): δ 7.23 (d, *J* = 8.8 Hz, 1H), 6.48 (d, *J* = 8.8 Hz, 1H), 6.44 (s, 1H), 4.02 (m, 2H), 3.92 (s, 3H), 3.91 (s, 3H), 3.86 (d, J_{AB} = 14.0 Hz, 1H), 3.46 (d, J_{AB} = 14.0 Hz, 1H), 3.44 (m, 2H), 3.10 (m, 1H), 2.79 (m, 1H), 2.32 (m, 1H), 1.35 (d, *J* = 6.8 Hz, 3H), 1.24 (t, *J* = 6.0 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 164.5, 160.1, 158.9, 131.1, 118.8, 104.1, 98.6, 60.4, 55.5, 55.4, 55.1, 51.4, 45.8, 45.6, 16.1, 14.5. This intermediate was used to ultimately prepare compounds **3**, **9-11** and **15-17**.

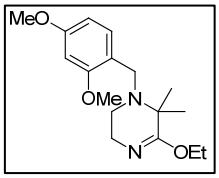
Step a$_2$. *tert*-Butyl 5-ethoxy-3,6-dihydropyrazine-1(2*H*)-carboxylate (22).



To a pre-made solution of Et_3OBF_4 (2.3 g, 12.1 mmol, 1.4 equiv.) in CH_2Cl_2 (20 mL) was added **19** (PG = Boc, R_1=R_2=H) (2 g, 8.8 mmol, 1.0 equiv.) at 0 °C. After the addition was completed, the ice bath was removed, and the reaction mixture was allowed to warm to room temperature and stirred for an additional hour. A saturated solution of $NaHCO_3$ (500 mL) was

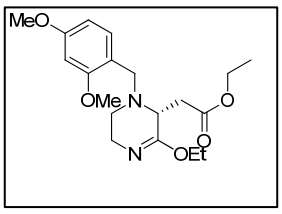
slowly added to the reaction mixture and it was stirred for 5 min. The organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (200 mL). The combined organic layers were subsequently washed with brine, dried over $MgSO_4$, filtered and dried *in vacuo* to dryness to obtain **22** as viscous yellow oil (2.03 g, 88 %). LCMS (Method B) *m/z* 246 (M + H_2O + 1). HPLC purity > 90% (254 nm). ^{1}H NMR ($CDCl_3$): δ: 4.1 (q, *J* = 7.1 Hz, 2H), 3.85 (s, 2H), 3.5 (m, 1H), 3.35 (t, *J* = 5.1 Hz, 2H), 1.45 (s, 9H), 1.3 (t, *J* = 7.1 Hz, 3H). This intermediate was used to ultimately prepare compound **12**.

Step a$_3$. 1-(2,4-Dimethoxybenzyl)-5-ethoxy-6,6-dimethyl-1,2,3,6-tetrahydropyrazine (23).



To a solution of 4-(2,4-dimethoxybenzyl)-3,3-dimethylpiperazin-2-one, **20** (285 mg, 1.02 mmol, 1 equiv.) in CH_2Cl_2 (3.4 mL) was added, at 0 °C, Na_2CO_3 (244 mg, 2.31 mmol, 2.25 equiv.), followed by Et_3OBF_4 (243 mg, 1.28 mmol, 1.25 equiv.). After stirring the reaction mixture 45 min further at room temperature, it was quenched with brine (10 mL) and diluted with CH_2Cl_2 (7 mL), the organic layer separated, dried ($MgSO_4$), filtered and concentrated in vacuo (1-2 mbar, 30 °C) to afford the crude product as a yellow oil. Purification by silica gel flash chromatography (EtOAc/MeOH: 99/1) afforded pure **23** as a colourless oil (0.3 g, 96%). LCMS (Method B) *m/z* 321 (M + H_2O + 1). HPLC purity > 95% (254 nm). This intermediate was used to ultimately prepare compound **14**.

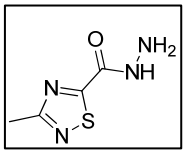
Step a$_4$. (*R*)-Ethyl 2-(1-(2,4-dimethoxybenzyl)-3-ethoxy-1,2,5,6-tetrahydropyrazin-2-yl)acetate (24).



To a solution of (*R*)-ethyl 2-(1-(2,4-dimethoxybenzyl)-3-oxopiperazin-2-yl)acetate, **21** (1.93 g, 5.74 mmol, 1 equiv.) in CH_2Cl_2 (19 mL) was added, at 0 °C, Na_2CO_3 (1.37 g, 12.91 mmol, 2.25 equiv.), followed by Et_3OBF_4 (1.36 g, 7.17 mmol, 1.25 equiv.). After stirring the reaction mixture 45 min further at room temperature, it was quenched with brine (40 mL) and the organic layer separated, dried ($MgSO_4$), filtered and concentrated in vacuo (1-2 mbar, 30 °C) to afford the crude product as a yellow oil. Purification by silica gel flash chromatography (EtOAc/MeOH: 99/1) afforded pure **24** as a colourless oil (1.3 g, 62%). LCMS (Method B) *m/z* 365/383 (M+1 / M + H_2O + 1 as 2 UV peaks because of partial hydratation). HPLC purity > 95% (254 nm).[3] This intermediate was used to ultimately prepare compound **18**.

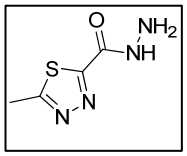
General Procedure A for the Synthesis of Intermediate Hydrazides 6, 25-28. An equivalent of the appropriate ester was dissolved in anhydrous EtOH (0.5 M) under Ar, and hydrazine monohydrate (1.15 equiv.) was added in one portion at room temperature. The resulting mixture was stirred for 30-45 min and thereafter the thick solid formed was filtered off. The solid obtained was washed with minimal EtOH volume and dried under high vacuum to dryness (40 °C, 0.2 mbar).

3-Methyl-1,2,4-thiadiazole-5-carbohydrazide (6):[4] Starting from the appropriate commercially



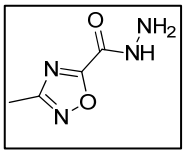
available ester as per General Procedure A to obtain 6.5 g (86 %). LCMS (Method B) *m/z* 159 (M + 1). HPLC purity > 95% (254 nm). ^{1}H-NMR ($CDCl_3$): δ 8.40 (bs, 1H), 4.14 (bs, 2H), 2.70 (s, 3H). This intermediate was used to ultimately prepare compounds **3, 12, 14, 15, 17** and **18**.

5-Methyl-1,3,4-thiadiazole-2-carbohydrazide (25)*:* Starting from the appropriate commercially



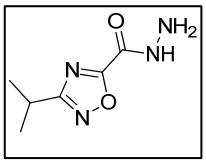
available ester as per General Procedure A to obtain 0.7 g (88 %). LCMS (Method B) *m/z* 159 (M + 1). HPLC purity > 95% (254 nm). This intermediate was used to ultimately prepare compound **9**.

3-Methyl-1,2,4-oxadiazole-5-carbohydrazide (26):[4] Starting from the appropriate commercially



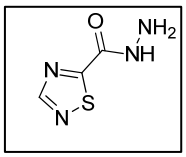
available ester as per General Procedure A to obtain 1.0 g (85 %). LCMS (Method B) *m/z* 143 (M + 1). HPLC purity > 95% (254 nm). ^{1}H-NMR ($CDCl_3$): δ 8.22 (bs, 1H), 4.21 (bs, 2H), 2.48 (s, 3H). This intermediate was used to ultimately prepare compound **10**.

3-*iso*Propyl-1,2,4-oxadiazole-5-carbohydrazide (27):[4] Starting from the appropriate commercially



available ester as per General Procedure A to obtain 1.2 g (91 %). LCMS (Method B) *m/z* 171 (M + 1). HPLC purity > 95% (254 nm). This intermediate was used to ultimately prepare compound **11**.

1,2,4-Thiadiazole-5-carbohydrazide (28):[4] Starting from the appropriate commercially available ester

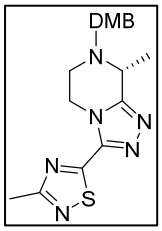


as per General Procedure A to obtain 1.1 g (85 %). LCMS (Method B) *m/z* 145 (M + 1). HPLC purity > 95% (254 nm). ^{1}H-NMR ($CDCl_3$): δ 8.76 (s, 1H), 8.45 (bs, 1H), 4.15 (bs, 2H). This intermediate was used to ultimately prepare compounds **13** and **16**.

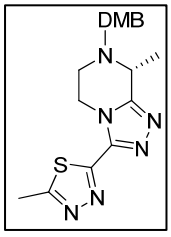
Step b. **General Procedure B for the Synthesis of Intermediates 25-32.** An equivalent of the appropriate iminoether (**5**, **22-24**) was dissolved in anhydrous MeOH (1 M) under Ar, and the appropriate hydrazide (**6**, **25-28**, 1.05 equiv.) was added in one portion. The resulting solution was stirred at 60-70 °C for 8 h whereupon it was allowed to reach room temperature and thereafter the volatiles were removed in vacuo (1-2 mbar, 30 °C). This residue obtained was then dissolved in CH_2Cl_2

(2.5 mL/mmol), washed with NaOH (1 M) and after separation, the organic layer was dried ($MgSO_4$), filtered and volatiles removed (1-2 mbar, 30 °C) to afford a crude residue which was used without further purification in the next step.

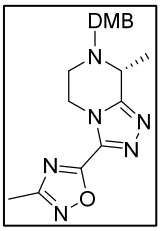
(*R*)-5-(7-(2,4-Dimethoxybenzyl)-8-methyl-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)-3-methyl-1,2,4-thiadiazole (29)*:*** Starting from iminoether **5** and hydrazide **6** as per General Procedure B to obtain 1.45 g (98 %). LCMS (Method B) *m/z* 387 (M + 1). HPLC purity 94% (254 nm). Chiral LC (Method D) 99.1 %ee ($t_{R(R)}$ = 25.1 min, $t_{R(S)}$ = 27.9 min). ^{1}H-NMR ($CDCl_3$): δ 7.26 (d, *J* = 8.4 Hz, 1H), 6.48 (d, *J* = 8.4 Hz, 1H), 6.47 (s, 1H), 4.50 (m, 1H), 4.30 (m, 1H), 4.09 (q, *J* = 6.6 Hz, 1H), 3.94 (t, *J* = 13.6 Hz, 1H), 3.80 (s, 6H), 3.61 (t, *J* = 13.6 Hz, 1H), 3.22 (m, 1H), 2.75 (m, 1H), 2.69 (s, 3H), 1.72 (d, *J* = 6.5 Hz, 3H). This intermediate was used to ultimately prepare compounds **3, 15** and **17**.



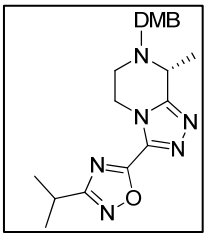
(*R*)-2-(7-(3,4-Dimethylbenzyl)-8-methyl-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)-5-methyl-1,3,4-thiadiazole (30)*:*** Starting from iminoether **5** and hydrazide **25** as per General Procedure B to obtain 450 mg (88 %). LCMS (Method B) *m/z* 387 (M + 1). HPLC purity > 95% (254 nm). This intermediate was used to ultimately prepare compound **9**.



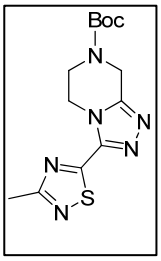
(*R*)-5-(7-(2,4-Dimethoxybenzyl)-8-methyl-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)-3-methyl-1,2,4-oxadiazole (31): Starting with iminoether **5** and hydrazide **26** as per General Procedure B to obtain 543 mg (87 %). LCMS (Method B) *m/z* 371 (M + 1). HPLC purity > 98% (254 nm). ^{1}H-NMR ($CDCl_3$): δ 7.25 (d, *J* = 8.4 Hz, 1H), 6.48 (d, *J* = 8.4 Hz, 1H), 6.47 (s, 1H), 4.43 (dt, *J* = 13.3, 4.6 Hz, 1H), 4.28 (m, 1H), 4.10 (q, *J* = 6.7 Hz, 1H), 3.94 (t, *J* = 13.6 Hz, 1H), 3.80 (2s, 6H), 3.63 (t, *J* = 13.6 Hz, 1H), 3.25 (dt, *J* = 13.1, 4.9 Hz, 1H), 2.77 (m, 1H), 2.49 (s, 3H), 1.74 (d, *J* = 6.6 Hz, 3H). This intermediate was used to ultimately prepare compound **10**.



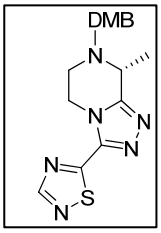
(*R*)-5-(7-(3,4-Dimethylbenzyl)-8-methyl-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)-3-isopropyl-1,2,4-oxadiazole (32)*:*** Starting with iminoether **5** and hydrazide **27** as per General Procedure B to obtain 653 mg (95 %). LCMS (Method B) *m/z* 399 (M + 1). HPLC purity = 84% (254 nm).[5] This intermediate was used to ultimately prepare compound **11**.



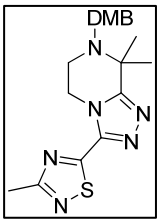
tert*-Butyl 3-(3-methyl-1,2,4-thiadiazol-5-yl)-5,6-dihydro-[1,2,4]triazolo[4,3-*a*]pyrazine-7(8*H*)-carboxylate (33):* Starting from iminoether **22** and hydrazide **6** as per General Procedure B with the following modification: reaction stirred at 120 °C (1M to neat reaction) for 16h to obtain 5.74 g (92 %). LCMS (Method B) *m/z* 323 (M + 1). HPLC purity 93% (254 nm). This intermediate was used to ultimately prepare compound **12**.



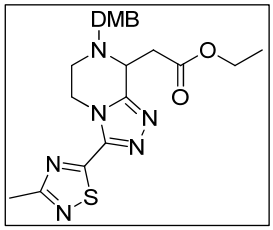
(*R*)-5-(7-(2,4-Dimethylbenzyl)-8-methyl-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)-1,2,4-thiadiazole (34)*:* Starting from iminoether **5** and hydrazide **28** as per General Procedure B to obtain 1.12 g (87 %). LCMS (Method B) *m/z* 373 (M + 1). HPLC purity > 95% (254 nm). ^{1}H-NMR ($CDCl_3$): δ 8.73 (s, 1H), 7.25 (d, *J* = 8.4 Hz, 1H), 6.49 (d, *J* = 8.4 Hz, 1H), 6.47 (s, 1H), 4.55 (dt, *J* = 4.6, 13.2 Hz, 1H), 4.29 (m, 1H), 4.11 (q, *J* = 6.6 Hz, 1H), 3.94 (t, *J* = 13.6 Hz, 1H), 3.81 (s, 6H), 3.63 (t, *J* = 13.6 Hz, 1H), 3.26 (dt, *J* = 4.6, 13.2 Hz, 1H), 2.78 (m, 1H), 1.74 (d, *J* = 6.5 Hz, 3H). This intermediate was used to ultimately prepare compounds **13** and **16**.



5-(7-(2,4-Dimethoxybenzyl)-8,8-dimethyl-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)-3-methyl-1,2,4-thiadiazole (35)*:* Starting from iminoether **23** and hydrazide **6** as per General Procedure B with the following modification: reaction stirred for 40 h to obtain 284 mg (72 %). LCMS (Method B) *m/z* 401 (M + 1). HPLC purity > 95% (254 nm). This intermediate was used to ultimately prepare compound **14**.



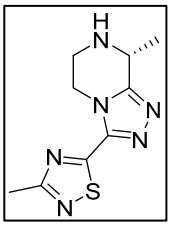
Ethyl 2-(7-(2,4-dimethoxybenzyl)-3-(3-methyl-1,2,4-thiadiazol-5-yl)-5,6,7,8-tetrahydro-[1,2,4]triazolo [4,3-*a*]pyrazin-8-yl)acetate (36)*:* Starting from iminoether **23** and hydrazide **6** as per General Procedure B with the following modification: reaction stirred for 40 h at 130 °C (neat) to obtain 2.19 g (87 %). LCMS (Method B) *m/z* 459 (M + 1). HPLC purity > 95% (254 nm). This intermediate was used to ultimately prepare compound **18**.



Step c. **General Procedure C for the Synthesis of Intermediates 7 and 37-43.** To a solution of intermediate **29-36** (1 equiv.) in CH_2Cl_2 (0.2 M solution) at 0 °C was added TFA (7.5 equiv.) in one portion. The ice bath was removed after 10 min and the reaction mixture stirred at room temperature for 15 min whereupon water was added, the suspension stirred for 30 min and filtered.[6] The aqueous layer

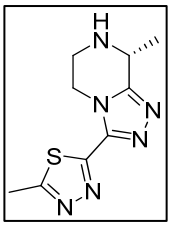
was adjusted to pH 14 by addition of NaOH (4M) and further extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$), filtered and volatiles removed to afford intermediates **7** and **37-43** as white solids.

(*R*)-3-Methyl-5-(8-methyl-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)-1,2,4-thiadiazole hydrochloride (7)*:*



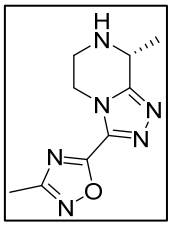
Starting from **29** as per General Procedure C to obtain 875 mg (91%). LCMS (Method B) *m/z* 237 (M + 1). HPLC purity 98% (215 nm). Chiral LC (Method E) 99.1 %ee ($t_{R(R)}$ = 11.1 min, $t_{R(S)}$ = 12.7 min). ^{1}H-NMR ($CDCl_3$): δ 4.66 (m, 1H), 4.33-4.21 (bm, 2H), 3.47 (m, 1H), 3.23 (m, 1H), 2.72 (s, 3H), 1.69 (d, *J* = 6.7 Hz, 3H). This intermediate was used to ultimately prepare compounds **3, 15 and 17.**

(*R*)-2-Methyl-5-(8-methyl-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)-1,3,4-thiadiazole (37):



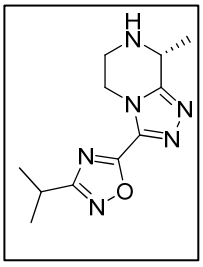
Starting from **30** as per General Procedure C to obtain 128 mg (47%). LCMS (Method B) *m/z* 221 (M + 1). HPLC purity 90% (254 nm). This intermediate was used to ultimately prepare compound **9.**

(*R*)-3-Methyl-5-(8-methyl-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)-1,2,4-oxadiazole (38):



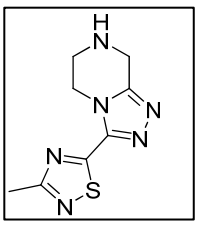
Starting from **31** as per General Procedure C to obtain 168 mg (57%). LCMS (Method B) *m/z* 221 (M + 1). HPLC purity > 98% (254 nm). This intermediate was used to ultimately prepare compound **10.**

(*R*)-3-Isopropyl-5-(8-methyl-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)-1,2,4-oxadiazole (39):



Starting from **32** as per General Procedure C with the proviso that the HCl salt formed by dissolving **32** in 2-propanol /HCl (4 M) (20 equiv.) was isolated. After 5 min stirring at room temperature, Et_2O was added and reaction further stirred for 1h before filtration to obtain 532 mg (quant). LCMS (Method B) *m/z* 249 (M + 1). HPLC purity > 98% (254 nm). This intermediate was used to ultimately prepare compound **11.**

3-Methyl-5-(5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)-1,2,4-thiadiazole (40)*:*



Starting from **33** as per General Procedure C with the modification that no filtration was performed during work-up to obtain 3.16 g (80%). LCMS (Method B) *m/z* 223 (M + 1). HPLC purity 95% (254 nm). This intermediate was used to ultimately prepare compound **12.**

(*R*)-5-(8-Methyl-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)-1,2,4-thiadiazole hydrochloride (41)*:* Starting from **34** as per General Procedure C with the proviso that the product was subjected to a silica gel flash chromatographic purification[7] (2 to 5% MeOH in CH_2Cl_2) to obtain 1.6 g (quant.). LCMS (Method A) *m/z* 223 (M + 1). HPLC purity > 98% (254 nm). ^{1}H-NMR (CD_3OD): δ 8.76 (s, 1H), 4.68 (m, 1H), 4.37-4.24 (bm, 2H), 3.49 (m, 1H), 3.23 (m, 1H), 1.70 (d, *J* = 6.7 Hz, 3H). This intermediate was used to ultimately prepare compounds **13** and **16.**

5-(8,8-Dimethyl-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)-3-methyl-1,2,4-thiadiazole (42): Starting from **35** as per General Procedure C to obtain 152 mg (86%). LCMS (Method B) *m/z* 251 (M + 1). HPLC purity > 98% (254 nm). This intermediate was used to ultimately prepare compound **14.**

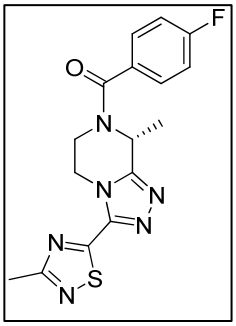
Ethyl 2-(3-(3-methyl-1,2,4-thiadiazol-5-yl)-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-*a*]pyrazin-8-yl)acetate (43)*:* To a solution of **36** (0.9 g, 1.963 mmol, 1 equiv.) in EtOH (3.9 mL) at room temperature was added HCl 4M in dioxane (2.45 mL, 9.81 mmol, 5 equiv.) in one portion. The reaction mixture was stirred at 60 °C for 3 h and then cooled to 0 °C with an ice bath. Et_2O (10 mL) was added and the solid was filtered off after 15 min stirring. After high vacuum drying, **43** was isolated as HCl salt to obtain 525 mg (78%). LCMS (Method B) *m/z* 309 (M + 1). HPLC purity > 98% (254 nm). This intermediate was used to ultimately prepare compound **18.**

Step c'. (*R*)-5-(8-Methyl-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl)-3-(trifluoromethyl)-1,2,4-thiadiazole (44). (The "innate C–H trifluoromethylation" reaction reported by Baran and co-workers was used in this preparation.[8]) To a solution of **41** (50 mg, 0.225 mmol, 1 equiv.) in CH_2Cl_2 (1 mL) and water (400 µL) was added sodium trifluoromethanesulfinate (100 mg, 0.641 mmol, 2.85 equiv) at room temperature, followed by 2-hydroperoxy-2-methylpropane (150 µL, 1.095 mmol, 4.85 equiv). Mixture was left unstirred for 4 days and fresh sodium trifluoromethanesulfinate (2.85 equiv) and 2-hydroperoxy-2-methylpropane (4.85 equiv) added each day. The reaction mixture was then taken up with EtOAc (100 mL) and water (20 mL) and carefully quenched with $NaHCO_3$ sat solution (~ 10 mL), then $NaHCO_3$ solid was added until pH 8 was reached. The organic phase was separated and the aqueous phase was extracted with EtOAc (2x 50 mL). The combined organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure to dryness to 2.56 g of crude yellow oil. This

residue was purified by silica gel flash chromatography (1 to 4 % MeOH in CH_2Cl_2) to afford 185 mg (11%). LCMS (Method B) *m/z* 291 (M + 1). HPLC purity 81% (215 nm). ^{1}H-NMR ($CDCl_3$): δ 4.69 (m, 1H), 4.39 (m, 2H), 3.53 (m, 1H), 3.27 (m, 1H), 1.75 (d, *J* = 6.7 Hz, 3H). This intermediate was used to ultimately prepare compound **16.**

Step d. **General Procedure D** for the Synthesis of compounds **3, 9-17 and 45.** To a solution of crude intermediates **7, 37-44** (1 equiv.) in CH_2Cl_2/$NaHCO_3$ sat. (2/1, 0.1 M) at room temperature was added corresponding acid chloride (1 equiv.) in one portion. The resulting mixture was then stirred for 1h at room temperature and the layers were then separated. The aq. layer was further extracted with CH_2Cl_2 and the combined organic layers were dried over $MgSO_4$, filtered and concentrated to dryness under reduced pressure to afford crude target, which was either purified by silica gel flash chromatography or crystallization.

(*R*)-(4-Fluorophenyl)(8-methyl-3-(3-methyl-1,2,4-thiadiazol-5-yl)-5,6-dihydro-[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl)methanone (3)*:*** Starting from intermediate **7** and 4-fluorobenzoyl chloride as per General Procedure D to obtain 1.35 g of crude **3** (111 %). This crude compound **3** was solubilized in boiling iPrOAc (18 mL) under stirring. The solution was allowed to reach room temperature whereupon TBME was added (9 mL). The white crystals formed after overnight standing at room temperature were filtered, washed with TBME (10 mL) and dried under high vacuum (< 0.2 mbar) to obtain **3** as a white crystalline solid (0.795 g, 65 %). LCMS (Method A) *m/z* 359 (M + 1). HPLC purity > 99% (254 nm). Chiral LC (Method F) > 99.8 %ee ($t_{R(S)}$ = 9.1 min, $t_{R(R)}$ = 11.7 min). ^{1}H-NMR ($CDCl_3$): δ 7.47 (m, 2H), 7.17 (t, *J* = 8.5 Hz, 2H), 5.77 (m, 1H), 4.90 (dd, *J* = 3.6, 13.5 Hz, 1H), 4.57 (m, 1H), 4.27 (td, *J* = 4.1, 13.6 Hz, 1H), 3.53 (m, 1H), 2.73 (s, 3H), 1.75 (d, *J* = 6.9 Hz, 3H); ^{19}F-NMR ($CDCl_3$): δ -98.4 (s, 1F); ^{13}C-NMR ($CDCl_3$): δ 174.6, 1 73.9, 170.1, 164.2 (d, *J* = 249 Hz), 154.0, 145.8, 131.0, 129.4 (d, *J* = 9 Hz), 116.2 (d, *J* = 22 Hz), 48.3, 45.2, 38.0, 19.8, 18.9. Elemental analysis: calc C, 53.62; H, 4.22; N, 23.45; S, 8.95; found C, 53.70; H, 4.55; N, 23.41; S, 8.87; mp. = 166.8 – 168.6°C. HRMS: calc 359.1091, found 359.1093, $C_{16}H_{16}FN_6OS$.



(*R*)-(4-Fluorophenyl)(8-methyl-3-(5-methyl-1,3,4-thiadiazol-2-yl)-5,6-dihydro-[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl)methanone (9): Starting from intermediate **37** and 4-fluorobenzoyl chloride as per General Procedure D to obtain 136 mg of crude **9**, which was purified by silica gel flash chromatography (0 to 2 % MeOH in EtOAc) to afford 20 mg as white solid (10 %). LCMS (Method A) *m/z* 359 (M + 1). HPLC purity 99% (254 nm). ^{1}H-NMR ($CDCl_3$): δ 7.47 (dd, *J* = 5.4, 8.4 Hz, 2H), 7.16 (t, *J* = 8.4 Hz, 2H), 5.80 (m, 1H), 4.94 (dd, *J* = 2.9, 13.3 Hz, 1H), 4.52 (m, 1H), 4.32 (td, *J* = 3.6, 13.0 Hz, 1H), 3.58 (m, 1H), 2.85 (s, 3H), 1.74 (d, *J* = 6.8 Hz, 3H); ^{19}F-NMR ($CDCl_3$): δ -98.4 (s, 1F); ^{13}C-NMR ($CDCl_3$): δ 170.1, 165.7, 164.0 (d, *J* = 252 Hz), 157.3, 153.9, 145.3, 130.7 (d, *J* = 30 Hz), 129.3 (d, *J* = 8 Hz), 116.3 (d, *J* = 22 Hz), 45.6,[10] 19.8, 15.6. HRMS: calc 359.1090, found 359.1104, $C_{16}H_{16}FN_6OS$.

(*R*)-(4-Fluorophenyl)(8-methyl-3-(3-methyl-1,2,4-oxadiazol-5-yl)-5,6-dihydro-[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl)methanone (10): Starting from intermediate **38** and 4-fluorobenzoyl chloride as per General Procedure D to obtain 1.03 g of crude **10**, which was purified by silica gel flash chromatography (EtOAc) to afford 0.75 g as white solid (97 %). LCMS (Method A) *m/z* 343 (M + 1). HPLC purity 99% (254 nm). Chiral LC (Method F) 98.8 %ee ($t_{R(R)}$ = 14.9 min, $t_{R(S)}$ = 17.3 min). ^{1}H-NMR ($CDCl_3$): δ 7.41 (m, 2H), 7.10 (t, *J* = 8.5 Hz, 2H), 5.72 (m, 1H), 4.74 (dd, *J* = 3.3, 13.5 Hz, 1H), 4.54 (m, 1H), 4.21 (td, *J* = 4.0, 12.8 Hz, 1H), 3.49 (m, 1H), 2.44 (s, 3H), 1.68 (d, *J* = 6.9 Hz, 3H); ^{19}F-NMR ($CDCl_3$): δ -98.6 (s, 1F); ^{13}C-NMR ($CDCl_3$): δ 170.1, 167.8, 164.7, 164.0 (d, *J* = 252 Hz), 130.4 (d, *J* = 3 Hz), 129.3 (d, *J* = 9 Hz), 116.2 (d, *J* = 23 Hz), 45.2,[10] 19.6, 11.6. HRMS: calc 343.1319, found 343.1321, $C_{16}H_{16}FN_6O_2$.

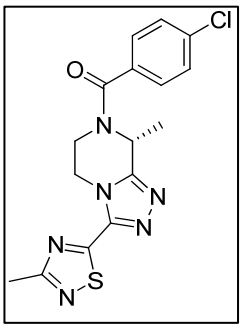
(*R*)-(4-Fluorophenyl)(3-(3-isopropyl-1,2,4-oxadiazol-5-yl)-8-methyl-5,6-dihydro-[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl)methanone (11): Starting from intermediate **39** and 4-fluorobenzoyl chloride as per General Procedure D to obtain 0.5 g of crude **11**, which was purified by silica gel flash chromatography (EtOAc) to afford 0.25 g as white solid (51 %). LCMS (Method A) *m/z* 371 (M + 1). HPLC purity > 99% (254 nm). Chiral LC (Method H) 98.6 %ee ($t_{R(S)}$ = 4.0 min, $t_{R(R)}$ = 4.2 min). ^{1}H-NMR ($CDCl_3$): δ 7.49 (dd, *J* = 5.5, 8.2 Hz, 2H), 7.17 (t, *J* = 8.2 Hz, 2H), 5.78 (m, 1H), 4.83 (dd, *J* = 3.5, 13.5 Hz, 1H), 4.67 (m, 1H), 4.29 (td, *J* = 4.0, 11.2 Hz, 1H), 3.58 (td, *J* = 4.4, 15.2 Hz, 1H), 3.20 (h, *J* = 6.9 Hz, 1H), 1.76 (d, *J* = 6.8 Hz, 3H), 1.40 (d, *J* = 6.9 Hz, 6H); ^{19}F-NMR ($CDCl_3$): δ -98.7 (s, 1F); ^{13}C-NMR ($CDCl_3$): δ 175.5, 170.0, 164.5, 163.9 (d, *J* = 252 Hz), 154.3, 140.9, 130.4 (d, *J* = 3 Hz), 129.3 (d, *J* = 9 Hz), 116.1 (d, *J* = 22 Hz), 45.1,[10] 26.7, 20.4, 19.5. HRMS: calc 371.1632, found 371.1634, $C_{18}H_{20}FN_6O_2$.

(4-Fluorophenyl)(3-(3-methyl-1,2,4-thiadiazol-5-yl)-5,6-dihydro-[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl)methanone (12)*:* Starting from intermediate **40** and 4-fluorobenzoyl chloride as per General Procedure D to obtain 3.5 g of crude **12**, which was crystallized by solubilized in boiling MeOH (130 mL) under stirring. The crystallization vessel was capped and left at 4°C for 14 h. The white crystals formed were filtered, washed with TBME (50 mL) and dried under high vacuum (< 0.2 mbar) to afford 2.08 g as white crystals (59 %). LCMS (Method A) *m/z* 345 (M + 1). HPLC purity 99% (254 nm). ^{1}H-NMR ($CDCl_3$): δ 7.51 (m, 2H), 7.18 (m, 2H), 5.28 (bs, 2H), 4.64 (m, 2H), 4.11 (m, 2H), 2.73 (s, 3H); ^{19}F-NMR ($CDCl_3$): δ -99.1 (s, 1F); ^{13}C-NMR ($CDCl_3$): δ 174.1, 173.9, 170.0, 164.1 (d, *J* = 252 Hz), 149.6, 145.8, 129.9, 129.8 (d, *J* = 8 Hz), 116.1 (d, *J* = 22 Hz), 44.8,[10] 18.9. HRMS: calc 345.0934, found 345.0934, $C_{15}H_{14}FN_6OS$.

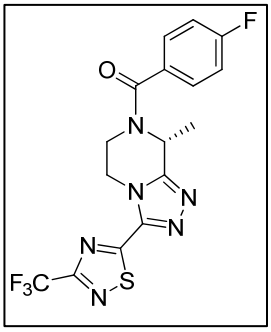
(*R*)-(4-Fluorophenyl)(8-methyl-3-(1,2,4-thiadiazol-5-yl)-5,6-dihydro-[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl)methanone (13)*:* Starting from intermediate **41** and 4-fluororbenzoyl chloride as per General Procedure D. The crude compound **13** obtained was purified by silica gel flash chromatography (1% MeOH in EtOAc) to afford 0.19 g as white solid (71 %). LCMS (Method A) *m/z* 345 (M + 1). HPLC purity 97% (254 nm). ^{1}H-NMR ($CDCl_3$): δ 8.77 (s, 1H), 7.48 (dd, *J* = 5.2, 13.8, 2H), 7.17 (m, 2H), 5.80 (m, 1H), 4.94 (dd, *J* = 3.5, 13.7 Hz, 1H), 4.59 (m, 1H), 4.31 (td, *J* = 4.6, 13.3 Hz, 1H), 3.55 (m, 1H), 1.76 (d, J = 6.9 Hz, 3H). ^{19}F-NMR ($CDCl_3$): δ -98.5 (s, 1F); ^{13}C-NMR ($CDCl_3$): δ 174.5, 170.1, 164.0 (d, *J* = 252 Hz), 162.9, 154.1, 145.5, 130.5, 129.2 (d, *J* = 8 Hz), 116.2 (d, *J* = 22 Hz), 45.1,[10] 19.7. HRMS: calc 345.0934, found 345.094, $C_{15}H_{14}FN_6OS$.

(8,8-Dimethyl-3-(3-methyl-1,2,4-thiadiazol-5-yl)-5,6-dihydro-[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl)(4-fluorophenyl)methanone (14)*:* Starting from intermediate **42** and 4-fluorobenzoyl chloride as per General Procedure D to obtain 80 mg as white solid (73 %). LCMS (Method A) *m/z* 373 (M + 1). HPLC purity 98% (254 nm). ^{1}H-NMR ($CDCl_3$): δ 7.54 (dd, *J* = 5.4, 8.3 Hz, 2H), 7.16 (t, *J* = 8.3 Hz, 2H), 4.62 (t, *J* = 4.9 Hz, 2H), 3.77 (t, *J* = 4.9 Hz, 2H), 2.73 (s, 3H), 2.12 (s, 6H); ^{19}F-NMR ($CDCl_3$): δ -98.5 (s, 1F); ^{13}C-NMR ($CDCl_3$): δ 174.5, 173.7, 171.8, 164.1 (d, *J* = 251 Hz), 159.8, 145.2, 132.5, 129.7 (d, *J* = 8 Hz), 116.2 (d, *J* = 22 Hz), 59.0, 45.4, 44.1, 26.2, 19.0 HRMS: calc 373.1247, found 373.1252, $C_{17}H_{18}FN_6OS$.

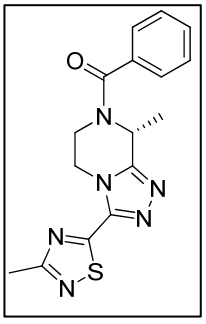
(*R*)-(4-Chlorophenyl)(8-methyl-3-(3-methyl-1,2,4-thiadiazol-5-yl)-5,6-dihydro-[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl)methanone (15)*:*** Starting from intermediate **7** and 4-chlorobenzoyl chloride as per General Procedure D to obtain crude **15**. This crude compound **15** was solubilized in boiling EtOAc (12 mL/mmol) under stirring. The solution was allowed to reach room temperature whereupon TBME was added (3 mL/mmol). The crystallization vessel was capped and left at room temperature for 14 h. The white crystals formed were filtered, washed with TBME and dried under high vacuum (< 0.2 mbar) to afford 1.01 g as white crystals (80 %). LCMS (Method A) *m/z* 375 (M + 1). HPLC purity 98% (254 nm). Chiral LC (Method G) > 99.8 %ee ($t_{R(S)}$ = 27.5 min, $t_{R(R)}$ = 34.6 min). ^{1}H-NMR ($CDCl_3$): δ 7.44 (m, 4H), 5.77 (m, 1H), 4.91 (dd, *J* = 3.6, 13.5 Hz, 1H), 4.59 (m, 1H), 4.25 (td, *J* = 4.1, 13.6 Hz, 1H), 3.54 (m, 1H), 2.73 (s, 3H), 1.75 (d, J = 6.9 Hz, 3H); ^{13}C-NMR ($CDCl_3$): δ 174.3, 173.8, 169.9, 153.8, 145.6, 137.0, 132.9, 129.3, 128.4, 45.1,[10] 19.7, 19.0. HRMS: calc 375.0795, found 375.0803, $C_{16}H_{16}ClN_6OS$.



(*R*)-(4-Fluorophenyl)(8-methyl-3-(3-(trifluoromethyl)-1,2,4-thiadiazol-5-yl)-5,6-dihydro-[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl)methanone (16)*:*** Starting from intermediate **44** and 4-fluorobenzoyl chloride as per General Procedure D. The crude compound **16** obtained was purified by silica gel flash chromatography (20 to 50 % EtOAc in cyclohexane) to afford 76 mg as white solid (27 %).[9] LCMS (Method A) *m/z* 413 (M + 1). HPLC purity 91% (254 nm). Chiral LC (Method F) 98.8 %ee ($t_{R(S)}$ = 6.3 min, $t_{R(R)}$ = 8.4 min). ^{1}H-NMR ($CDCl_3$): δ 7.48 (m, 2H), 7.18 (m, 2H), 5.84 (m, 1H), 4.92 (dd, *J* = 3.1, 13.6 Hz, 1H), 4.60 (m, 1H), 4.32 (td, *J* = 4.2, 12.8 Hz, 1H), 3.55 (m, 1H), 1.77 (d, *J* = 6.9 Hz, 3H); ^{19}F-NMR ($CDCl_3$): δ -62.9 (s, 3F), -98.7 (s, 1F); ^{13}C-NMR ($CDCl_3$): δ 177.5, 170.1, 164.1 (d, *J* = 252 Hz), 163.0 (d, *J* = 39 Hz), 154.8, 144.8, 130.4 (d, *J* = 4 Hz), 129.4 (d, *J* = 8 Hz), 119.8, 116.3 (d, *J* = 22 Hz), 45.2,[10] 19.7. HRMS: calc 413.0808, found 413.0811, $C_{16}H_{13}F_4N_6OS$.

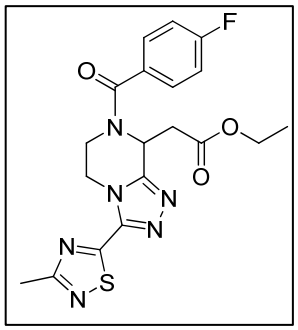


(*R*)-(8-Methyl-3-(3-methyl-1,2,4-thiadiazol-5-yl)-5,6-dihydro-[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl)(phenyl)methanone (17)*:*** Starting from intermediate **7** and benzoyl chloride as per General Procedure D. The crude compound **17** obtained was solubilized in boiling EtOAc (13 mL/mmol) under stirring. The solution was allowed to reach room temperature whereupon TBME was added (3 mL/mmol). The crystallization vessel was capped and left at room temperature for 14 h. The white crystals formed were

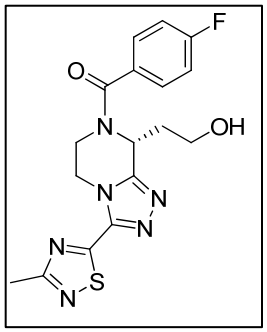


filtered, washed with TBME and dried under high vacuum (< 0.2 mbar) to afford 0.73 g as white crystals (63 %). LCMS (Method A) *m/z* 341 (M + 1). HPLC purity 98% (254 nm). Chiral LC (Method G) 94.4 %ee ($t_{R(R)}$ = 23.1 min, $t_{R(S)}$ = 34.8 min). ^{1}H-NMR ($CDCl_3$): δ 7.46 (m, 5H), 5.77 (m, 1H), 4.89 (dd, *J* = 3.6, 13.5 Hz, 1H), 4.60 (m, 1H), 4.26 (td, *J* = 4.1, 13.6 Hz, 1H), 3.52 (m, 1H), 2.73 (s, 3H), 1.74 (d, J = 6.9 Hz, 3H); ^{13}C-NMR ($CDCl_3$): δ 174.5, 173.9, 154.1, 145.6, 134.6, 130.8, 129.1, 126.9, 45.3,[10] 19.0. HRMS: calc 341.1184, found 341.1201, $C_{16}H_{17}N_6OS$.

Ethyl 2-(7-(4-fluorobenzoyl)-3-(3-methyl-1,2,4-thiadiazol-5-yl)-5,6,7,8-tetrahydro-[1,2,4]triazolo [4,3-*a*]pyrazin-8-yl)acetate (45)*:*** Starting from intermediate **43** and 4-fluorobenzoyl chloride as per General Procedure D to obtain crude **45** (1.9 g), which was solubilized in boiling EtOAc (15 mL) under stirring. The solution was allowed to reach room temperature whereupon TBME was added (45 mL). The crystallization vessel was capped and left at room temperature for 14 h. The white crystals formed were filtered, washed with TBME (10 mL) and dried under high vacuum (< 0.2 mbar) to afford 1.35 g as white crystals (72 %). LCMS (Method A) *m/z* 431 (M + 1). HPLC purity 98% (254 nm).



Step e. **(*R*)-(4-Fluorophenyl)(8-(2-hydroxyethyl)-3-(3-methyl-1,2,4-thiadiazol-5-yl)-5,6-dihydro-[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl)methanone (18).** To a solution of **45** (1.3 g, 3.02 mmol, 1 equiv.) in anhydrous THF (13 mL) at -40°C under N_2 was added lithium aluminum hydride (0.115 g, 3.02 mmol, 1 equiv.) in one portion. The mixture was stirred for 10 min. at -40°C and then quenched with NaOH 1M (5 mL), and the mixture was stirred for 45 min at room temperature. The reaction mixture was extracted with CH_2Cl_2 (2x 50 mL) and combined organic layer was dried over $MgSO_4$, filtered and concentrated to dryness to afford 1.1 g of crude **18**. This residue was purified by silica gel flash chromatography (0 to 5 % MeOH in CH_2Cl_2) to afford 770 mg (66%).[11] LCMS (Method A) *m/z* 389 (M + 1). HPLC purity 98% (254 nm). This product was then purified by preparative chiral HPLC purification (Chiralpak IA 5µ 20x250mm, Hex/EtOH (1:1 v/v) + 0.1% DEA, 20 mL/min) to afford 245 mg (37%). LCMS (Method A) *m/z* 389 (M + 1). HPLC purity 99% (254 nm).Chiral LC (Method I) 97. %ee ($t_{R(S)}$ = 7.2 min, $t_{R(R)}$ = 13.1 min). ^{1}H-NMR ($CDCl_3$): δ 7.49 (m, 2H), 7.19 (m, 2H), 6.15 (m, 1H), 4.92 (m, 1H), 4.26 (m, 2H), 3.85 (m, 2H), 3.60 (m, 1H), 3.35 (m, 1H), 2.72 (s, 3H), 2.42 (m, 1H), 2.21 (m, 1H); ^{19}F-NMR ($CDCl_3$): δ -98.7 (s, 1F); ^{13}C-NMR ($CDCl_3$): δ 174.0, 173.9, 170.6, 163.9 (d, *J* = 252 Hz), 153.2, 145.6, 130.3 (d, *J* = 3 Hz), 129.4 (d, *J* = 8 Hz), 116.2 (d, *J* = 22 Hz), 58.2, 45.3,[10] 36.3, 18.9. HRMS: calc 389.1196, found 389.1194, $C_{17}H_{18}FN_6O_2S$.



2. X-ray crystallography reports on compound 3: Accesion code CCDC 1052911.

We are grateful to Dr. Koen Robeyns at Univeristé Catholique de Louvain for X-ray crystallographic characterization detailed here.

All data were recorded on a MAR345 image plate (MARRESEARCH) using MoKα radiation (λ=0.71073). X-rays were generated on a RIGAKU rotating anode generator with power settings of 50KV and 70mA. A Zr filter is used to eliminate the MoKβ radiation.

A suitable crystal was chosen under a microscope, mounted in a nylon loop and aligned on the goniometer prior to the x-ray experiment. A total of 200 images corresponding to a 1.5° phi rotation were collected at room temperature.

The reflections on de diffraction images were indexed and integrated using the Crysalis data processing suite - Version 1.171.35.19 (Agilent). During the integration the friedel pairs were kept unmerged in order to preserve the anomalous signal needed for absolute structure determination.

Data were corrected for absorption "Empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm"

Structure solution was performed by SHELXS and the refinement was done by SHELXL ("A short history of SHELX". Sheldrick, G.M. (2008). Acta Cryst. A64, 112-122).

Chirality:

The chirality around atom C11 is R.

This is based on the Flack parameter (H. D. Flack (1983). "On Enantiomorph-Polarity Estimation". Acta Cryst A39: 876–881.) as well as the Hooft parameter (J. Appl. Cryst. (2008), 41, 96-103, [Hooft, Straver & Spek].)

Flack -0.02(11)

Hooft -0.01(6) ; P2(tr) 1.000, P3(tr) 1.000, Bijvoet Pair Coverage (Perc) = 98

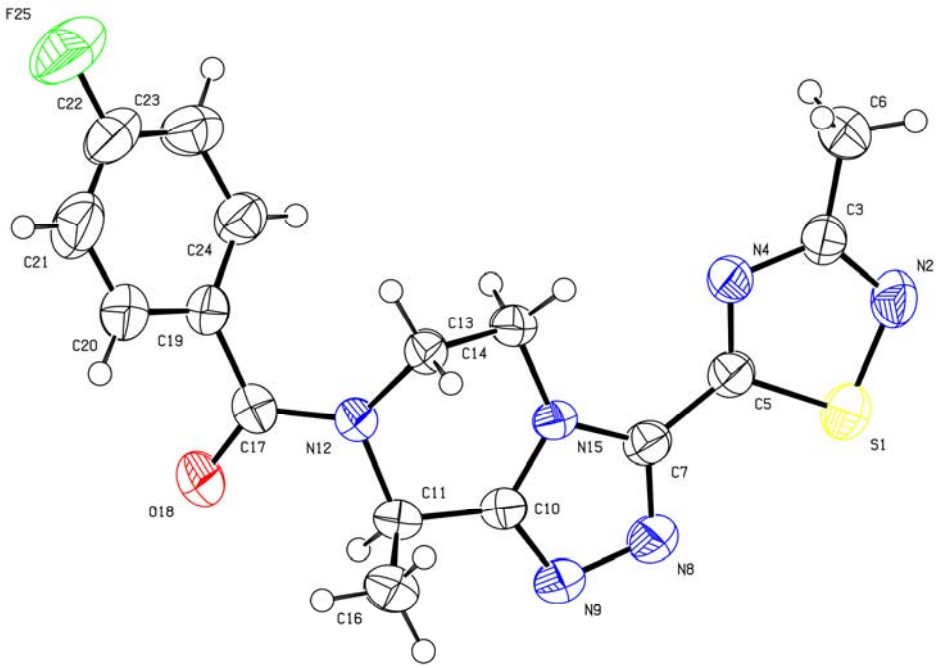


Figure S1: compound **3** with thermal displacement ellipsoids drawn at the 50% probability level.

3. In vitro pharmacology characterization of analogues (RLB and Ca^{2+} aequorin assays)

The radioligand binding (RLB) assay was performed through displacement of [^{3}H]-labeled SB222200 from recombinant human NK_3R in CHO cells. (See: Sarau, H. M.; Griswold, D. E.; Potts, W. ; Foley, J. J.; Schmidt, D. B.; Webb, E. F.; Martin, L. D.; Brawner, M. E.; Elshourbagy, N. A.; Medhurst, A. D.; Giardina, G. M.; Hay, D. W. P. Nonpeptide tachykinin receptor antagonists: I. Pharmacological and pharmacokinetic characterization of SB223412, a novel, potent and selective neurokinin-3 receptor antagonist. *J. Pharmacol. Exp. Ther.* **1997**, *281*, 1303-1311.)

Confirmation of NK_3R antagonist activity was made through aequorin Ca^{2+} bioluminescence functional assay measuring inhibition of NKB-induced Ca^{2+} signaling in CHO cells expressing recombinant human NK_3R (See: Le Poul, E.; Hisada, S.; Mizuguchi, Y.; Dupriez, V. J. ; Burgeon, E. ; Detheux, M. Adaptation of aequorin functional assay to high throughput screening *J. Biomol. Screening* **2002**, *7,* 57-65.)

The functional assay data established the foregoing compounds as antagonists of the human NK_3R receptor as with the reference compound SB222200.

Linear regression analysis for RLB versus aequorin functional data displayed an excellent fit with r^2 = 0.96.

The RLB (pK_i) and functional aequorin Ca^{2+} (pIC_{50}) data reported were obtained with the assays performed on each compound at least in triplicate with standard deviation ≤ 0.3.

4. hERG Patch-Clamp Assay.

The assay was outsourced to IPS Thérapeutique (Sherbrooke, Québec, Canada) and was run as follows. This assay quantifies the *in vitro* effects of the test compound on the potassium-selective (IK_r) current generated in normoxic conditions in stably transfected HEK 293 cells with the human ether-a-go-go-related gene (hERG). Whole-cell currents (acquisition by manual patch-clamp) elicited during a voltage pulse were recorded in baseline conditions and following application of the test compound after 5 min exposure at 0.3, 3, 10 and 30 µM concentrations.. The pulses protocol applied is as follow: the holding potential (every 3 seconds) was stepped from −80 mV to a maximum value of +40 mV, starting with −40 mV, in eight increments of +10 mV, for a period of 1 second. The membrane potential was then returned to −55 mV, after each of these incremented steps, for 1 second and finally repolarized to −80 mV for 1 second. The current density recorded were normalized against the baseline conditions and corrected for solvent effect. Inhibition curves were obtained for compounds and the concentrations which decreased about 50% of the current density determined in the baseline conditions (IC_{50}) were determined. hERG assays were carried out in triplicate (N = 3) with percent coefficient of variation (%CV) of < 6%.

5. CYP P450 Profiling.

P450-Glo™ Screening assay (Promega) was used to evaluate the potential of the test compounds to inhibit CYP P450 isoforms, specifically 1A2, 2C9, 2C19, 2D6 and 3A4. These assays employ luminogenic CYP450 probe substrates that are derivatives of beetle luciferin, a substrate for luciferase enzymes. The derivatives are converted by P450s cytochrome to luciferin, which in turn reacts with luciferase to produce an amount of light that is directly proportional to the activity of the P450. P450-Glo™ assays are performed in two steps, the P450-Glo™ subtrates are first converted by cytochrome P450 enzyme to a luciferin product which is then detected as a luminescent signal from a luciferase reaction. To perform the assay, the cytochrome P450 mixture with cytochrome P450 enzyme and a P450- Glo™ substrate is prepared at pH 7.4 in a phosphate buffer at the optimal concentration for each cytochrome P450 isoform. The test compounds are measure in duplicates over a concentration range from 30 nM to 100 µM. Luciferin-free water + 0.1% DMSO was used as negative control and a known inhibitor as positive control. The reactions are initiated by adding the NADPH regeneration system and are performed at 37 °C. Luciferin detection reagent is added to stop cytochrome P450 activity and initiate the D-luciferin detection reaction. The IC_{50} value of the compound was then determined based on analysis of the data thus obtained.

6. Pharmacokinetic (PK) Studies.

Rat PK studies were conducted on male Sprague-Dawley rats (250 ± 20 g). Three to four rats were housed per cage in a temperature-controlled room (22 ± 2°C) and 50 ± 10% relative humidity with a 12 h/12 h light/dark cycles. For iv bolus administration, the formulated test compound was administrated at a single dose (1 mg/kg; N = 3) with blood samples drawn into tubes containing K_3EDTA at the following time points post-dosing: T = 1, 5, 15, 90, 150, 210, 300, 390 min and 24 h. For oral administration, the formulated test compound was administrated by gavage at 3 mg/kg (N = 4 rats) with blood samples drawn in EDTA tubes at the following time points post-dosing: T = 5, 15, 45, 90, 150, 210, 300, 390 min and 24 h. Plasma samples were then immediately isolated by centrifugation and stored at –20 °C prior to analysis. Plasma concentrations were determined by LCMS/MS and data were analyzed by non-compartmental methods using PK Solutions 2.0 software (Summit Research Services, CO, USA).

Monkey PK studies were outsourced to PHARMARON (China) and performed on male cynomolgus monkeys castrated at sexual maturity and tested > 6 months after castration (4.5 ± 0.5 kg; N = 4); monkeys tested repeatedly under all conditions with a minimum of 1-week washout period between treatments. Intravenous (bolus) dosing 10 mg/kg formulated in physiological saline with 9% 2-hydroxypropyl-β-cyclodextrin. Orally doses compounds were formulated in 0.5% methylcellulose/water. Over the 8h test period, at the timepoints indicated in Fig. 6, blood samples were collected into centrifuge tubes containing K_3EDTA and plasma samples were then immediately isolated by centrifugation and stored at –20 °C prior to analysis. Plasma concentrations were determined by LCMS/MS and data were analyzed by non-compartmental methods using WinNonlin Pro (Pharsight Corp., Mountain View, CA).

7. Plasma Protein (f_u) & Brain Non-specific Binding (bf_u) Determinations.

Equilibrium dialysis approach was employed to determine plasma protein binding (f_u) and non-specific brain binding (bf_u). Using a RED (Rapid Equilibrium Dialysis) device insert, which is made of two side-by-side chambers separated by an O-ring-sealed vertical cylinder of dialysis membrane (MW cut-off ca 8000 Da), plasma or brain homogenate containing test compound (at 5 μM) is added to one chamber, while PBS pH 7.4 buffer is added to the second. After 4 h incubation at 37 °C with agitation, aliquots from both chambers are then analyzed using LCMS/MS to determine both free and bound levels.

8. Caco2 Permeability Determination

Based on the relatively high solubility and low log$D_{7.4}$ for the analogues in this report, i.e. high mass balance compound for absorption projection, the apparent permeability coefficient (P_{app}) was determined *without* addition of BSA in the receiver compartement. To recall, the P_{app} values, reported in Part I to this report were obtained in the presence of 0.1% BSA,[1] given that the majority of compounds therein were more lipophilic,. Comparison for Caco-2 values obtained with (Table 5 in the Part I report[1]) and without (herein) BSA for the two analogues that appeared in both reports is shown below. Our findings are consistent the fact that BSA is known to improve permeability from both transport directions with minimal effect on efflux substrate identification.[12]

Cpd (Part I[1] /this work)	P_{app} (nm/sec) with 0.1% BSA (Part I)[1]			P_{app} (nm/sec) without BSA (this work)		
	AB	BA	ER	AB	BA	ER
31 / **2**	482	529	0.9	339	224	0.7
39 / **8**	609	628	1.0	360	221	0.6

Method. Caco-2 cells provided from ATCC (CRL-2102; C2BBe1 clone) were cultured in DMEM containing 1% penicillin-streptomycin and supplemented with 10% fetal bovine serum. The Caco-2 cells were seeded on polycarbonate membrane filters in a 24 transwell plate with cell density = 85000 cells/cm^2. The cells were cultured at 37 ºC in an atmosphere of 5% CO_2 with a relative humidity of 95% over a 21-day period with the culture media from both apical and basolateral compartments are refreshed 3 times per week. On the day of the experiment, the monolayers were washed twice for 30 min with pre-warmed (37°C) Hanks Balanced Salt Solution (HBSS-HEPES, pH 7.4) to stabilize physiological parameters. The dosing solutions were prepared from DMSO stock solution in the assay buffer with 1% final DMSO concentration. Test compound permeability was assessed in duplicate.

For assessment of the apical to basolateral (AB) permeability, the HBSS is removed from both compartments and the dosing solution was added to the apical side whereas fresh buffer assay was added to the basolateral side. For assessment of BA permeability, HBSS is removed from both compartments and replaced by test compound dosing solution in basolateral side or fresh buffer assay to the apical side. The starting concentration (C_0) is determined from the dosing solution. The assay plate was incubated at 37 °C without shaking, for 45 min in the AB permeability assessment and for 15 min in the BA permeability assessment to assume sink conditions for highly permeable compounds. At the end of the incubation, samples were taken from both the donor and receiver sides after separation of the apical compartment inserts from the companion plates. Samples with appropriate dilution were

quantified by LC-MS/MS using 8-point calibration curve. The apparent permeability value P_{app} expressed as nm/sec is calculated based on the appearance rate of compound in the receiver side from the following equation: P_{app} = (dQ/dt)/ (C_0 × A) where dQ/dt is the rate of permeation of the drug across the cells, C_0 is the donor compartment concentration at time zero and A is the area of the cell monolayer. The efflux ratio (ER) was determined as follows: ER = (P_{app} BA) / (P_{app} AB)**.** The experimental recovery was calculated from C_0 and both the apical and basolateral compartment concentrations and the results expressed as %cpd initially added to the donor compartment. The integrity of the monolayer throughout the experiment was verified by monitoring fluoresceine permeation after 1 h at 37 °C using UV spectrometer at 490 nm. To assess good integrity membrane, the %fluorescein in the basolateral compartment was required to be lower than 4% versus the reference solution corresponding to a 100% permeability of fluorescein from A to B compartments.

9. Log$D_{7.4}$ Determination[13]

A 20 µL aliquot of 10 mM DMSO solution of the test compound was evaporated to dryness (Genevac at 40°C), whereupon 440 µL of 1-octanol (pre-saturated with phosphate buffer) was added. After sonication and vortexing the sample solution, 440 µL of pH 7.4 phosphate buffer (0.01M, pre-saturated with 1-octanol) was added. The capped vial was agitated during 5 h using an orbital shaker at 40 rpm and thereafter centrifuged during 15 min at 3000 rpm. The compound distribution between 1-octanol and phosphate buffer phases was determined by HPLC/UV analysis.

The following equation was used to calculate the log$D_{7.4}$ value in each case:

$$logD_{7.4} = LOG_{10}\left(\frac{v_{buf}}{v_{oct}} \times \frac{AREA_{oct}}{AREA_{buf}}\right)$$

wherein, $AREA_{buf}$ and $AREA_{oct}$ correspond to peak area for injection from phosphate buffer or 1-octanol phase and v_{buf} and v_{oct} correspond to injection volume from phosphate buffer or 1-octanol phase (i.e., 2µL and 20µL for the compounds in this paper, respectively).

For each compound log$D_{7.4}$ measurement was carried out in triplicate and the average value is reported.

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[1] Hoveyda, H. R.; Fraser, G. L.; Roy, M.-O. ; Dutheuil, G.; Batt, F.; El Bousmaqui, Korac, J.; Lenoir, F.; Lapin, A.; Noël, S.; Blanc, S. *J. Med. Chem*., **2015**, *58*, 3060-3082.

[2] Commercial grade reagent did not always afford the best chiral purity in the imidate formation step. Freshly prepared Et_3OBF_4 is a free flowing white powder (transparent pasty solid to be avoided!); it was stored under anhydrous inert N_2 (or Ar) atmosphere at –20 °C. An appropriate test of the suitability of this reagent is its solubility in CH_2Cl_2: fresh reagent is more soluble (200 mg in 2 mL) vs partially degraded reagent requiring 10 mL or more to dissolve. Et_3OBF_4 can be easily prepared from epichlorohydrin and $BF_3{\cdot}OEt_2$ as described in: Meerwein, H. Triethyloxonium flurorborate. *Org. Synth.* **1966**, *46*, 113. The quality of the Et_3OBF_4 was also an important parameter in ensuring the best chiral purity during the imidate formation step.

[3] No chiral analytical conditions were found for this substrate.

[4] Hydrazide hydrate is commercially available.

[5] This purity was inferior to our standard criteria and typical results. However, it was sufficiently pure to afford the final target with high purity. As with all other intermediates, i.e. **29-36**, silica gel flash chromatography could be performed easily to isolate purer intermediates.

[6] The white solid filtered at this stage is the by-product of DMB deprotection, which is likely polymeric in nature based on its insoluble nature and related obseravtions in the literature. For example, it is reported by Nussbaumer et al. that DMB-trifluoroacetamide is prone to polymerization "with a few drops of acid (TFA, 1N HCl)" (Nussbaumer, P.; Baumann, K.; Dechat, T.; Harasek, M. *Tetrahedron*, **1991**, *47*, 4591-4602.) Moreover, the reactivity of the 2,4-DMB cation generated transiently during the deprotection has been noted by Wood et al., who used a large excess of thioanisole, as a cation scavenger, to suppress formation of further by-products derived from alkylation of their indolocarbazole substrate with the benzyl carbocation (See Scheme 13 and footnote 45 in Wood, J. L.; Stoltz, B. M.; Diterich, H.-J.; Pflum, D. A.; Petsch, D.

T. *J. Am. Chem. Soc.* **1997**, *119*, 9641-9651). Thioanisole scavenger was not very effective in our hands in suppressing the formation of the insoluble by-product during deprotection and since alkylation side-product formation was not a caveat herein, we opted to use TFA without thioanisole scavenger.

[7] The additional purification step was warranted for the subsequent so-called innate C–H trifluoromethylation reaction (see reference 8) to prepare intermediate **44**.

[8] Ji, Y.; Burecki, T.; Baxter, R. D.; Fujiwara, Y.; Seiple, I. B.; Su, S.; Blackmond, D. G.; Baran, P. S. *Proc. Natl. Acad. Sci. U. S. A* **2011**, *108*, 14411-14415.

[9] A less pure fraction was collected too to afford further 80 mg (28%) with 80% purity, which was then purified by preparative RP –HPLC to furnish extra pure sample (HPLC purity > 98% (254 nm)).

[10] Due to slow relaxation, several Csp^3 carbons in the piperazine Ring B were not readily detectable.

[11] This alcohol has the tendency to undergo amide bond cleavage under the reaction conditions. Corresponding amino alcohol was isolated in 31% yield (250 mg).

[12] See for instance: Liu, T. ; Chang, L.-J. ; Uss, A. ; Chu, I. ; Morrison, R. A. ; Wang, L. ; Prelusky, D. ; Cheng, K.-C.; Li, C. *J. Pharm. Biomed. Anal.* **2010**, *51*, 1069-1077.

[13] The procedure used in our manuscript is adapted from the following reference: Alelyunas, Y. W. ; Kilby-Pelosi, L.; Turcotte, P.; Kary, M.-B.; Spreen, R. C. *J. Chromatog. A.* **2010,** *1217*, 1950-1955.