Volumes of interest (VOIs) were drawn manually as spheres in brain regions guided by high resolution MR structural images and summed PET data, with a radius no less than 4mm. A common VOI mask was applied to both baboon scans. Time-activity curves (TACs) were exported in terms of decay corrected activity per unit volume at specified time points with gradually increasing intervals. The TACs were expressed as percent injected dose per unit volume for analysis.

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Supporting Information

Evaluation of Potential PET Imaging Probes for the Orexin 2 Receptors

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Chemical synthesis

Synthesis of (2-hydroxy-6-methoxyphenyl)(4-(quinolin-2-yl)-1,4-diazepan-1-yl)methanone (**4**).
To a solution of 2-chloroquinoline (**1**) (4.0 g, 24 mmol) and 1, 4-diazepane (**2**) (7.2 g, 72 mmol) in 20 mL DMSO, sodium carbonate (3.8 g, 36 mmol) was added. The mixture was stirred at 120 °C for 10 h. After cooling down to room temperature, the reaction mixture was diluted with water and extracted with ethyl acetate (30 mL x 3), the combined organic phase was washed with brine, dried over magnesium sulfate, filtered, and concentrated. Crude intermediate **3** was used for next step without purification. Solid **3** (1.0 g, 4.4 mmol) was dissolved in DCM, 2-hydroxy-6-methoxybenzoic acid (0.74 g, 4.4 mmol) and EDC·HCl (0.92 g, 4.8 mmol) were added into the solution. The mixture was stirred at room temperature for 1 h, then water was added. The mixture was extracted with DCM (20 mL x 3). The organic layer was separated and combined, dried over magnesium sulfate, filtered, and the solvent was evaporated to dryness. The product was purified by chromatography (hexane/ethyl acetate = 1:3) and afforded white solid (0.66 g, 24 % for two steps).^{1}H-NMR (500 MHz, DMSO-d_6): δ 9.66 (s, 1H), 8.01 (d, J = 9.0 Hz, 1H), 7.67 (m, 1H), 7.48 (m, 2H), 7.11 (m, 3H), 6.49 (m, 2H), 3.93 (m, 6H), 3.53 (m, 2H), 3.44 (s, 3H), 1.99 (m, 1H), 1.68 (m, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 156.8, 156.0, 154.5, 148.2, 137.7, 130.0, 129.6, 127.8, 126.3, 122.9, 121.9, 114.1, 109.9, 108.9, 102.3, 55.6, 49.3, 47.8, 45.7, 27.0, 25.4. LC-MS calculated for $C_{22}H_{23}N_3O_3$ expected [M]: 377.2; found $[M+H]^+$: 378.2. HPLC purity: 96.1%.

Synthesis of (2,6-dimethoxyphenyl)(4-(quinolin-2-yl)-1,4-diazepan-1-yl)methanone (**5**, CW3).
Solid **3** (1.0 g, 4.4 mmol) was dissolved in DCM, 2,6-dimethoxybenzoic acid (0.80 g, 4.4 mmol) and EDC·HCl (0.92 g, 4.8 mmol) were added into the solution. The mixture was stirred at room temperature for 1 h, then water was added. The mixture was extracted with DCM (20 mL x 3). The organic layer was separated and combined, dried over magnesium sulfate, filtered, and the solvent was evaporated to dryness. The product was purified by chromatography (hexane/ethyl

acetate = 1:2) and afforded white solid (0.86 g, 30 % for two steps).^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.00 (m, 1H), 7.67 (m, 1H), 7.50 (m, 2H), 7.27 (m, 1H), 7.18 (m, 1H), 7.10 (m, 1H), 6.64 (d, J = 8.5 Hz, 1H), 6.60 (d, J = 8.5 Hz, 1H), 3.93 (m, 1H), 3.81 (m, 6H), 3.58 (s, 3H), 3.50 (s, 3H), 3.13 (m, 1H), 1.95 (m, 1H), 1.60 (m, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 165.7, 156.3, 148.2, 137.7, 130.5, 129.6, 127.8, 126.3, 122.9, 122.8, 121.8, 115.0, 109.9, 109.7, 104.5, 104.4, 55.9, 48.5, 47.5, 45.9, 45.5, 27.1, 26.0. LC-MS calculated for $C_{23}H_{25}N_3O_3$ expected [M]: 391.2; found $[M+H]^+$: 392.4. HPLC purity: 97.2%.

Synthesis of (4-(6-chlorobenzo[d]thiazol-2-yl)-1, 4-diazepan-1-yl)(2-hydroxyphenyl)methanone (**8**).

To a solution of 2, 6-dichlorobenzo[d]thiazole (4.9 g, 24 mmol) and 1, 4-diazepane (7.2 g, 72 mmol) in 20 mL DMSO, sodium carbonate (3.8 g, 36 mmol) was added. The mixture was stirred at 120 °C for 10 h. After cooling down to room temperature, the reaction mixture was diluted with water and extracted with ethyl acetate (30 mL x 3), the combined organic phase was washed with brine, dried over magnesium sulfate, filtered, and concentrated. Crude intermediate **7** was used for next step without purification. Solid **7** (1.0 g, 3.8 mmol) was then dissolved in DCM, 2-hydroxybenzoic acid (0.53 g, 3.8 mmol) and EDC.HCl (0.73 g, 3.8 mmol) were added into the solution. The mixture was stirred at room for 1h, then water was added. The mixture was extracted with DCM (20 mLx3). The organic layer was separated and combined, dried over magnesium sulfate, filtered, and the solvent was evaporated to dryness. The product was purified by chromatography (hexane/ethyl acetate = 1:3) and afforded white solid (1.0 g, 34 % for two steps).).^{1}H-NMR (500 MHz, DMSO-d_6): δ 9.75 (s, 1H), 7.89 (s, 1H), 7.42 (d, J = 8.5 Hz, 1H), 7.29 (d, J = 2.0 Hz, 1H), 7.17 (m, 1H), 6.84 (d, J = 8.0 Hz, 1H), 6.78 (m, 1H), 6.60 (m, 1H), 3.76 (m, 4H), 3.51 (m, 2H), 1.98 (m, 2H), 1.70 (m, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 169.0, 153.3, 149.8, 130.3, 126.4, 121.2, 120.5, 120.3, 119.7, 119.2, 116.1, 115.6, 110.0, 109.8, 50.3, 47.5, 43.1, 25.4, 22.1. LC-MS calculated for $C_{19}H_{18}ClN_3O_2S$ expected [M]: 387.1; found $[M+H]^+$: 388.5. HPLC purity: 98.6%.

Synthesis of (4-(6-chlorobenzo[d]thiazol-2-yl)-1, 4-diazepan-1-yl)(2-methoxyphenyl)methanone (**9**, CW4).

Solid **7** (1.0 g, 3.8 mmol) was dissolved in DCM, 2-methoxybenzoic acid (0.53 g, 3.8 mmol) and EDC·HCl (0.73 g, 3.8 mmol) were added into the solution. The mixture was stirred at room temperature for 1 h, then water was added. The mixture was extracted with DCM (20 mL x 3). The organic layer was separated and combined, dried over magnesium sulfate, filtered, and the solvent was evaporated to dryness. The product was purified by chromatography (hexane/ethyl acetate = 1:2) and afforded white solid (1.2 g, 40 % for two steps). ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.90 (s, 1H), 7.43 (d, J = 8.5 Hz, 1H), 7.33 (M, 1H), 7.29 (d, J = 2.0 Hz, 1H), 7.02 (m, 1H), 6.94 (m, 1H), 6.73 (m, 1H), 3.76 (m, 6H), 3.60 (s, 3H), 1.90 (m, 2H), 1.70 (m, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 168.3, 155.1, 152.2, 132.4, 130.5, 127.7, 127.4, 126.3, 125.0, 121.2, 120.7, 119.6, 111.8, 110.0, 55.6, 47.2, 45.0, 43.7, 27.2, 25.8. LC-MS calculated for $C_{20}H_{20}ClN_3O_2S$ expected [M]: 401.1; found $[M+H]^+$: 402.5. HPLC purity: 97.5%.

Synthesis of (4-(6-chlorobenzo[d]thiazol-2-yl)-1, 4-diazepan-1-yl)(2-hydroxy-6-methoxyphenyl) methanone (**10**)

Solid **7** (1.0 g, 3.8 mmol) was dissolved in DCM, 2-hydroxy-6-methoxybenzoic acid (0.74g, 4.4 mmol) and EDC.HCl (0.92g, 4.8 mmol) were added into the solution. The mixture was stirred at room temperature for 1 h, then water was added. The mixture was extracted with DCM (20 mLx3). The organic layer was separated and combined, dried over magnesium sulfate, filtered, and the solvent was evaporated to dryness. The product was purified by chromatography (hexane/ethyl acetate = 1:3) and afforded white solid (0.86 g, 27 % for two steps).^{1}H-NMR (500 MHz, DMSO-d_6): δ 9.62 (s, 1H), 7.89 (d, J = 2.0 Hz, 1H), 7.42 (d, J = 8.5 Hz, 1H), 7.27 (m, 1H), 7.11 (m, 1H), 6.47 (m, 2H), 3.80 (m, 6H), 3.72 (m, 2H), 3.46 (s, 3H), 1.97 (m, 1H), 1.65 (m, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 168.0, 166.2, 156.8, 154.5, 152.2, 132.4, 130.2, 126.4, 124.9, 121.2, 119.5, 113.7, 108.8, 102.3, 55.6, 48.1, 44.8, 43.5, 27.3, 25.8. LC-MS calculated for $C_{20}H_{20}ClN_3O_3S$ expected [M]: 417.1; found $[M+H]^+$: 418.5. HPLC purity: 98.6%; t_R: 1.7 min; condition: Agilent Eclipse XDB-C8, 150 mm x 4.6 mm, 2.0 mL/min, 30% H_2O + TFA (0.01% v/v)/ 70% CH_3CN + TFA (0.01% v/v). HPLC purity: 94.3%.

Synthesis of (4-(6-chlorobenzo[d]thiazol-2-yl)-1, 4-diazepan-1-yl)(2, 6-dimethoxyphenyl) methanone (**11**, CW6)

Solid **7** (1.0 g, 3.8 mmol) was dissolved in DCM, 2,6-dimethoxybenzoic acid (0.80 g, 4.4 mmol) and EDC·HCl (0.92 g, 4.8 mmol) were added into the solution. The mixture was stirred at room temperature for 1 h, and then water was added. The mixture was extracted with DCM (30 mL x 3). The organic layer was separated and combined, dried over magnesium sulfate, filtered, and the solvent was evaporated to dryness. The product was purified by chromatography (hexane/ethyl acetate = 1:2) and afforded white solid (1.1 g, 33 % for two steps).^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.90 (d, J = 2.0 Hz, 1H), 7.41 (d, J = 8.5 Hz, 1H), 7.28 (m, 3H), 6.63 (d, J = 8.5 Hz, 1H), 3.70 (m, 4H), 3.52 (s, 3H), 3.49 (s, 3H), 3.40 (m, 1H), 3.21 (m, 1H), 1.92 (m, 1H), 1.62 (m, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 167.3, 156.3, 152.3, 144.0, 141.7, 132.4, 130.6, 130.0, 129.8, 126.1, 124.6, 119.5, 104.5, 104.4, 55.8, 50.4, 48.3, 45.0, 43.5, 27.3, 25.1. LC-MS calculated for $C_{21}H_{22}ClN_3O_3S$ expected [M]: 431.1; found $[M+H]^+$: 432.5. HPLC purity: 95.1%.

References

1. de Lecea, L., et al., *The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity.* Proc Natl Acad Sci U S A, 1998. **95**(1): p. 322-7.
2. Alexander, S.P., A. Mathie, and J.A. Peters, *Guide to Receptors and Channels (GRAC), 3rd edition.* Br J Pharmacol, 2008. **153 Suppl 2**: p. S1-209.
3. Siegel, J.M., *Hypocretin (orexin): role in normal behavior and neuropathology.* Annu Rev Psychol, 2004. **55**: p. 125-48.
4. Marcus, J.N., et al., *Differential expression of orexin receptors 1 and 2 in the rat brain.* J Comp Neurol, 2001. **435**(1): p. 6-25.
5. Nishino, S., *The hypocretin/orexin receptor: therapeutic prospective in sleep disorders.* Expert Opin Investig Drugs, 2007. **16**(11): p. 1785-97.
6. Hirose, M., et al., *N-acyl 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline: the first orexin-2 receptor selective non-peptidic antagonist.* Bioorg Med Chem Lett, 2003. **13**(24): p. 4497-9.
7. Brisbare-Roch, C., et al., *Promotion of sleep by targeting the orexin system in rats, dogs and humans.* Nat Med, 2007. **13**(2): p. 150-5.
8. Winrow, C.J., et al., *Promotion of sleep by suvorexant-a novel dual orexin receptor antagonist.* J Neurogenet, 2011. **25**(1-2): p. 52-61.
9. Herring, W.J., et al., *Orexin receptor antagonism for treatment of insomnia: A randomized clinical trial of suvorexant.* Neurology, 2012. **79**(23): p. 2265-74.
10. Bettica, P., et al., *The orexin antagonist SB-649868 promotes and maintains sleep in men with primary insomnia.* Sleep, 2012. **35**(8): p. 1097-104.