A New Positron Emission Tomography Probe for Orexin Receptors Neuroimaging

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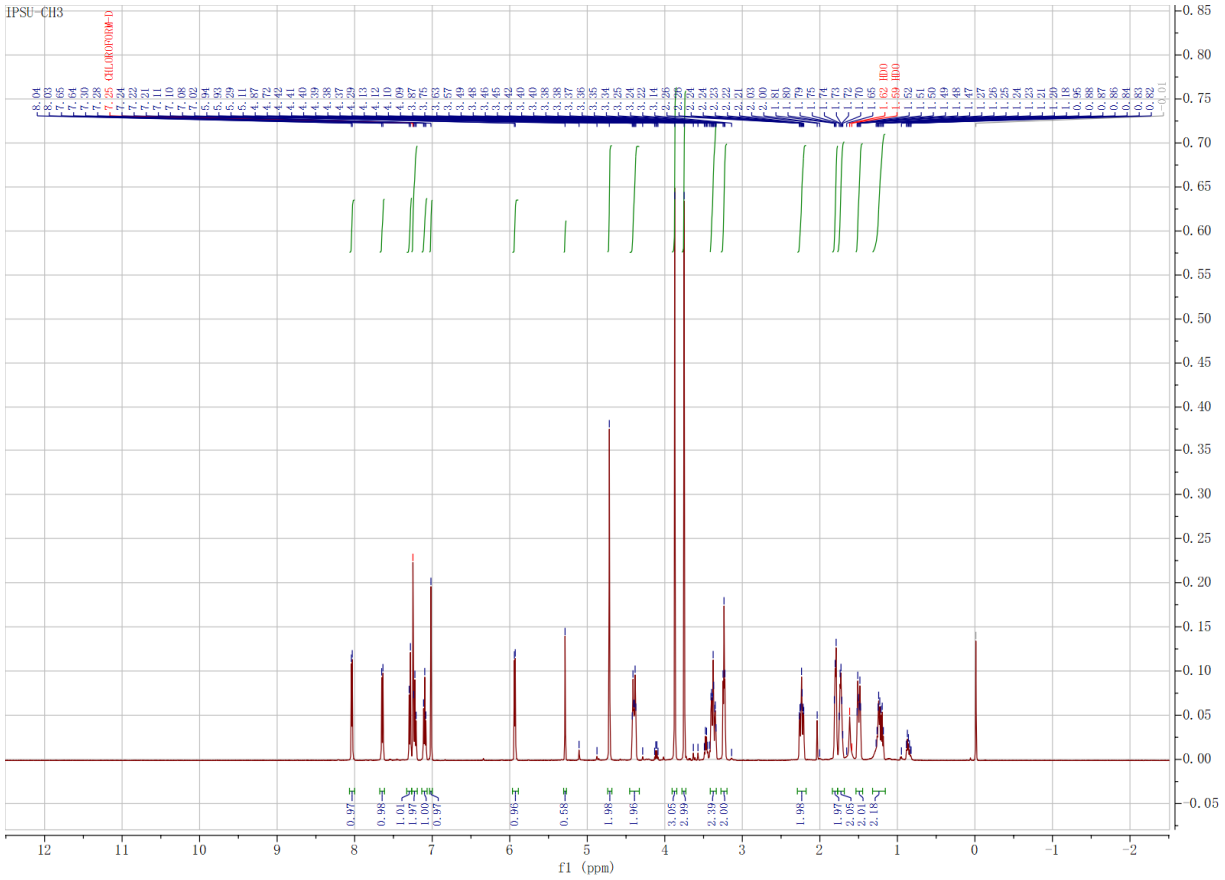
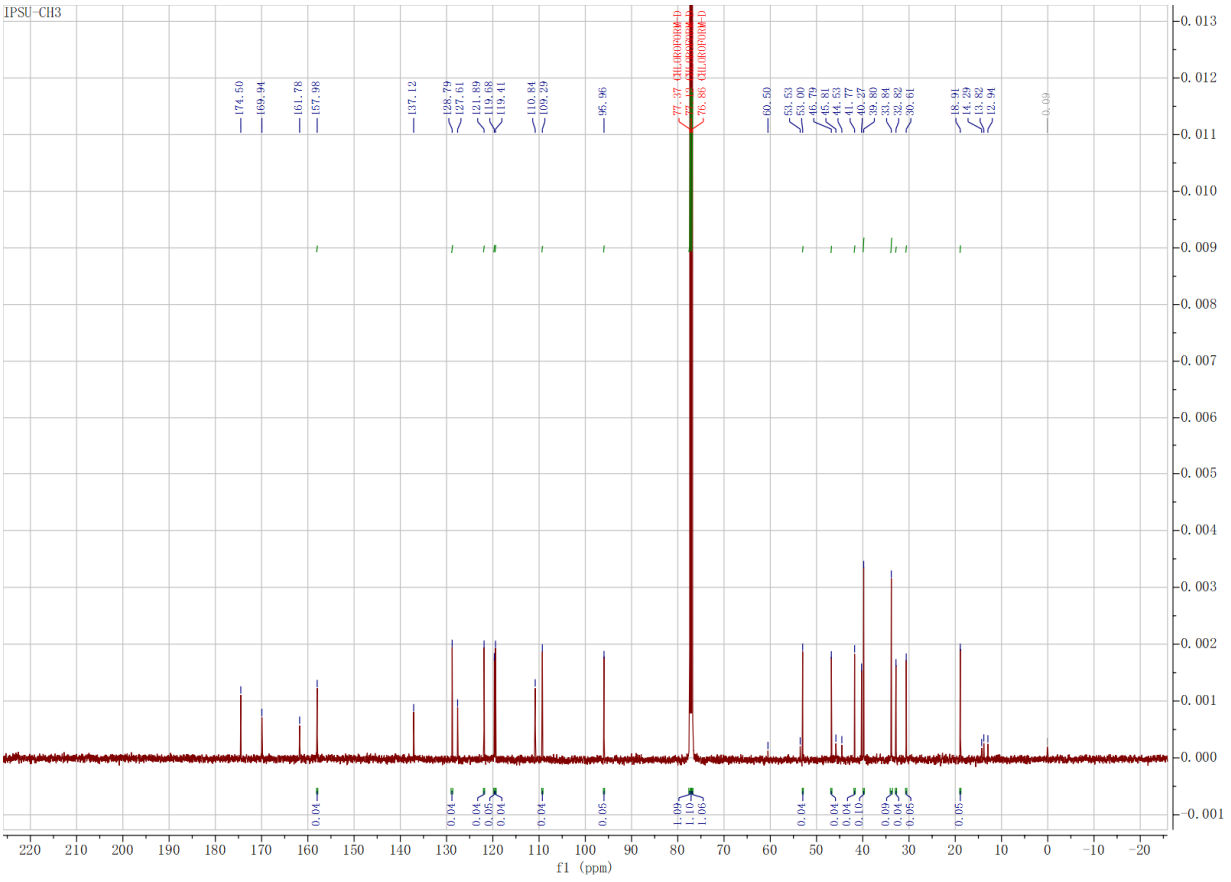
Fig. S1. Representative ^{1}H, ^{13}C NMR and HPLC data for CW24.


IPSU-CH3
f1 (ppm)



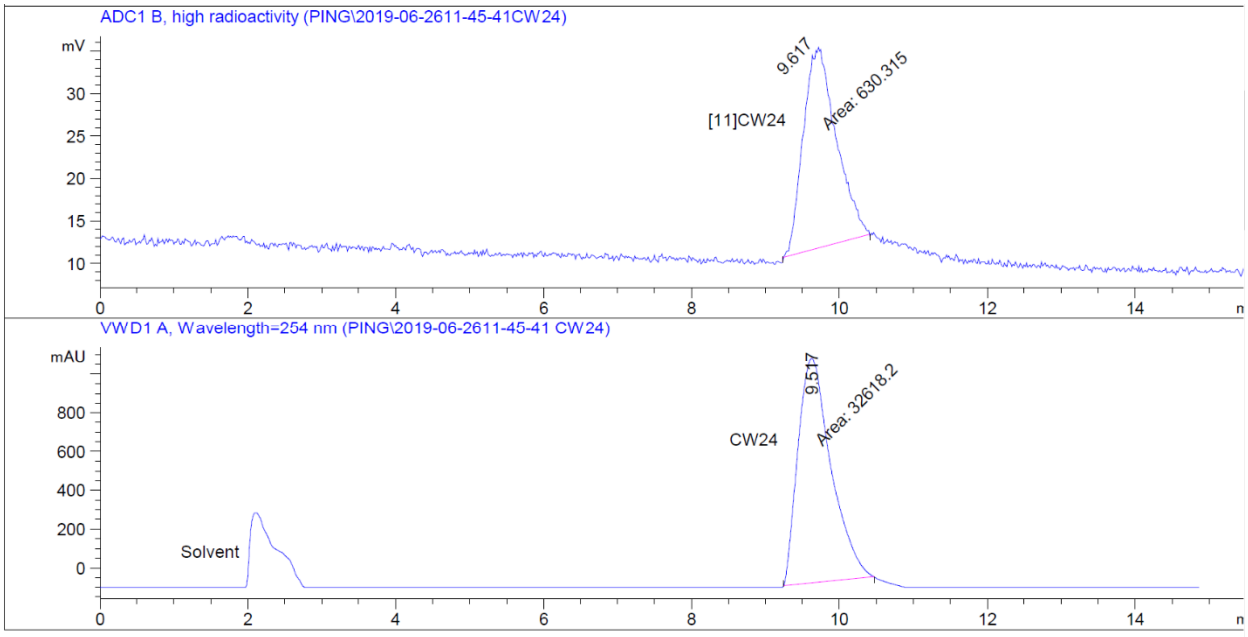


Fig. S2. The HPLC profile of CW24 (HPLC conditions: 60% H_2O + 0.1M AF/40% CH_3CN, at the flow rate of 5.0 mL/min.)

Table S1. Off binding data of CW24.

The off-target binding assays were performed at PDSP with their standard protocol (https://pdspdb.unc.edu/pdspWeb/content/UNC-CH%20Protocol%20Book.pdf). In radioligand binding assays, CW24 was tested at a single concentration (10 μM) and in quadruplicate in 96-well plates. The binding assays were carried out in a final of volume of 125 μl per well in appropriate binding buffers. The hot ligand concentration is usually at a concentration close to the Kd (unless otherwise indicated). Total binding and nonspecific binding are determined in the absence and presence of 10 μM of the appropriate reference compound, respectively. In brief, plates are usually incubated at room temperature and in the dark for 90 mins. Reactions are stopped by vacuum filtration onto 0.3% polyethyleneimine (PEI) soaked 96-well filter mats using a 96-well Filtermate harvester, followed by three washes with cold wash buffers (for details, see Tables 2 to 24). Scintillation cocktail is then melted onto the microwave-dried filters on a hot plate and radioactivity is counted in a Microbeta counter.

Receptor	% Inhibition 1	% Inhibition 2	% Inhibition 3	% Inhibition 4	Mean±SD % inhibition
5-HT1A	-4.55	-4.08	-22.12	-9.84	-10.15 ± 8.4
5-HT1B	18.96	17.99	6.23	10.41	13.4 ± 6.1
5-HT1D	32.06	19.98	11.86	30.24	23.54 ± 9.4
5-HT1E	31.33	19.5	22.46	2.07	18.84 ± 12.2
5-HT2A	25.7	20.15	12.75	-9.45	12.29 ± 15.4
5-HT2B	45.92	11.35	11.15	17.78	21.55 ± 7.7
5-HT2C	28.83	-38.5	8.5	58.2	14.26 ± 40.6
5-HT3	-7.18	2.23	-23.93	12.89	-4 ± 15.6
5-HT5A	5.59	-6.33	-13.11	-3.89	-4.44 ± 7.73
5-HT6	17.7	23.24	-12.28	-5.73	5.73 ± 17.3

5-HT7A	-92.69	-50.78	-54.31	-21.23	-54.75 ± 29.3
Alpha1A	17	-16.24	-17.64	-15.07	-7.99 ± 16.7
Alpha1B	-6.11	-16.27	-36.74	-23.55	-20.67 ± 12.8
Alpha1D	6.91	-32.75	-9.43	-19.42	-13.67 ± 16.7
Alpha2B	-8.15	-23.65	-14.5	8.29	-9.5 ± 13.4
Alpha2C	38.57	2.3	-9.91	13.27	11.06 ± 20.6
Beta2	-12.23	-5.09	-7.41	-17.26	-10.5 ± 5.4
D1	22.73	-0.84	21.85	28.02	17.94 ± 12.8
D2	-8.12	21.42	-24.23	4.13	-1.7 ± 19.3
D3	3.09	-9.41	-6.79	-6.5	-4.9 ± 5.5
D4	73.21	69.99	60.34	65.33	67.22 ± 5.6
D5	4.57	-5.32	7.05	55.75	15.51 ± 27.3
DAT	-0.27	-4.72	8.47	14.9	4.6 ± 8.8
DOR	41.02	8.06	10.63	-3.98	13.93 ± 19.1
GABAA	24.87	0.04	4	17.31	11.56 ± 11.5
H1	13	-0.52	1.94	27.5	10.48 ± 12.7
H2	25.4	24.43	24.78	22.12	24.18 ± 1.43
H3	-25	-12.5	0	0	-9.38 ± 11.92
H4	-8.25	9.91	8.32	3.76	3.44 ± 8.21
M1	17.45	-8.79	-6.38	-1.13	0.29 ± 11.93
M2	-1.12	-4.61	-17.35	22.75	-0.08 ± 16.71
M3	-5.69	-3.65	-12.4	-16.48	-9.56 ± 5.94
M4	0.88	-23.89	-3.92	11.5	-3.86 ± 14.83
M5	15.35	31.89	40.55	21.65	27.36 ± 11.13
MOR	5.06	-11.35	8.74	-10.61	-2.04 ± 10.43

SERT	10.62	-3.59	10.62	25.65	10.83 ± 11.94
Sigma 1	8.39	1.56	8.99	30.38	12.33 ± 12.50
Sigma 2	42.39	19.45	26.56	20.46	27.22 ±10.59