SUPPLEMENTARY MATERIALS AND METHODS

Article title: The use of physiology-based PK and PD modeling in the discovery of the dual orexin receptor antagonist ACT-541468

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Table S1 **Mean apparent K_b values for selected dual orexin receptor antagonists in calcium release assays determined in CHO-ratOX_1 and CHO-ratOX_2 cells ($n \geq 3$)**

Receptor	K_b [σ_g] (nM)*				
	Suvorexant	**Almorexant**	**ACT-541468**	**ACT-605143**	**ACT-658090**
Rat OX_1	0.84 [1.4]	5.7 [1.3]	0.62 [2.1]	0.75 [2.3]	8.8 [2.1]
Rat OX_2	1.1 [1.5]	0.50 [1.6]	0.82 [2.3]	1.3 [1.5]	13 [2.2]

*K_b values were derived from IC_{50} values using the generalized Cheng-Prusoff equation, and their geometric mean is shown.

Table S2 **Biochemical and physico-chemical input data for the human PBPK models of almorexant and suvorexant**

Parameter	**almorexant**	**suvorexant**
logD	4.2	4.0
pK_a	5.9	neutral
Molecular weight (g/mol)	513	451
$f_{u,plasma}$	0.6	0.1
$f_{u,OX2\ assay}$	0.35	0.54
Blood/plasma ratio	0.55	0.61

Biochemical data are means of at least triplicate experiments.

Table S3 Plasma protein binding of ACT-541468 in rat and man

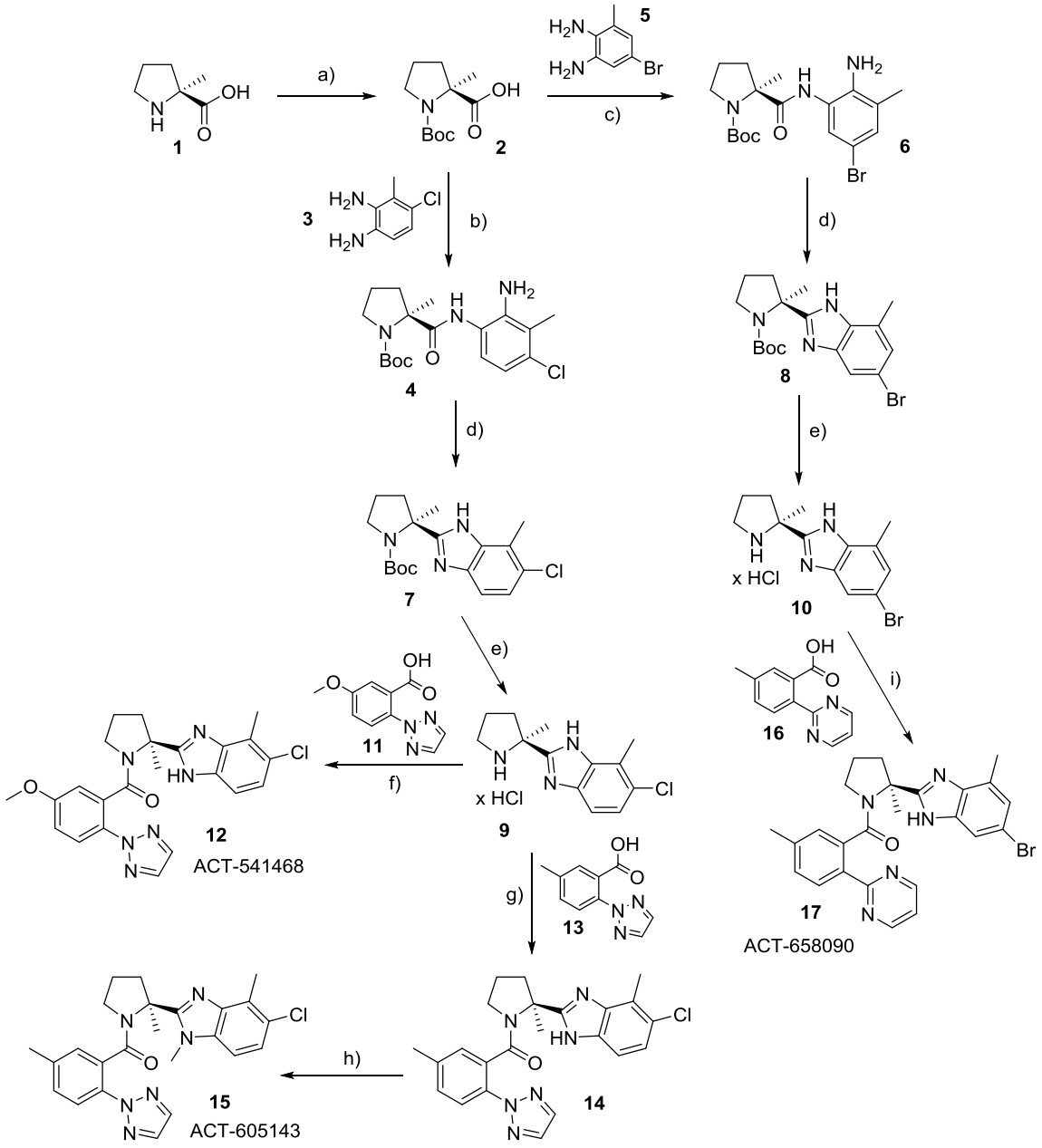
Concentration (μg/mL)	Rat (%)	Human (%)
0.5	2.8	0.3
1.5	2.8	0.3
5	3.3	0.5
15	4.2	1.6

Plasma protein binding was determined by equilibrium dialysis using ^{14}C-labeled ACT-541468.

Data represent unbound fractions and are means of n=3.

SUPPLEMENTARY FIGURES

Figure S1 Synthesis of benzimidazole DORA compounds



ACT-541468, ACT-658090 and ACT-605143 were synthesized according to procedures outlined in detail in patents WO2013/182972; WO2015/083094; WO2015/083070. The preparation started with (S)-2-methylpyrrolidine-2-carboxylic acid hydrochloride (**1**) which was reacted with Boc-anhydride in a 1:1 acetonitrile/water mixture in the presence of triethylamine, to obtain (S)-1-(tert-butoxycarbonyl)-2-methylpyrrolidine-2-carboxylic acid

(**2**) in good yield. The carboxylic acid **2** was then either reacted with 4-chloro-3-methylbenzene-1,2-diamine (**3**) or with 5-bromo-3-methylbenzene-1,2-diamine (**5**) in a 1:1 mixture of dichloromethane/dimethylformamide (DMF) in the presence of excess Hünig's base and O-(7-azabenzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium-hexafluorphosphate (HATU) as the coupling agent to give either tert-butyl (S)-2-((2-amino-4-chloro-3-methylphenyl)carbamoyl)-2-methylpyrrolidine-1-carboxylate (**4**) or tert-butyl (S)-2-((2-amino-5-bromo-3-methylphenyl)carbamoyl)-2-methylpyrrolidine-1-carboxylate (**6**) after aqueous work-up. Compounds **4** and **6** were further used without purification. The cyclization to the benzimidazole derivatives tert-butyl (S)-2-(6-chloro-7-methyl-1H-benzo[d]imidazol-2-yl)-2-methylpyrrolidine-1-carboxylate (7) and tert-butyl (S)-2-(5-bromo-7-methyl-1H-benzo[d]imidazol-2-yl)-2-methylpyrrolidine-1-carboxylate (**8**) was achieved by reacting the precursors **4** and **6** in pure acetic acid at 60°C for 3 hours. Subsequent Boc-deprotection by dissolving **7** and **8** in methanol and addition of a 4 M solution of hydrochloric acid in dioxane resulted in (S)-6-chloro-7-methyl-2-(2-methylpyrrolidin-2-yl)-1H-benzo[d]imidazole hydrochloride **9** and (S)-5-bromo-7-methyl-2-(2-methylpyrrolidin-2-yl)-1H-benzo[d] imidazole hydrochloride **10**. Precursors **9** and **10** were reacted under peptide coupling reaction conditions using dichloromethane as the solvent, DIPEA as the base, HATU as coupling reagent, and addition of a small amount of DMF containing the respective benzoic acid derivative **11**, **13** or **16** to give (S)-(2-(5-chloro-4-methyl-1H-benzo[d]imidazol-2-yl)-2-methylpyrrolidin-1-yl)(5-methoxy-2-(2H-1,2,3-triazol-2-yl)phenyl)methanone (**12 = ACT-541468**), (S)-(2-(6-bromo-4-methyl-1H-benzo[d]imidazol-2-yl)-2-methylpyrrolidin-1-yl)(5-methyl-2-(pyrimidin-2-yl)phenyl)methanone (**17 = ACT-658090**) and (S)-(2-(5-chloro-4-methyl-1H-benzo[d]imidazol-2-yl)-2-methylpyrrolidin-1-yl)(5-methyl-2-(2H-1,2,3-triazol-2-yl)phenyl)methanone (**14**). Compound **14** was dissolved in DMF and reacted with methyliodide in the presence of caesium carbonate at 0°C to give (S)-(2-(5-chloro-1,4-

dimethyl-1H-benzo[d]imidazol-2-yl)-2-methylpyrrolidin-1-yl)(5-methyl-2-(2H-1,2,3-triazol-2-yl)phenyl)methanone (**15 = ACT-605143)**. The final compounds **12**, **17** and **15** were purified by preparative high performance liquid chromatography (HPLC). The benzoic acids **11** (Boss et al., 2015a; Boss et al., 2015b), **13** (Baxter et al., 2011; Mangion et al., 2012) or **16** (Girardin et al., 2013; Chung et al., 2014) were prepared as described in the literature. Reaction conditions were as follows: a) MeCN/H_2O = 1/1; TEA; Boc_2O; RT; 3 h; b) **3**, DMF/DCM = 1/1; DIPEA; HATU; RT; 17 h; c) **5**, DMF/DCM = 1/1; DIPEA; HATU; RT; 17 h; d) AcOH; 60°C; 3 h; e) MeOH; 4 M HCl in dioxane; RT; 2 h; f) **11**, DCM; DIPEA; then HATU; DIPEA in DMF; RT 16 h; g) **13**, DCM; DIPEA; then HATU; DIPEA in DMF; RT 16 h; h) DMF; Cs_2CO_3; CH_3I; 0°C; 60 min; i) **16**, DCM; DIPEA; then HATU; DIPEA in DMF; RT 16 h.

Figure S2 **Dose-response of almorexant or suvorexant on total sleep time over the 6 h night period in telemetrized rats.**

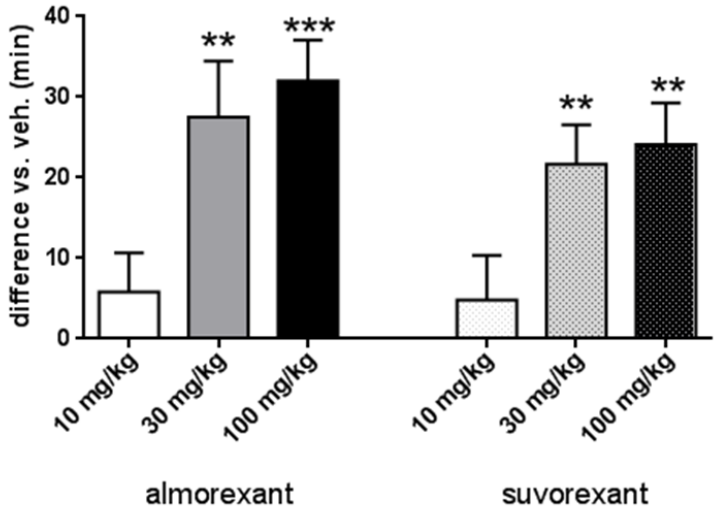
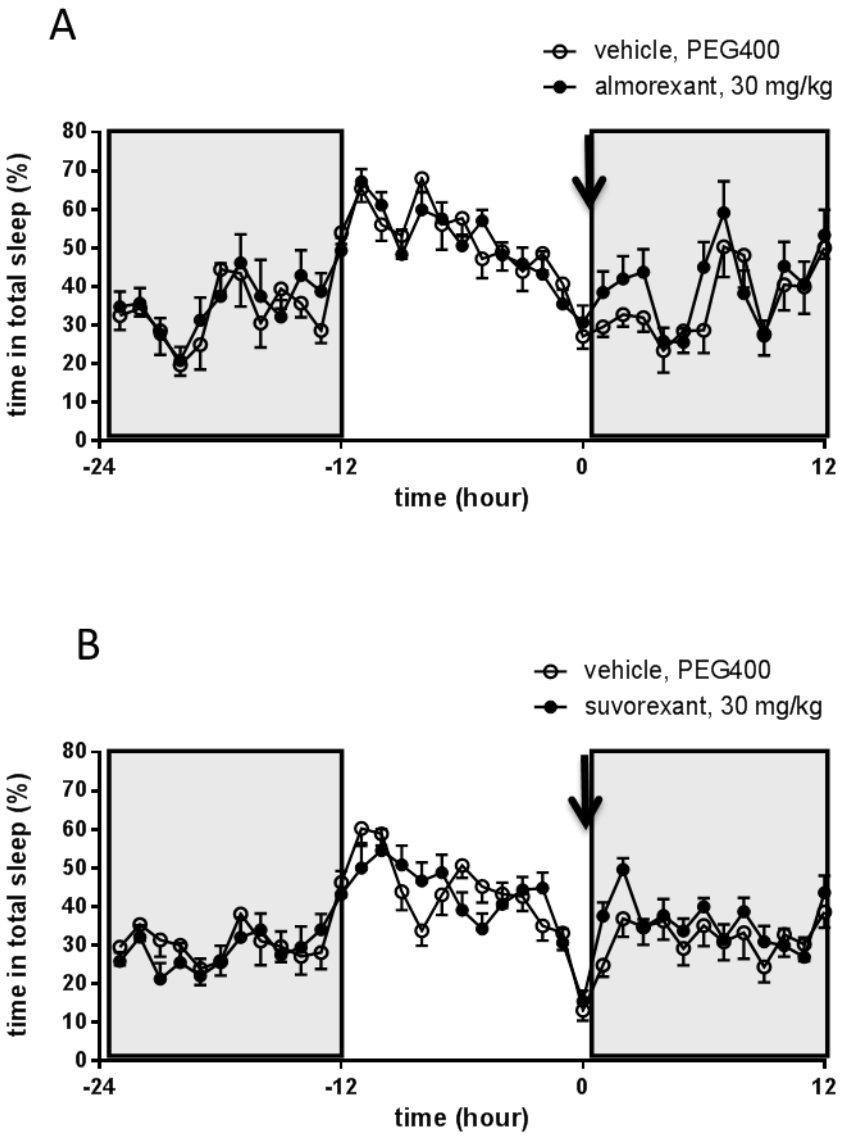


Figure S3 **Time spent on sleep after treatment with almorexant (panel A) or suvorexant (panel B) at 30 mg/kg in telemetrized rats.**



Binding experiments

Binding to plasma and microsomal proteins was determined using a Pierce rapid equilibrium dialysis device from Thermo Fisher Scientific (Lausanne, Switzerland) on an orbital shaker for 4 h at 37°C in an atmosphere containing 5% CO_2. The device contained a protein compartment and a buffer compartment separated by a dialysis membrane with a molecular weight cut-off of 8 kDa, which allowed unbound test compound but not proteins to traverse the membrane and equilibrate between the two compartments (Wan and Rehngren, 2006; Kochansky et al., 2008; Waters et al., 2008). The donor compartment was either plasma, fortified with EDTA as anti-coagulant, or 100 mM phosphate buffer solution (pH 7.4) fortified with 0.5 mg/mL microsomal protein, while the receiver compartment consisted of 100 mM phosphate buffer (pH 7.4). Following dialysis, 50-μL aliquots of the donor compartment were diluted with buffer, while 50 μL of the receiver compartment were diluted with 50 μL of plasma or 100 mM phosphate buffer containing microsomal protein, in order to generate samples with the same analytical matrix. Proteins were precipitated with 300 μL of methanol containing the internal standard, centrifuged at 3220 *g* for 20 min at 4°C, and compound concentrations were quantified using LC-MS/MS.

Blood/plasma partitioning

Blood to plasma partitioning of all compounds in rat and dog was determined *ex vivo* with blood samples taken during the conduct of pharmacokinetic experiments. Blood/plasma partitioning in man was determined *in vitro* at 1 μM for all compounds. For the latter purpose, triplicate samples of human blood were fortified with compound dissolved in DMSO, samples split into two aliquots, and incubated at 37°C on a rotary mixer. After 2 h incubation, one aliquot was centrifuged at 3220 *g* at 4°C for 20 min to prepare plasma, while the second blood sample was stored at 4°C pending analysis. Compound concentrations in

blood and plasma were then quantified by LC-MS/MS after protein precipitation with methanol.

Metabolic turnover in liver microsomes

Intrinsic clearance (CL_{int}) was determined in a substrate depletion assay with liver microsomes of rat, dog and man at 1 μM substrate concentration (Obach and Reed-Hagen, 2002). Compounds were incubated at 37°C in a 100 mM phosphate buffer containing 0.5 mg/mL microsomal protein. The reaction was started by addition of the NADPH-regenerating system and stopped at pre-defined times over a period of 20 min by addition of two volume equivalents of methanol. After removal of proteins by centrifugation, residual drug concentrations were determined by LC/MS-MS analysis. CL_{int} was calculated from the apparent first-order elimination rate constant and incubation volume, and normalized for protein content.