Electronic Supplementary Material (ESI) for MedChemComm.

Discovery of 2-(5,6-dimethoxypyridin-3-yl)-4-(2,4,6-trifluorobenzyl)-2*H*-pyrido[2,3-*e*][1,2,4]thiadiazin-3(4*H*)-one 1,1-dioxide (HTL6641), a dual orexin receptor antagonist with differentiated pharmacodynamic properties.

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Table 1. Commercial sources of **6**, **7**, **10**, **11**, **28**.[a]

Compound	Source	Supplier ID
6	ChemDiv	C200-9385
7	ChemDiv	K261-2686
10	ChemDiv	K261-2683
11	ChemDiv	K261-2679
28	ChemDiv	C260-0428

[a]All other compounds were synthesized according to the details in Table 3.

Table 2. LCMS and ^{1}H NMR QC data for **6-28**.

Compound	MW	LCMS purity[a]	MS data m/z (ESI +)	Retention Time (min)	LCMS method[b]	NMR data[c]
6	468.5	95%	469.3	3.97	Method 1	n/d
7	424.5	95%	425.1	4.21	Method 1	n/d

8	442.5	> 98%	442.9	3.98	Method 2	($CDCl_3$) δ: 3.91 (s, 3H), 3.93 (s, 3H), 5.44 (s, 2H), 6.96-6.98 (m, 2H), 7.05-7.13 (m, 3H), 7.19-7.29 (m, 3H), 7.33 (t, *J*=7.5 Hz, 1H), 7.60 (t, *J*=7.3 Hz, 1H), 7.96 (d, *J*=7.8 Hz, 1H).
9	476.9	> 98%	476.9, 478.8	4.50	Method 2	($CDCl_3$) δ: 3.90 (s, 3H), 3.93 (s, 3H), 5.39 (s, 2H), 6.94-6.97 (m, 2H), 7.04-7.10 (m, 2H), 7.14-7.20 (m, 3H), 7.35 (t, *J*=7.8 Hz, 1H), 7.62 (t, *J*=8.5 Hz, 1H), 7.96 (t, *J*=7.8 Hz, 1H).
10	476.9	95%	477.1, 479.1	4.74	Method 1	n/d
11	476.9	95%	477.1, 479.3	4.39	Method 1	n/d
12	478.4	> 98%	478.9	4.43	Method 2	(DMSO) δ: 3.72 (s, 3H), 3.82 (s, 3H), 5.46 (s, 2H), 6.87 (d, *J*=2.3 Hz, 1H), 6.92 (dd, *J*=8.5, 2.3 Hz, 1H), 7.08 (d, *J*=8.8 Hz, 1H), 7.22 (t, *J*=8.8 Hz, 2H), 7.45 (t, *J*=7.5 Hz, 1H), 7.73 (d, *J*=8.5 Hz, 1H), 7.82-7.90 (m, 1H), 7.94 (dd, *J*=8.0, 1.3 Hz, 1H).
13	494.9	> 98%	495.0, 497.0	4.59	Method 2	(DMSO) δ: 3.72 (s, 3H), 3.82 (s, 3H), 5.46 (s, 2H), 6.86 (d, *J*=2.3 Hz, 1H), 6.91 (dd, *J*=8.5, 2.3 Hz, 1H), 7.70 (d, *J*=8.5 Hz, 1H), 7.33-7.47 (m, 3H), 7.71 (d, 8.5, 1H), 7.85 (td, *J*=8.7, 1.4 Hz, 1H), 7.95 (dd, *J*=7.9, 1.4 Hz, 1H).

14	490.5	95 %	491.0	4.46	Method 2	(DMSO) δ: 3.73 (s, 3H), 3.79 (s, 3H), 3.83 (s, 3H), 5.37 (s, 2H), 6.76-6.92 (m, 3H), 6.96 (dd, *J*=8.5, 2.5 Hz, 1H), 7.10 (d, *J*=8.5 Hz, 1H), 7.39 (t, *J*=7.7 Hz, 1H), 7.65 (d, *J*=8.5 Hz, 1H), 7.75-7.85 (m, 1H), 7.89 (dd, *J*=7.9, 1.1 Hz, 1H).
15	490.9	> 98%	490.9, 492.9	4.79	Method 2	(DMSO) δ: 2.28 (s, 3H), 3.72 (s, 3H), 3.82 (s, 3H), 5.46 (s, 2H), 6.87 (d, *J*=2.3 Hz, 1H), 6.93 (dd, *J*=8.5, 2.5 Hz, 1H), 7.06-7.09 (m, 2H), 7.19 (br. s, 1H), 7.42 (t, *J*=7.7 Hz, 1H), 7.67 (d, *J*=8.5 Hz, 1H), 7.83 (td, *J*=7.9, 1.3 Hz, 1H), 7.94 (dd, *J*=7.8, 1.3 Hz, 1H).
16	490.5	> 98%	491.1	4.47	Method 2	(DMSO) δ: 3.73 (s, 3H), 3.75 (s, 3H), 3.82 (s, 3H), 5.42 (s, 2H), 6.74 (d, *J*=10.3 Hz, 2H), 6.87 (d, *J*=2.3 Hz, 1H), 6.93 (dd, *J*=8.5, 2.3 Hz, 1H), 7.09 (d, *J*=8.5 Hz, 1H), 7.43 (t, *J*=7.7 Hz, 1H), 7.70 (d, *J*=8.3 Hz, 1H), 7.81-7.88 (m, 1H), 7.93 (dd, *J*=7.8, 1.3 Hz, 1H).
17	506.9	> 98%	507.1, 509.1	4.59	Method 2	(DMSO) δ: 3.72 (s, 3H), 3.77 (s, 3H), 3.82 (s, 3H), 5.43 (s, 2H), 6.86–6.96 (m, 4H), 7.08 (d, *J*=8.8 Hz, 1H), 7.43 (t, *J*=7.7 Hz, 1H), 7.68 (d, *J*=8.3 Hz, 1H), 7.78-7.88 (m, 1H), 7.93 (dd, *J*=7.8, 1.3 Hz, 1H).
18	479.4	> 98%	480.1	4.25	Method 2	(DMSO) δ: 3.78 (s, 3H), 3.94 (s, 3H), 5.47 (s, 2H), 7.22 (t, *J*=8.8 Hz, 2H), 7.27 (d, *J*=2.3 Hz, 1H), 7.47 (t, *J*=7.5 Hz, 1H), 7.72 (d, *J*=2.0 Hz, 1H), 7.76 (d, *J*=8.5 Hz, 1H), 7.85-7.93 (m, 1H), 7.97 (dd, *J*=7.8 Hz, 1.3, 1H).
19	479.4	> 98%	480.1	4.95	Method 2	(DMSO) δ: 3.91 (s, 6H), 5.45 (s, 2H), 6.38 (s, 2H), 7.21 (t, *J*=8.8 Hz, 2H), 7.47 (t, *J*=7.7 Hz, 1H), 7.76 (d, *J*=8.5 Hz, 1H), 7.84-7.92 (m, 1H), 7.95 (dd, *J*=7.9, 1.1 Hz, 1H).

20	495.9	> 98%	496.1, 498.1	4.58	Method 2	(DMSO) δ: 3.77 (s, 3H), 3.93 (s, 3H), 5.47 (s, 2H), 7.26 (d, *J*=2.0 Hz, 1H), 7.37 (ddd, *J*=11.5, 9.3, 2.5 Hz, 1H), 7.41-7.52 (m, 2H), 7.71 (d, *J*=2.3 Hz, 1H), 7.74 (d, *J*=8.3 Hz, 1H), 7.83-7.92 (m, 1H), 7.98 (dd, *J*=7.9, 1.4 Hz, 1H).
21	449.4	> 98%	450.1	4.34	Method 1	(DMSO) δ: 3.91 (s, 3H), 5.45 (s, 2H), 6.85 (dd, *J*=1.8, 0.6 Hz, 1H), 7.03 (dd, *J*=5.5, 1.8 Hz, 1H), 7.22 (t, *J*=8.7 Hz, 2H), 7.45-7.50 (m, 1H), 7.77 (d, *J*=8.2 Hz, 1H), 7.87-7.98 (m, 2H), 8.33 (d, *J*=5.5 Hz, 1H).
22	491.5	> 98%	492.2	4.98	Method 2	(DMSO) δ: 3.75 (s, 3H), 3.91 (s, 6H), 5.41 (s, 2H), 6.39 (s, 2H), 6.74 (d, *J*=10.3 Hz, 2H), 7.45 (t, *J*=7.7 Hz, 1H), 7.73 (d, *J*=8.5 Hz, 1H), 7.83-7.90 (m, 1H), 7.94 (dd, *J*=7.8, 1.3 Hz, 1H).
23	491.5	> 98%	492.2	4.39	Method 2	(DMSO) δ: 3.75 (s, 3H), 3.78 (s, 3H), 3.94 (s, 3H), 5.43 (s, 2H), 6.74 (d, *J*=10.0 Hz, 2H), 7.27 (d, *J*=2.0 Hz, 1H), 7.45 (t, *J*=7.7 Hz, 1H), 7.70-7.77 (m, 2H), 7.82-7.91 (m, 1H), 7.95 (dd, *J*=7.8, 1.3 Hz, 1H).
24	491.5	> 98%	492.2	4.26	Method 2	(DMSO) δ: 3.75 (s, 3H), 3.80 (s, 3H), 3.86 (s, 3H), 5.41 (s, 2H), 6.73 (d, *J*=10.3 Hz, 2H), 7.03 (d, *J*=8.0 Hz, 1H), 7.40-7.50 (m, 2H), 7.73 (d, *J*=8.5 Hz, 1H), 7.82-7.88 (m, 1H), 7.91 (d, *J*=7.8 Hz, 1H).
25	491.5	> 98%	492.0	4.53	Method 2	(DMSO) δ: 2.61 (d, *J*=5.0 Hz, 3H), 3.71 (s, 3H), 3.89 (s, 3H), 5.50 (s, 2H), 5.70 (d, *J*=5.0 Hz, 1H), 6.57 (d, *J*=1.8 Hz, 1H), 6.67 (d, *J*=10.1 Hz, 2H), 7.29 (d, *J*=2.3 Hz, 1H), 7.45 (dd, *J*=7.8, 4.6 Hz, 1H), 8.42 (dd, *J*=8.0, 1.6 Hz, 1H), 8.78 (dd, *J*=4.8, 1.6 Hz, 1H).

26	480.4	> 98%	481.1	4.47	Method 2	(DMSO) δ: 3.82 (s, 3H), 3.85 (s, 3H), 5.58 (s, 2H), 7.08 (d, *J*=8.0 Hz, 1H), 7.19 (t, *J*=8.8 Hz, 2H), 7.46 (d, *J*=8.3 Hz, 1H), 7.52 (dd, *J*=7.9, 4.9 Hz, 1H), 8.47 (dd, *J*=7.9, 1.6 Hz, 1H), 8.83 (dd, *J*=4.9, 1.6 Hz, 1H).
27	480.4	> 98%	481.1	4.49	Method 2	(DMSO) δ: 3.77 (s, 3H), 3.93 (s, 3H), 5.59 (s, 2H), 7.19 (t, *J*=8.8 Hz, 2H), 7.35 (d, *J*=2.0 Hz, 1H), 7.53 (dd, *J*=7.8 Hz, 4.8, 1H), 7.75 (d, *J*=2.0 Hz, 1H), 8.51 (dd, *J*=7.8 Hz, 1.8, 1H), 8.85 (dd, *J*=4.9, 1.6 Hz, 1H).
28	422.9	95%	423.1	4.27	Method 1	n/d

[a] Data generated by Heptares. [b] QC Method 1: LCMS data with electrospray ionisation were generated under the following conditions. Instruments: HP 1100 with G1315A diode array detector, Micromass ZQ; Column: Waters X-Bridge C-18, 2.5 micron, 2.1 x 20 mm or Phenomenex Gemini-NX C-18, 3 micron, 2.0 x 30 mm; Gradient [time (min)/solvent D in C (%)]:0.00/2, 0.10/2, 8.40/95, 9.40/95; Solvents: solvent C = 2.5 L H_2O + 2.5 mL 28% aqueous ammonia solution; solvent D = 2.5 L MeCN + 135 mL H_2O + 2.5 mL 28% aqueous ammonia solution); Injection volume 1 uL; UV detection 230 to 400 nM; column temperature 45^oC; Flow rate 1.5 mL/min. QC Method 2: LCMS data with electrospray ionisation were generated under the following conditions. Instruments: Waters Alliance 2795, Waters 2996 PDA detector, Micromass ZQ; Column: Waters X-Bridge C-18, 2.5 micron, 2.1 x 20 mm or Phenomenex Gemini-NX C-18, 3 micron, 2.0 x 30 mm; Gradient [time (min)/solvent D in C (%)]: 0.00/2, 0.10/2, 8.40/95, 9.40/95, 9.50/2, 10.00/2;

Solvents: solvent C = 2.5 L H_2O + 2.5 mL ammonia solution; solvent D = 2.5 L MeCN + 135 mL H_2O + 2.5 mL ammonia solution); Injection volume 3 uL; UV detection 230 to 400 nM; column temperature 45°C; Flow rate 1.5 mL/min. [c] n/d = not determined. All ^1H NMR data are at 400 MHz.

Table 3: Synthetic routes for **8**, **9**, **12-26**.

Compound	Route	Compound	Route
8	A	**19**	A
9	A	**20**	B
12	A	**21**	B
13	B	**22**	A
14	B	**23**	A
15	B	**24**	A
16	A	**25**	C
17	B	**26**	C
18	A		

Synthetic details for preparation of intermediates and final compounds.

Unless otherwise stated, all reagents were commercially available and were used as supplied, without further purification. Chromatography refers to column chromatography performed using 60 - 120 mesh silica gel and executed under positive pressure (flash chromatography) conditions.

Synthetic routes A and B: Synthesis of **8**, **9**, **12**-**24** by alkylation (A) or Mitsonobu coupling (B).

(1) Typical procedures for the preparation of cyclized intermediates, exemplified by the preparation of 2-(3,4-dimethoxyphenyl)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (Intermediate 1) and 2-(5,6-Dimethoxypyridin-3-yl)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (Intermediate 2).

Preparation of 2-(3,4-dimethoxyphenyl)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (Intermediate 1).

***N*-(3,4-dimethoxyphenyl)-2-nitrobenzenesulfonamide**. A mixture of 2-nitrobenzenesulfonyl chloride (3.0 g, 13.5 mmol) and 3,4-dimethoxyaniline (2.1 g, 14.9 mmol) in 1,4-dioxane (30 mL) in a sealed reaction tube was heated at 80ºC for 24 h with TLC monitoring (hexane:EtOAc, 1:1). The reaction mixture was diluted with H_2O (30 mL), extracted with EtOAc (3 x 100 mL) and the combined organic phases dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by gradient flash chromatography, eluting with 20-25% EtOAc in hexane yielded the title compound (2.5 g, 7.39 mmol). Mass spectroscopy: *m/z* 339 $[M+H]^+$.

2-Amino-*N*-(3,4-dimethoxyphenyl)benzenesulfonamide. A mixture of *N*-(3,4-dimethoxyphenyl)-2-nitrobenzenesulfonamide (2.5 g, 7.39 mmol) and $SnCl_2$ (8.34 g, 36.9 mmol) in ethanol was heated at 100ºC for 5 h in a sealed tube with TLC monitoring (hexane:EtOAc, 1:1). After concentration *in vacuo* H_2O (30 mL) was added and the mixture was extracted with EtOAc (3 x 100 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by gradient flash chromatography, eluting with 20-25% EtOAc in hexane yielded the title compound (2.0 g, 6.49 mmol). Mass spectroscopy: *m/z* 309.1 $[M+H]^+$.

2-(3,4-Dimethoxyphenyl)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (Intermediate 1). A mixture of 2-amino-*N*-(3,4-dimethoxyphenyl)benzenesulfonamide (0.8 g, 2.6 mmol) and triphosgene (0.99 g, 3.4 mmol) in anhydrous 1,4-dioxane was heated in a sealed tube at 100ºC overnight with TLC monitoring (hexane:EtOAc, 1:1). After concentration *in vacuo* H_2O (30 mL) was added and the mixture was extracted with EtOAc (3 x 100 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by gradient flash chromatography, eluting with 20-25% EtOAc in hexane yielded the title compound (0.4 g, 1.20 mmol). Mass spectroscopy: *m/z* 335.1 $[M+H]^+$; ^1H NMR (400 MHz, DMSO) δ: 3.73 (s, 3H), 3.82 (s, 3H), 6.89-7.02 (m, 2H), 7.08 (d, *J*=8.5 Hz, 1H), 7.30-7.41 (m, 2H), 7.71-7.81 (m, 1H), 7.89 (d, *J*=7.3 Hz, 1H), 11.53 (s, 1H).

Preparation of 2-(5,6-dimethoxypyridin-3-yl)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (Intermediate 2).

***N*-(5,6-Dimethoxypyridin-3-yl)-2-nitrobenzenesulfonamide**. The title compound (1.56 g, 4.60 mmol) was prepared from 2-nitrobenzenesulfonyl chloride (2.66 g, 12.0 mmol), 5,6-dimethoxy-3-pyridinamine (2.04 g, 13.2 mmol) and pyridine (2.9 mL, 36.0

mmol) using the methods of Intermediate 1, step 1. Mass spectroscopy: *m/z* 340.1 $(M+H)^+$; ^{1}H NMR (400 MHz, DMSO) δ: 3.72 (s, 3H), 3.79 (s, 3H), 7.03 (d, *J*=2.3 Hz, 1H), 7.39 (d, *J*=2.0 Hz, 1H), 7-81-7.89 (m, 2H), 7.97 (ddd, *J*=7.3, 5.4, 1.9 Hz, 2H), 10.52 (s, 1H).

2-Amino-*N*-(5,6-dimethoxypyridin-3-yl)benzenesulfonamide. A mixture of *N*-(5,6-dimethoxypyridin-3-yl)-2-nitrobenzenesulfonamide (509 mg, 1.5 mmol), Fe powder (559 mg, 15.0 mmol) and acetic acid (10 mL) was heated at 60º for 45 min before cooling to rt and concentration *in vacuo*. After partitioning between EtOAc and H_2O, the aqueous phase was extracted with EtOAc and the combined organic phases were concentrated *in vacuo* to yield the title compound (486 mg) as a brown oil which was used without further purification. Mass spectroscopy: *m/z* 310.1 $(M+H)^+$; ^{1}H NMR (400 MHz, DMSO) δ: 3.66 (s, 3H), 3.76 (s, 3H), 6.00 (br. s, 2H), 6.54 (t, *J*=7.2 Hz, 1H), 6.77 (d, *J*=8.3 Hz, 1H), 6.91 (d, *J*=2.3 Hz, 1H), 7.17-7.28 (m, 1H), 7.33 (d, *J*=2.3 Hz, 1H), 7.40 (dd, *J*=8.0, 1.3 Hz, 1H), 10.00 (br. s, 1H).

2-(5,6-Dimethoxypyridin-3-yl)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (Intermediate 2). A mixture of 2-amino-*N*-(5,6-dimethoxypyridin-3-yl)benzenesulfonamide (486 mg, 1.57 mmol), 1,1′-carbonyldiimidazole (1.02 g, 6.29 mmol) and triethylamine (0.44 mL, 3.14 mmol) in DMF (3 mL) was heated in a sealed tube for 90 min at approximately 100ºC. After cooling to rt and concentration *in vacuo* the residue was partitioned between DCM and 1M (aq) HCl, and the organic phase was washed with brine. After standing, an initial crop of the title compound was isolated by filtration; this was combined with a further crop which was isolated by concentration of the filtrate *in vacuo* and trituration with Et_2O, to yield the title compound (257 mg, 0.77 mmol) as a pale brown solid. Mass spectroscopy: *m/z* 336.1 $(M+H)^+$; ^{1}H NMR (400 MHz, DMSO) δ: 3.79 (s, 3H), 3.94 (s, 3H), 7.18-7.52 (m, 3H), 7.63-7.85 (m, 2H), 7.93 (dd, *J*=8.2, 1.1 Hz, 1H), 11.65 (br. s, 1H).

The following cyclized intermediates were prepared according to the above procedures:

Intermediate	Product (yield)	Prepared from	Analytical data
Intermediate 3	2-(2,6-Dimethoxypyridin-4-yl)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (0.40 g, 1.19 mmol).	Prepared in three steps from 2-nitrobenzenesulfonyl chloride (2.66 g, 12.0 mmol), 2,6-dimethoxypyridin-4-amine (2.04 g, 13.2 mmol) and pyridine (2.9 mL, 36.0 mmol) in 1,4-dioxane (48 mL) using the methods of Intermediate 2.	*m/z* 336.1 $(M+H)^+$; ^{1}H NMR (400 MHz, DMSO) δ: 3.91 (s, 6H), 6.47 (s, 2H), 7.36 (t, *J*=8.2 Hz, 2H), 7.78 (dt, *J*=8.2, 0.9 Hz, 1H), 7.92 (d, *J*=7.8 Hz, 1H), 11.66 (br. s, 1H).
Intermediate 4	2-(5,6-Dimethoxypyridin-2-yl)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (0.30 g, 0.89 mmol).	Prepared in three steps from 2-nitrobenzenesulfonyl chloride (1.05 g, 4.72 mmol), 5,6-dimethoxypyridin-2-amine (0.80 g, 5.19 mmol) and pyridine (1.15 mL, 14.2 mmol) in 1,4-dioxane (19 mL) using the methods of Intermediate 2.	*m/z* 334.2 $(M-H)^-$; ^{1}H NMR (400 MHz, DMSO) δ: 3.82 (s, 3H), 3.86 (s, 3H), 7.10 (d, *J*=8.0 Hz, 1H), 7.32-7.41 (m, 2H), 7.46 (d, *J*=8.0 Hz, 1H), 7.72-7.82 (m, 1H), 7.89 (dd, *J*=8.0, 1.3 Hz, 1H), 11.59 (s, 1H).
Intermediate 5	2-(2-Methoxypyridin-4-yl)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (2.2 g, 7.21 mmol).	Prepared in three steps from 2-nitrobenzenesulfonyl chloride (16.0 g, 72.5 mmol), 2-methoxy-4-aminopyridine (10.0 g, 80.6 mmol) and triethylamine (8.95 g, 88.6 mmol) using the methods of Intermediate 1.	*m/z* 305.9 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO) δ: 3.92 (s, 3H), 6.94 (d, *J*=1.5 Hz, 1H), 7.11 (dd, *J*=5.3, 1.7 Hz, 1H), 7.30-7.45 (m, 2H), 7.72-7.85 (m, 1H), 7.93 (d, *J*=7.6 Hz, 1H), 8.34 (d, *J*=5.5 Hz, 1H), 11.75 (s, 1H).

(2) Preparation of benzyl alcohol intermediates:

Preparation of (2-chloro-4,6-difluorophenyl)methanol (Intermediate 6).

(4-Chloro-2,6-difluorophenyl)trimethylsilane. A solution of 2.5M n-BuLi in hexanes (60 mL, 0.15 mol) was added dropwise to a solution of 1-chloro-3,5-difluorobenzene (19.2 g, 0.13 mol) in THF (200 mL) cooled to -70°C under Ar. After the addition was complete, the mixture was stirred for 1 h before a solution of chlorotrimethylsilane (21.7 g, 0.2 mol) in THF (25 mL) was added dropwise. The reaction was allowed to warm to rt with stirring overnight before cooling and quenching with H_2O. The phases were separated and pentane (200 mL) was added; after washing with H_2O the solution was dried over $MgSO_4$ and carefully concentrated *in vacuo* to yield the title compound as a solution in THF (46 g) which was used in the next step without further purification.

6-Chloro-2,4-difluoro-3-(trimethylsilyl)benzaldehyde. A solution of 2.5M n-BuLi in hexanes (60 mL, 0.15 mol) was added dropwise to a solution of (4-chloro-2,6-difluorophenyl)trimethylsilane (assumed 0.13 mol) in THF (180 mL) cooled to -70°C under Ar. After the addition was complete, the mixture was stirred for 1 h before a solution of *N*-formylmorpholine (20 mL, 0.2 mol) in THF (25 mL) was added dropwise. The reaction was allowed to warm to rt with stirring and then cooled to 0°C and quenched with H_2O. Ether was added, the phases were separated, the aqueous phase was re-extracted with ether and the combined organics washed with brine, dried over $MgSO_4$ and concentrated *in vacuo* to give the title compound (35 g) as an orange oil which was used without further purification.

2-Chloro-4,6-difluorobenzaldehyde. CsF (approximately 0.2 g) was added to a mixture of crude 6-chloro-2,4-difluoro-3-(trimethylsilyl)benzaldehyde (10.5 g, approximately 42 mmol) in DMF (15 mL) / H_2O (2 mL). After stirring for 10 min the reaction was diluted with heptane and H_2O. The phases were separated and the aqueous extracted with further heptane. The combined organic layers were washed with H_2O, dried over $MgSO_4$ and concentrated *in vacuo* to a yellow oil. Purification by column chromatography (1:4 EtOAc : heptane) followed by trituration with pentane gave the title compound as a white solid (3.0 g , 17.0 mmol). TLC: Rf = 0.54 (8:7 heptane:EtOAc).

(2-Chloro-4,6-difluorophenyl)methanol (Intermediate 6). Sodium borohydride (1.42 g, 37.5 mmol) was added in portions to an ice-cooled solution of 2-chloro-4,6-difluorobenzaldehyde (6.70 g, 38.0 mmol) in methanol (56 mL); once addition was complete the reaction was warmed to rt over 1 h and then concentrated *in vacuo*. The residue was partitioned between ether and saturated sodium bicarbonate solution, the phases were separated and the aqueous phase extracted with more ether. The combined organic phases were washed with H_2O, dried with $MgSO_4$ and concentrated *in vacuo* to yield the crude product as an oil which was diluted with pentane (20 mL) and allowed to stand in a freezer overnight. The liquors were decanted from the resultant white crystals which were then dried *in vacuo* to yield the title compound (5.60 g, 31.4 mmol). ^{1}H NMR (400 MHz, DMSO) δ: 4.54 (dd, *J*=5.5, 2.3 Hz, 2H), 5.26 (t, *J*=5.5 Hz, 1H), 7.17-7.46 (m, 2H).

Preparation of (2,4-difluoro-6-methoxyphenyl)methanol (Intermediate 7).

Methyl 2,4-difluoro-6-methoxybenzoate. Methyl iodide (1.97 mL, 31.6 mmol) was added to a suspension of 2,4-difluoro-6-hydroxybenzoic acid (2.50 g, 14.4 mmol) and K_2CO_3 (5.96 g, 43.1 mmol) in acetone (50 mL) and the mixture heated at reflux for 12 h. After concentration in vacuo the material was suspended in DCM (50 mL) and filtered, rinsing the residue with DCM (2 x 50 mL). The combined filtrates were concentrated *in vacuo* and purified by gradient flash chromatography eluting with 5-40% EtOAc in iso-hexane to yield the title compound (1.15 g, 5.71 mmol). ^{1}H NMR (400 MHz, DMSO) δ: 3.83 (s, 3H), 3.84 (s, 3H), 6.92-7.03 (m, 2H).

(2,4-Difluoro-6-methoxyphenyl)methanol (Intermediate 7). A solution of $LiBH_4$ (2M in THF, 6.28 mL, 12.6 mmol) was added to a solution of methyl 2,4-difluoro-6-methoxybenzoate (1.15 g, 5.71 mmol) in THF (30 mL). The solution was stirred at rt for 23 h, then at 60ºC for 4.5 h, and reflux for 18.5 h. After cooling to rt, H_2O (20 mL) was added dropwise and the mixture stirred for 5 min before being partially concentrated *in vacuo*. DCM (20 mL) was added, the phases were separated, and the aqueous phase was extracted with DCM (2 x 10 mL). The organic layers were concentrated *in vacuo* to yield the title compound (978 mg, 5.62 mmol). ^{1}H NMR (400 MHz, DMSO) δ: 3.83 (s, 3H), 4.41 (dd, *J*=5.4, 1.9 Hz, 2H), 4.85 (t, *J*=5.5 Hz, 1H), 6.69-6.86 (m, 2H).

Preparation of (2-chloro-6-fluoro-4-methylphenyl)methanol (Intermediate 8).

2-Chloro-6-fluoro-4-methylbenzaldehyde. Under Ar, a 2.5M solution of n-BuLi (6.0 mL, 15.0 mmol) was added slowly to a solution of 3-chloro-5-fluorotoluene (2.0 g, 13.8 mmol) in THF (20 mL) cooled in a dry ice / acetone bath at approximately -70°C. After the addition was complete, the reaction was stirred for 1 h at approximately -70°C and a solution of *N*-formylmorpholine (2.3 g,

2.0 mmol) in THF (10 mL) was added dropwise. After stirring for 10 min, the reaction was allowed to warm to rt with stirring over 1 h, then cooled to -30°C and quenched by the addition of H_2O then 1M citric acid. The mixture was allowed to warm to rt with stirring and the phases were separated. The aqueous phase was extracted with EtOAc and the combined organic phases were washed with H_2O, dried with MgSO4 and concentrated to yield the title compound as an oil which solidified on standing (2.2 g, 12.8 mmol). TLC: Rf = 0.22 (4:1 heptane:EtOAc).

(2-Chloro-6-fluoro-4-methylphenyl)methanol (Intermediate 8). Sodium borohydride (1.42 g, 37.5 mmol) was added in portions to an ice-cooled solution of 2-chloro-6-fluoro-4-methylbenzaldehyde (6.25 g, 38.2 mmol) in methanol (56 mL); once addition was complete the reaction was allowed to warm rt with stirring over 1 h and then concentrated *in vacuo*. The residue was partitioned between ether and saturated aqueous sodium bicarbonate solution. The layers were separated and the aqueous re-extracted with ether. The organic layers were combined, washed with H_2O, dried with $MgSO_4$ and concentrated *in vacuo* to yield the crude product as an oil. This oil was diluted with pentane, cooled and stirred which resulted in a white solid. Filtration and drying in vacuo yielded the title compound (5.10 g, 29.2 mmol). ^{1}H NMR (400 MHz, DMSO) δ: 2.36 (s, 3H), 4.58 (d, *J*=3.3 Hz, 2H), 5.20 (t, *J*=5.1 Hz, 1H), 7.10 (d, *J*=10.5 Hz, 1H), 7.21 (s, 1H).

Preparation of 2-chloro-6-fluoro-4-methoxybenzyl alcohol (Intermediate 9).

3-Chloro-5-fluoro-4-(hydroxymethyl)phenol. 3-Chloro-5-fluorophenol (703 mg, 4.8 mmol) was added to a solution of potassium hydroxide (297 mg, 5.3 mmol) in H_2O (1.45 mL) and heated at 60°C. Formaldehyde (37 wt% in H_2O, 0.74 mL, 9.12 mmol) in H_2O

(1.45 mL) was added dropwise and the reaction mixture left to stir at 40°C overnight. The reaction mixture was cooled to rt and conc. HCl (approximately 6 mL) was added. The resultant precipitate was filtered, washed with H_2O and dried to yield the title compound as a cream solid (334 mg, 1.89 mmol). Mass spectroscopy: *m/z* 175, 177 $(M-H)^-$.

2-Chloro-6-fluoro-4-methoxybenzyl alcohol (Intermediate 9). Methyl iodide (0.13 mL, 2.08 mmol) was added dropwise to a solution of 3-chloro-5-fluoro-4-(hydroxymethyl)phenol (334 mg, 1.89 mmol) and potassium carbonate (287 mg, 2.08 mmol) in DMF (5 mL) and the reaction mixture stirred for 4 h at rt. The reaction mixture was partitioned between DCM and H_2O, the organic layer separated and the aqueous further extracted with DCM. The combined organic phases were concentrated *in vacuo* to yield the title compound as a yellow oil (360 mg, 1.89 mmol). ^{1}H NMR (400 MHz, DMSO) δ 3.79 (s, 3H), 4.50 (dd, *J*=5.3, 2.0 Hz, 2H), 5.07 (t, *J*=5.5 Hz, 1H), 6.86 (dd, *J*=11.5, 2.5 Hz, 1H), 6.90-6.95 (m, 1H).

(3) Typical procedure for the preparation of examples by *N*-alkylation of cyclised intermediates, as exemplified by the preparation of 2-(3,4-dimethoxyphenyl)-4-(2-fluorobenzyl)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (8). A mixture of 2-(3,4-dimethoxyphenyl)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (Intermediate 1, 67 mg, 0.20 mmol), 2-fluorobenzyl bromide (45 mg, 0.24 mmol) and K_2CO_3 (83 mg, 0.60 mmol) in DMF (2 mL) was heated at 100ºC overnight in a sealed vial. After cooling to rt, H_2O (3 mL) and EtOAc (9 mL) were added and the phases were separated. The aqueous phase was extracted with EtOAc (3 mL) and the combined organic phases were washed with H_2O (2 x 2 mL) and brine (2 mL). After concentration *in vacuo* purification by

gradient column chromatography, eluting with eluting with 7-60% EtOAc in iso-hexane yielded the title compound (64 mg, 0.14 mmol, 72%) as a white solid. LCMS and ^{1}H NMR data are detailed in Table 2.

The following compounds were prepared according to the above procedures: [a]

Compound	Product (yield)	Prepared from
9	4-(4-Chloro-2-fluorobenzyl)-2-(3,4-dimethoxyphenyl)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (43 mg, 0.09 mmol).	Prepared from Intermediate 1 (50 mg, 0.15 mmol), K_2CO_3 (62 mg, 0.45 mmol) and 4-chloro-2-fluorobenzyl bromide (40 mg, 0.18 mmol) in DMF (2 mL) using the methods of **8**.
12	2-(3,4-Dimethoxyphenyl)-4-(2,4,6-trifluorobenzyl)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (29 mg, 0.06 mmol).	Prepared from Intermediate 1 (84 mg, 0.25 mmol), K_2CO_3 (104 mg, 0.75 mmol) and 2,4,6-trifluorobenzyl bromide (68 µL, 0.30 mmol) in DMF (2 mL) using the methods of **8**.
16	2-(3,4-Dimethoxyphenyl)-4-(2,6-difluoro-4-methoxybenzyl)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (59 mg, 0.12 mmol).	Prepared from Intermediate 1 (84 mg, 0.25 mmol), K_2CO_3 (104 mg, 0.75 mmol) and 2,6-difluoro-4-methoxybenzyl bromide (71 mg, 0.30 mmol) in DMF (2 mL) using the methods of **8**.
18	2-(5,6-Dimethoxypyridin-3-yl)-4-(2,4,6-trifluorobenzyl)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (103 mg, 0.21 mmol).	Prepared from Intermediate 2 (84 mg, 0.25 mmol), K_2CO_3 (104 mg, 0.75 mmol) and 2,4,6-trifluorobenzyl bromide (68 mg, 0.30 mmol) in DMF (2 mL) using the methods of **8**.
19	2-(2,6-Dimethoxypyridin-4-yl)-4-(2,4,6-trifluorobenzyl)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (103 mg, 0.21 mmol).	Prepared from Intermediate 3 (84 mg, 0.25 mmol), 2,4,6-trifluorobenzyl bromide (68 mg, 0.30 mmol) and K_2CO_3 (104 mg, 0.75 mmol) using the methods of **8**.
22	4-(2,6-Difluoro-4-methoxybenzyl)-2-(2,6-dimethoxypyridin-4-yl)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (92 mg, 0.19 mmol)	Prepared from Intermediate 3 (84 mg, 0.25 mmol), 2,6-difluoro-4-methoxybenzyl bromide (71 mg, 0.30 mmol) and K_2CO_3 (104 mg, 0.75 mmol) using the methods of **8**.
23	4-(2,6-Difluoro-4-methoxybenzyl)-2-(5,6-dimethoxypyridin-3-	Prepared from Intermediate 2 (84 mg, 0.25 mmol), K_2CO_3 (104

	yl)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (38 mg, 0.08 mmol).	mg, 0.75 mmol) and 2,6-difluoro-4-methoxybenzyl bromide (71 mg, 0.30 mmol) in DMF (2 mL) using the methods of **8**.
24	4-(2,6-Difluoro-4-methoxybenzyl)-2-(5,6-dimethoxypyridin-2-yl)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (38 mg, 0.08 mmol).	Prepared from Intermediate 4 (84 mg, 0.25 mmol), 2,6-difluoro-4-methoxybenzyl bromide (71 mg, 0.30 mmol) and K_2CO_3 (104 mg, 0.75 mmol) using the methods of **8**.

[a] LCMS and ^{1}H NMR data of final compounds are detailed in Table 2.

(4) Typical procedure for the preparation of examples by Mitsonobu coupling of substituted benzyl alcohols with cyclized intermediates, exemplified by the preparation of 4-(2-chloro-4,6-difluorobenzyl)-2-(3,4-dimethoxyphenyl)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (13).

Diisopropyl azodicarboxylate (131 μL, 0.67 mmol) was added to a solution of Intermediate 1 (148 mg, 0.44 mmol), Intermediate 6 (118 mg, 0.66 mmol) and triphenylphosphine (174 mg, 0.66 mmol) in THF (5 mL) and the mixture was stirred at rt for 17 h. After concentration *in vacuo*, purification by gradient flash chromatography, eluting with 20-100% EtOAc in iso-hexane, yielded the title compound (176 mg, 0.36 mmol) as a white solid. LCMS and ^{1}H NMR data are detailed in Table 2.

The following compounds were prepared according to the above procedures: [a]

Compound	Product (yield)	Prepared from
14	4-(2,4-Difluoro-6-methoxybenzyl)-2-(3,4-dimethoxyphenyl)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (212 mg, 0.43 mmol).	Prepared from Intermediate 1 (179 mg, 0.54 mmol) and Intermediate 7 (140 mg, 0.80 mmol) using the methods of **13**.

15	4-(2-Chloro-6-fluoro-4-methylbenzyl)-2-(3,4-dimethoxyphenyl)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (199 mg, 0.41 mmol).	Prepared from Intermediate 1 (149 mg, 0.44 mmol) and Intermediate 8 (116 mg, 0.66 mmol) using the methods of **13**.
17	4-(2-Chloro-6-fluoro-4-methoxybenzyl)-2-(3,4-dimethoxyphenyl)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (63 mg, 0.12 mmol).	Prepared from Intermediate 1 (84 mg, 0.25 mmol) and Intermediate 9 (52 mg, 0.28 mmol) using the methods of **13**.
20	4-(2-Chloro-4,6-difluorobenzyl)-2-(5,6-dimethoxypyridin-3-yl)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (28 mg, 0.06 mmol).	Prepared from Intermediate 2 (84 mg, 0.25 mmol) and Intermediate 6 (49 mg, 0.28 mmol) using the methods of **13**.
21	2-(2-Methoxypyridin-4-yl)-4-(2,4,6-trifluorobenzyl)-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxide (60 mg, 0.13 mmol).	Prepared from Intermediate 5 (200 mg, 0.59 mmol) and 2,4,6-trifluorobenzylalcohol (100 mg, 0.65 mmol) using the methods of **13**.

[a] LCMS and ^{1}H NMR data of final compounds are detailed in Table 2.

Synthetic route C: Synthesis of 25 and 26.

Preparation of 4-(2,6-difluoro-4-methoxybenzyl)-2-[6-methoxy-5-(methylamino)pyridin-3-yl]-2*H*-pyrido[2,3-e][1,2,4]thiadiazin-3(4*H*)-one 1,1-dioxide (25).

***tert*-Butyl (5-{[(2-chloropyridin-3-yl)sulfonyl]amino}-2-methoxypyridin-3-yl)methylcarbamate.** The title compound (2.12 g, 4.94 mmol) was prepared from 2-chloropyridine-3-sulfonyl chloride (1.10 g, 5.20 mmol), *tert*-butyl (5-amino-2-methoxypyridin-3-yl)methylcarbamate (1.45 g, 5.72 mmol) and pyridine (1.26 mL, 15.6 mmol) in DCM (20 mL) at rt using the methods of **27**. Mass

spectroscopy: *m/z* 429.1, 431.1 $(M+H)^+$; ^{1}H NMR (400 MHz, DMSO) δ: 1.20 (s, 9H), 2.89 (s, 3H), 3.77 (s, 3H), 7.29 (s, 1H), 7.57 (dd, *J*=6.9, 5.0 Hz, 1H), 7.76 (s, 1H), 8.33 (d, *J*=7.8 Hz, 1H), 8.59 (d, *J*=2.7 Hz, 1H), 10.76 (br. s, 1H).

2-{5-[(1-tert-butoxyethyl)(methyl)amino]-6-methoxypyridin-3-yl}-4-(2,6-difluoro-4-methoxybenzyl)-2*H*-pyrido[2,3-e][1,2,4]thiadiazin-3(4*H*)-one 1,1-dioxide. The title compound (226 mg, 0.38 mmol) was prepared in two steps from *tert*-butyl (5-{[(2-chloropyridin-3-yl)sulfonyl]amino}-2-methoxypyridin-3-yl)methylcarbamate (214 mg, 0.5 mmol), 2,6-difluoro-4-methoxybenzylamine (0.28 mL, 2.0 mmol) and *N*,*N*-diisopropylethylamine (0.18 mL, 1.0 mmol) in MeCN (3 mL) at 120ºC for 6 hours; followed by 1,1′-carbonyldiimidazole (350 mg, 2.16 mmol) and triethylamine (0.15 mL, 1.08 mmol) using the methods of **27**; Mass spectroscopy: *m/z* 592.2 $(M+H)^+$; ^{1}H NMR (400 MHz, DMSO) δ: 1.29 (s, 9H), 3.01 (s, 3H), 3.71 (s, 3H), 3.93 (s, 3H), 5.50 (s, 2H), 6.67 (d, *J*=9.6 Hz, 2H), 7.48 (dd, *J* =7.8, 5.5 Hz, 1H), 7.67 (d, *J*=1.8 Hz, 1H), 8.07 (d, *J*=2.8 Hz, 1H), 8.46 (dd, *J*=7.3, 1.4 Hz, 1H), 8.80 (dd, *J*=5.0, 1.8 Hz, 1H).

4-(2,6-Difluoro-4-methoxybenzyl)-2-[6-methoxy-5-(methylamino)pyridin-3-yl]-2*H*-pyrido[2,3-e][1,2,4]thiadiazin-3(4*H*)-one 1,1-dioxide (25). TFA (2 mL) was added to a solution of 2-{5-[(1-tert-butoxyethyl)(methyl)amino]-6-methoxypyridin-3-yl}-4-(2,6-difluoro-4-methoxybenzyl)-2*H*-pyrido[2,3-e][1,2,4]thiadiazin-3(4*H*)-one 1,1-dioxide (226 mg, 0.38 mmol) in DCM (3 mL) at rt. After stirring at rt for 3 h the reaction mixture was concentrated *in vacuo*, partitioned between DCM and saturated aqueous $NaHCO_3$ solution, and the phases were separated. After extraction with DCM the combined organic phases were concentrated *in vacuo* and purified by gradient flash chromatography, eluting with 10-60% EtOAc in iso-hexane to yield the title compound (155 mg, 0.32 mmol) as a white solid. LCMS and ^{1}H NMR data are detailed in table 2.

Preparation of 2-(5,6-dimethoxypyridin-2-yl)-4-(2,4,6-trifluorobenzyl)-2*H*-pyrido[2,3-e][1,2,4]thiadiazin-3(4*H*)-one 1,1-dioxide (26).

2-Chloro-*N*-(5,6-dimethoxypyridin-2-yl)pyridine-3-sulfonamide. The title compound (549 mg, 1.66 mmol) was prepared from 2-chloropyridine-3-sulfonyl chloride (530 mg, 2.5 mmol), 5,6-dimethoxypyridin-2-amine (424 mg, 2.8 mmol) and pyridine (0.6 mL, 7.5 mmol) in DCM (10 mL) using the methods of **27**. Mass spectroscopy: *m/z* 328, 330 $(M-H)^-$, 330, 332 $(M+H)^+$; ^{1}H NMR (400 MHz, DMSO) δ: 3.42 (s, 3H), 3.68 (s, 3H), 6.52 (d, *J*=8.3 Hz, 1H), 7.26 (d, *J*=8.3 Hz, 1H), 7.67 (dd, *J*=7.8, 4.8 Hz, 1H), 8.58 (dd, *J*=7.9, 1.9 Hz, 1H), 8.64 (dd, *J*=4.8, 1.8 Hz, 1H), 11.22 (s, 1H).

2-(5,6-Dimethoxypyridin-2-yl)-4-(2,4,6-trifluorobenzyl)-2*H*-pyrido[2,3-e][1,2,4]thiadiazin-3(4*H*)-one 1,1-dioxide (26). The title compound (118 mg, 0.25 mmol) was prepared in two steps from 2-chloro-*N*-(5,6-dimethoxypyridin-2-yl)pyridine-3-sulfonamide (165 mg, 0.5 mmol), 2,4,6-trifluorobenzylamine (0.18 mL, 1.5 mmol); followed by 1,1′-carbonyldiimidazole (246 mg, 1.52 mmol), triethylamine (0.11 mL, 0.76 mmol) in DMF (1.5 mL) using the methods of **27**. LCMS and ^{1}H NMR data are detailed in table 2.

OX_1 receptor expression and membrane preparation. Sf21 cells were infected with a P1 phage virus containing OX_1 WT DNA. Protein expression was carried out over 48 hours after which time the cell cultures were harvested and washed twice with ice cold phosphate-buffered saline. The pellet was subjected to three freeze-thaw cycles using dry ice and then resuspended in ice-cold buffer containing 20 mM Tris-HCl, pH 7.4, 1 mM EDTA and homogenised with an Ultraturax for 30 s at maximum speed. After centrifugation at 48,000 g for 30 min at 4ºC, the pellet was resuspended and spun again. The final pellet was resuspended and frozen at -80ºC before use. Protein concentration was determined using the BCA protein assay method.

OX_1 radioligand binding assay. After thawing, membrane homogenates were re-suspended in the binding buffer (8.5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.4, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 118 mM NaCl, 4.7 mM KCl, 4 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 11 mM Glucose) to a final assay concentration of 4.0 μg protein per well. Saturation isotherms were determined by the addition of various concentrations of [^{3}H]-**23** in a total reaction volume of 250 μL for 90 min at rt. At the end of the incubation, membranes were filtered onto a unifilter, a 96-well white microplate with bonded GF/B filter pre-incubated with 0.5% polyethylenimine, with a Tomtec cell harvester and washed 4 times with distilled water. Non-specific binding (NSB) was measured in the presence of 10 μM suvorexant. Radioactivity on the filter was counted (1 min) on a microbeta counter after addition of 50 μL of scintillation fluid. For inhibition experiments, membranes were incubated with [^{3}H]-**23** at a concentration equal to the K_D value of the radioligand and 10 concentrations of the inhibitory compound (0.001-10 μM). IC_{50} values were derived from the inhibition curve and the equilibrium dissociation constant (K_i) values were calculated using the Cheng-Prussoff equation.[1]

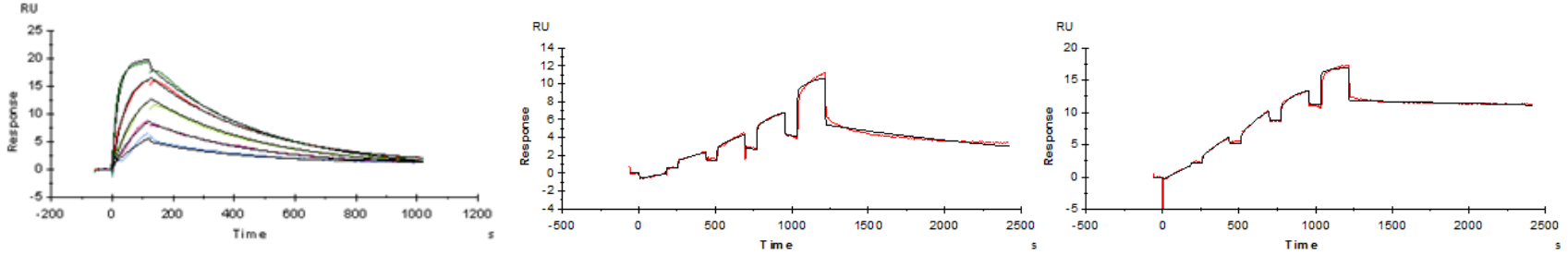
OX_2 receptor expression and membrane preparation. cDNA encoding the human OX_2 receptor was transfected into HEK293 cells using the transfection reagent Genejuice (Novagen). Forty-eight hours after transfection, cells were harvested and washed twice with ice cold phosphate-buffered saline. The pellet was resuspended in ice-cold buffer containing 20 mM Tris-HCl, pH7.4, 1 mM EDTA and homogenised with an Ultraturax for 30 s at maximum speed. After centrifugation at 48,000 g for 30 min at 4ºC, the pellet was resuspended and spun again. The final pellet was resuspended and frozen at -80ºC before use. Protein concentration was determined using the BCA protein assay method.

OX_2 radioligand binding assay. After thawing, membrane homogenates were re-suspended in the binding buffer (8.5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.4, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 118 mM NaCl, 4.7 mM KCl, 4 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 11 mM Glucose) to a final assay concentration of 2.5 μg protein per well. Saturation isotherms were determined by the addition of various concentrations of [^{3}H]EMPA[28] in a total reaction volume of 250 μL for 90 min at rt. At the end of the incubation, membranes were filtered onto a unifilter, a 96-well white microplate with bonded GF/B filter pre-incubated with 0.5% polyethylenimine, with a Tomtec cell harvester and washed 4 times with distilled water. Non-specific binding (NSB) was measured in the presence of 10 μM (2*S*)-1-(3,4-dihydro-6,7-dimethoxy-2(1*H*)-isoquinolinyl)-3,3-dimethyl-2-[(4-pyridinylmethyl)amino]-1-butanone hydrochloride (TCS OX2 29, Tocris bioscience, catalogue number 3371). Radioactivity on the filter was counted (1 min) on a microbeta counter after addition of 50 μL of scintillation fluid. For inhibition experiments, membranes

were incubated with [^{3}H]EMPA at a concentration equal to the K_D value of the radioligand and 10 concentrations of the inhibitory compound (0.001-10 μM). IC_{50} values were derived from the inhibition curve and the equilibrium dissociation constant (K_i) values were calculated using the Cheng-Prussoff equation.[1]

OX_1 and OX_2 functional assays. Receptor-stimulated ERK1/2 phosphorylation was determined using the AlphaScreen™ ERK1/2 SureFire™ assay. CHO cells stably expressing the human OX_1 or OX_2 receptor were seeded at 25,000 cells/well in solid-walled 96-well half-area plates (Costar). After 4 h, cells were washed with PBS and maintained in DMEM/F12 without any serum for 16 h at 37^{o}C. For functional antagonism studies increasing concentrations of test antagonist were added and incubated at 37 °C for 30 min. Following this cells were challenged with an EC_{80} concentration of orexin-A for a further 5 min at 37 °C and terminated by the removal of media and addition of SureFire lysis buffer. The plate was then agitated for 30 min before transfer of 4 μL of lysate into a white opaque 384-well Proxiplate. Addition of the AlphaScreen beads/activated lysate mixture, diluted as per manufacturer's instructions, was then performed in diminished light, and the plate was then incubated in the dark at room temperature for 2 h after which time the fluorescence signal was measured by a PheraStar fluorescence plate reader (BMG LabTech, Germany) using standard AlphaScreen settings. All data were expressed as a percentage of ERK1/2 phosphorylation mediated by a maximal concentration to orexin-A. Antagonist pIC_{50} values were converted to functional pK_b using the Cheng Prusoff equation.[1]

SPR protocol, OX_2 sensorgrams for 18, OX_1 and OX_2 sensorgrams for suvorexant. Kinetic analyses were run on a Biacore T200 instrument at 10ºC using PBS, 0.05 mM EDTA, 0.1% *n*-dodecyl-*β*-D-maltopyranoside (DDM), 5% DMSO, pH 7.5 as the running buffer. The purified OX_1 or OX_2 stabilized receptor (100 nM in running buffer) was captured on a Ni^{2+} loaded chip NTA (GE Healthcare). Twofold dilution series of each compound (five concentrations, in the range 0.1-1.6 µM for **18**, 0.13-2.0 µM for **27** and 0.25-4.0 µM for suvorexant) were injected. The assay was run in multicycle format with **18** and single cycle format with suvorexant and **27**, due to the different kinetic behaviour of the compounds. Blank-subtracted data were fitted to 1:1 interaction model to obtain kinetic and affinity constants.



OX_2 SPR sensorgram for **18**, OX_1 and OX_2 sensorgrams for suvorexant respectively.

Selectivity data for 27 and 18.

Table 4: Selectivity of **27** in radioligand binding assays. Data for M_1, M_2, M_3, M_4, CGRP and GLP-1 assays are expressed as pK_i values (data generated at Heptares); data for the remainder are expressed as percent inhibition of radioligand binding at 10 μM concentration of **27** (data generated at Eurofins, www.eurofinspanlabs.com/Panlabs).

M_1	< 5.0	Cannabinoid CB_1	44%
M_2	< 4.5	Dopamine D_3	26%
M_3	< 4.3	$GABA_A$, Flunitrazepam, Central	-14%
M_4	< 5.1	Glucocorticoid	9%
CGRP	< 5.8	Histamine H_1	10%
GLP-1	< 4.7	Neuropeptide Y Y_1	-3%
$mGlu_5$	< 4.6	Neurotensin NT_1	2%
Adenosine A_{2A}	13%	Prostanoid EP_4	34%
Adrenergic β_1	-1%	Serotonin $5HT_{1A}$	3%
Androgen (Testosterone) AR	57%	Tachykinin NK_1	6%
Calcium Channel L-type, Benzothiazepine	23%		

Table 5: Selectivity of **18**, as percent inhibition of radioligand binding at 10 μM concentration of **18** (data generated at Eurofins, www.eurofinspanlabs.com/Panlabs).

Adenosine A_1	30%	Glucocorticoid	1%
Adenosine A_{2A}	32%	Glutamate, Kainate	8%

Adenosine A_3	38%	Glutamate, NMDA, Agonism	3%
Adrenergic α_{1A}	-5%	Leukotriene, Cysteinyl $CysLT_1$	17%
Adrenergic α_{1B}	4%	Melatonin MT_1	16%
Adrenergic α_{1D}	0%	Muscarinic M_1	1%
Adrenergic α_{2A}	9%	Muscarinic M_2	4%
Adrenergic β_1	1%	Muscarinic M_3	2%
Adrenergic β_2	7%	Neuropeptide Y Y_1	-1%
Androgen (Testosterone) AR	60%	Neuropeptide Y Y_2	8%
Bradykinin B_1	10%	Nicotinic Acetylcholine	-4%
Bradykinin B_2	-3%	Nicotinic Acetylcholine α1, Bungarotoxin	1%
Calcium Channel L-Type, Benzothiazepine	26%	Opiate δ_1 (OP1, DOP)	-8%
Calcium Channel L-Type, Dihydropyridine	17%	Opiate κ (OP2, KOP)	13%
Calcium Channel N-Type	-3%	Opiate μ (OP3, MOP)	7%
Cannabinoid CB_1	24%	Phorbol Ester	0%
Dopamine D_1	-5%	Platelet Activating Factor (PAF)	30%
Dopamine D_{2S}	9%	Potassium Channel [K_{ATP}]	10%
Dopamine D_3	7%	Potassium Channel hERG	21%
Dopamine $D_{4.2}$	9%	Prostanoid EP_4	52%
Endothelin ET_A	-20%	Purinergic P_{2X}	10%
Endothelin ET_B	2%	Purinergic P_{2Y}	0%
Epidernal Growth Factor (EGF)	-1%	Rolipram	13%
Estrogen ERα	-6%	Serotonin $5HT_{1A}$	3%
$GABA_A$, Flunitrazepam, Central	-16%	Serotonin $5HT_{2B}$	4%
$GABA_A$, Muscimol, Central	-3%	Serotonin $5HT_3$	-5%
$GABA_{B1A}$	8%	Sigma σ_1	12%

Glutamate, NMDA, Glycine	3%	Sodium Channel, Site 2	17%
Glutamate, NMDA, Phencyclidine	5%	Tachykinin NK_1	1%
Histamine H_1	10%	Thyroid Hormone	10%
Histamine H_2	-3%	Transporter, Dopamine (DAT)	5%
Histamine H_3	2%	Transporter, GABA	2%
Imidazoline I_2, Central	-1%	Transporter, Norepinephrine (NET)	17%
Interleukin IL-1	5%	Transporter, Serotonin (SERT)	6%

Computational Chemistry details. The OX_2 experimentally enabled (site-directed mutagenesis, SDM) homology model was developed based upon the adenosine A_{2A} structure 3PWH,[2] with ~33% sequence identity within the TM helices. The model was built within the Schrödinger Maestro package using the Prime module,[3] with manual readjustment of the automated alignment where necessary. The alignment was checked to ensure consistency with known GPCR conserved motifs and particularly the conserved disulfide bond, common to family A GPCRs, which is located between the top of helix 3 and the extracellular loop 2. The model was then further evaluated using two different approaches. First, SDM data, both from the literature[4] and in-house (data not shown), was mapped onto the modelled protein structure. The majority of these residues lined the anticipated ligand binding site in the model. Second, a small number of known OX_2 antagonists were docked into the structure using Glide to ensure the docked poses were consistent with known SAR for these compounds, further final refinements of residue side chains were made at this stage in line with the Schrödinger rotamer library conformations.

1 Y.-C. Cheng and W. H. Prusoff, W. H. *Biochem. Pharmacol*., 1973, **22**, 3099.

2 A. S. Doré, N. Robertson, J. C. Errey, I. Ng, K. Hollenstein, B. Tehan, E. Hurrell, K. Bennett, M. Congreve, F. Magnani, C. G. Tate, M. Weir and F. H. Marshall, *Structure*, 2011, **19**, 1283.

3 Maestro (version 9.8), Schrödinger, LLC, New York.

4 P. Malherbe, O. Roche, A. Marcuz, C. Kratzeisen, J. G. Wettstein, and C. Bissantz, *Mol. Pharmacol*., 2010, **78**, 81.