DISCOVERY AND PRECLINICAL CHARACTERIZATION OF THE PHENYLALKYLAMINE, CYB210010, A POTENT AND LONG-ACTING SEROTONIN 5-HT$_2$ RECEPTOR AGONIST

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Introduction

The last decade has witnessed a revival of interest in serotonin (5-HT)-based psychedelics as pharmacotherapeutics for neuropsychiatric disorders.

Most of the clinical focus has been on the classic psychedelic tryptamines, including psilocybin and N,N-dimethyltryptamine (DMT), and results from several clinical trials suggest that psychedelics could result in a paradigm shift in psychiatric medicine.

Despite encouraging anecdotal human data and some academic explorations, there has been little focus on the development of another class of psychedelics, the 2C-X phenethylamines.

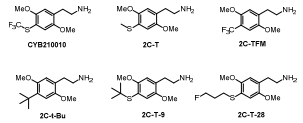
Given their clinical potential, the purpose of these studies was to explore the structure-activity relationships (SAR) of a broader range of 2C-X analogs to identify novel 5-HT$_2$ receptor ligands with unique pharmacodynamic (PD) and pharmacokinetic (PK) properties that may have therapeutic utility.

A particular focus of this work was the introduction of lipophilic 4-fluoroalkyl substitutions for enabling enhanced CNS penetration and improved receptor affinity/potency (Shah and Westwell, 2006; Hagmann, 2008; Gillis et al., 2015). These substitutions were tethered to the aryl ring by oxygen or sulfur heteroatoms with a selection of the latter leading to a cluster of the highly active molecules with attractive drug-like characteristics.

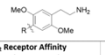
Screening Cascade

- Novel compounds were synthesized and tested for their potency at the 5-HT$_{2A}$ receptor using in vitro binding ([^{3}H]LSD or *[^{125}I]DOI competition binding) and/or functional assays (IP1 production).
- Compounds exhibiting <100 nM affinity (Ki) or functional potency (EC_{50}) at the 5-HT$_{2A}$ receptor were screened in the C57BL/6J mouse head twitch response (HTR) model of 5-HT$_{2A}$ receptor activation (@3 mg/kg, SC). The HTR model was adapted to evaluate the duration of activity.
- Additional in vitro competitive binding assays were conducted on specific compounds of interest to measure binding affinity at the 5-HT$_{2B}$ and/or 5-HT$_{2C}$ receptor subtypes.
- A highly potent 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor agonist, CYB210010 (compound 1) was identified that produced a robust and long-acting HTR. This compound was further evaluated for its activity at other serotonin receptors, as well as at >70 other proteins.
- Additional HTR studies were conducted and included SC and PO dose/response studies, and studies to measure the duration of effect and the impact of repeat dosing.
- The PK profile of CYB210010 was established in three species, mouse, rat and dog, following either IV, SC, or PO administration.
- Finally, the ability of CYB210010, dosed acutely or sub-chronically, to modulate genes implicated in neuroplasticity in the mouse frontal cortex and hippocampus, was tested.

CYB210010 and benchmark 2C-X phenethylamine psychedelics



5-HT$_2$ Receptor Affinities and Mouse HTR of 4-Thio- and 4-Oxy-fluoroalkyl-2,5-Dimethoxy-Substituted Phenethylamines



#	R	5-HT$_2$ Receptor Affinity K_i, nM (pK$_i$; 95 % CI) 5-HT$_{2A}$	5-HT$_{2B}$	5-HT$_{2C}$	5-HT$_{2A}$: 5-HT$_{2B}$ Selectivity	HTRs/10 min (3 mg/kg) 5-25** min	160-170 min
1		0.35* (9.46; 9.53-9.49) 4.1 (8.39; 8.45-8.35)	6.7* (8.17; 8.24-8.11) 10 (7.99; 8.07-7.93)	0.55* (9.26; 9.29-9.23) 6.7 (8.17; 8.25-8.10)	19* 2.5	21 ± 2.1	30 ± 4.1
2		6.3 (8.19; 8.25-8.15)	24 (7.63; 7.71-7.55)	16 (7.78; 7.85-7.72)	3.8	33 ± 4.1	17 ± 2.4
3		19 (7.73; 7.79-7.65)	6.5 (8.19; 8.33-8.05)	21 (7.68; 7.83-7.53)	0.37	17 ± 1.1	18 ± 1.1
4		1.9 (8.72; 8.76-8.6)	8.4 (8.07; 8.13-8.02)	13 (7.87; 7.95-7.79)	4.2	26 ± 2.3	28 ± 1.7
5		27 (7.56; 7.66-7.45)	26 (7.59; 7.77-7.41)	82 (7.08; 7.25-6.92)	0.96	3 ± 0.8	4 ± 0.6
6		11 (7.98; 8.04-7.91)	20 (7.69; 7.91-7.47)	83 (7.08; 7.27-6.89)	1.8	5 ± 1.7	1.7 ± 0.4
7		5.9 (8.22; 8.35-8.09)	28 (7.56; 7.68-7.44)	26 (7.59; 7.79-7.40)	4.7	1.7 ± 0.4	2.3 ± 0.4
8		78 (7.11; 7.19-7.02)	66 (7.18; 7.32-7.04)	67 (7.18; 7.32-7.04)	0.84	1.8 ± 0.2	3.4 ± 1
9		59 (7.23; 7.36-7.10)	10 (7.99; 8.14-7.86)	26 (7.59; 7.68-7.51)	0.17	0.6 ± 0.2	4.8 ± 0.6
10		10 (8.00; 8.06-7.95)	5.5 (8.26; 8.37-8.15)	11 (7.67; 8.06-7.87)	0.60	3.3 ± 0.8	6.1 ± 1
11		23 (7.64; 7.70-7.57)	6.8 (8.17; 8.25-8.09)	8.9 (8.05; 8.14-7.96)	0.29	5 ± 0.8	2.6 ± 0.7
12		33.7 (7.42; 7.53-7.41)	4.5 (8.35; 8.41-8.28)	10.8 (7.97; 8.07-7.87)	0.13	3.1 ± 0.5	5.6 ± 0.9
13		477 (6.32; 6.48-6.17)	ND	ND	ND	ND	ND
14		387 (6.41; 6.52-6.30)	ND	ND	ND	ND	ND
15		1636 (5.77; 5.92-5.67)	ND	ND	ND	ND	ND

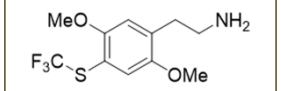
Binding assay: [^{3}H]LSD competition binding except for *[^{125}I]DOI competition binding data from Eurofins.
Total number of HTRs per time period; **early observations were performed from 5-15 min or 15-25 min post injections, and data reported are mean HTRs/10 min ± SEM. HTR studies included vehicle control and DOI (positive control) groups.
ND = Not Determined.

In Vitro Receptor Pharmacology

Serotonergic Receptors	% Inhibition @ 10 µM (Ki, nM) CYB210010	DOI
5-HT$_{1A}$	98 (32)	81
5-HT$_{1B}$	83 (949)	53
5-HT$_{2A}$	100 (0.35)	100 (0.28)
5-HT$_{2B}$	100 (6.7)	100 (1.5)
5-HT$_{2C}$	99 (0.55)	100 (0.48)
5-HT$_3$	0	20
5-HT$_{3A}$	20	6
5-HT$_6$	98 (65)	64
5-HT$_7$	98 (4918)	58

Secondary Targets	% Inhibition @ 10 µM CYB210010	DOI
Adrenergic α_2	97	82
Adrenergic β_2	52	82
L-Type Ca^{2+} channel	85	56
Na+ channel (Site 2)	68	54
Dopamine D_2	70	38
Muscarinic M_1	88	72
Muscarinic M_3	79	60
Muscarinic M_4	86	76
Muscarinic M_5	84	63
µ Opioid	49	65
Sigma	64	62
5-HT transporter	30	76

CYB210010



5-HT$_{2A}$ EC_{50} = 4.1 nM
5-HT$_{2B}$ EC_{50} = 88 nM
5-HT$_{2C}$ EC_{50} = 7.3 nM

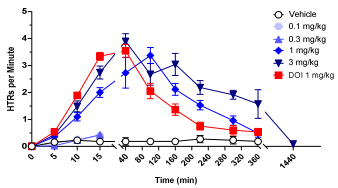
Ex Vivo 5-HT$_{2A}$ Receptor Binding

	Specific [^{3}H]Cimbi-36 Binding	% Inhibition of [^{3}H]Cimbi-36 Binding	P value
Vehicle	1092 ± 53	0	
CYB210010 5 min	947 ± 48	13	0.141
CYB210010 10 min	993 ± 38	9	0.519
CYB210010 15 min	971 ± 66	11	0.297
CYB210010 40 min	732 ± 15	33	<0.001
CYB210010 100 min	820 ± 28	25	<0.001
CYB210010 160 min	736 ± 30	33	<0.001
CYB210010 220 min	796 ± 35	27	<0.001
CYB210010 300 min	857 ± 33	22	0.003
CYB210010 360 min	820 ± 44	25	<0.001

Competitive binding profile of CYB210010 in the mouse frontal cortex.
Values are mean (± SEM) . N=5 per group/time point.

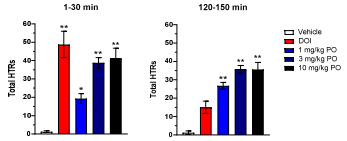
Mouse Head Twitch Response

CYB210010 (SC) dose/response and time-course



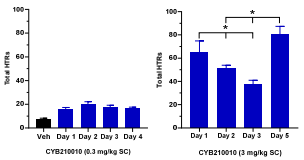
Effects of CYB210010 (0.1-3 mg/kg, SC) on the HTR in mice.
Values are mean ± SEM . N=7 (CYB210010 at each dose) to 36 (DOI 1 mg/kg, IP) per group.

CYB210010 (PO) dose/response



Effects of CYB210010 and DOI on the HTR in mice.
Values are mean ± SEM. *p<0.05; **p<0.01 vs. Vehicle, N=10 per group. DOI 1 mg/kg, IP

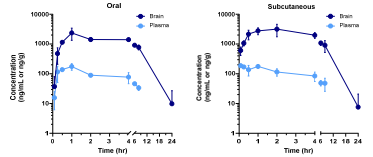
CYB210010 (SC) tachyphylaxis study



Effects of repeat subcutaneous dosing of CYB210010 on the mouse HTR.
CYB210010 dosed at 0.3 mg/kg (left) and at 3 mg/kg once a day for 3 days, followed by a 48 hr washout period and then a Day 5 dose at 3 mg/kg (right). Values are mean ± SEM. *p<0.01 vs. Day 1 or Day 5. N=6 per group.

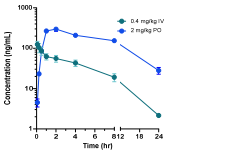
In Vivo Pharmacokinetics

Mouse SC/PO PK Study



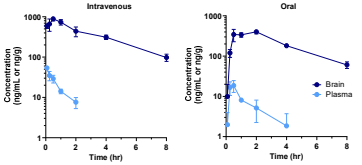
CYB210010 plasma and brain concentrations in C57/BL6 mice after SC (3 mg/kg) and PO (3 mg/kg) administration. Values are mean ± SEM. N=3 per route.

Dog IV/PO PK Study



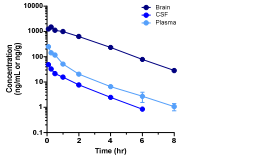
CYB210010 plasma concentrations in the dog after IV and PO administration. Values are mean ± SEM. N=3 per route.

Rat IV/PO PK Study



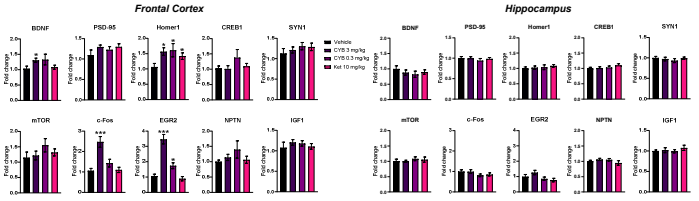
CYB210010 plasma and brain concentrations in Wistar rat after IV (1 mg/kg) and PO (5 mg/kg) administration. Values are mean ± SEM. N=3 per route.

Rat IV (1 mg/kg) 3-Compartment PK Study



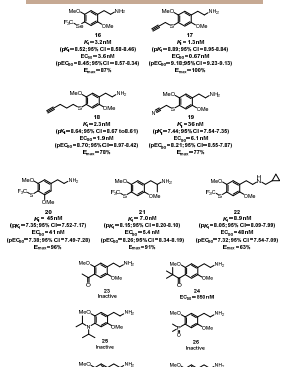
CYB210010 concentrations in Sprague Dawley rat plasma, brain and CSF after IV administration at 1 mg/kg. Values are mean ± SEM. N=5.

Gene Expression Study



CYB210010-induced changes in expression of genes associated with neurogenesis and neuroplasticity in mouse frontal cortex and hippocampus.
CYB210010 dosed at 3 mg/kg (SC) or for 4 consecutive days at 0.3 mg/kg (SC). Ketamine administered at 10 mg/kg (IP). Tissue collected 2 hr after final dose. N=9-10 per group; *p<0.05, ***p<0.001 vs. Vehicle.

Additional active and inactive molecules



Exploring the effect of deuteration

#	R		R_1	R_2	R_3	R_4	5-HT$_{2A}$ Affinity (K_i, nM)	RLM $T_{1/2}$ (min)
		2C-T	Me	Me	H	H	296	6.2
29		2C-T-d2	Me	Me	D	D	357	6.6
30		2C-T-d6	CD_3	CD_3	H	H	174	6.9
31		*R*-ALEPH-1	Me	Me	Me	H	7	15
32		*S*-ALEPH-1	Me	Me	H	Me	18	4.5
33		*R*-ALEPH-1-d6	CD_3	CD_3	Me	H	26	16.6
34		*S*-ALEPH-1-d6	CD_3	CD_3	H	Me	56	6.3
		2C-TFM	Me	Me	H	H	3.5	7.7
35		2C-TFM-d2	Me	Me	D	D	42	11.1
36		2C-TFM-d6	CD_3	CD_3	H	H	55	21.4
		2C-t-butyl	Me	Me	H	H	9.9	2.4
37		2C-t-butyl-d6	CD_3	CD_3	H	H	11	2.4
38		2C-t-butyl-d9	Me	Me	H	H	10	2.5
		2C-Cp	Me	Me	H	H	134	7.8
39		2C-Cp-d6	CD_3	CD_3	H	H	212	7.5

RLM: Rat Liver Microsomes; $T_{1/2}$: half-life

Results and Discussion

- Structure-Activity studies focused on the 2C-X scaffold identified the novel, potent, and orally active serotonin 5-HT$_{2A}$/5-HT$_{2C}$ receptor agonist, CYB210010, as well as other potent agonists that require further profiling.
- Strategies such as deuteration had little to no effect on 5-HT$_{2A}$ receptor binding affinity or metabolism.
- CYB210010 exhibits modest selectivity over the 5-HT$_{1A}$, 5-HT$_{2B}$, and 5-HT$_6$ subtypes.
- CYB210010 had modest potency at adrenergic α_{2A} (72 nM) and α_{2C} (108 nM) receptors but low potency at α_{2B} receptors (1052 nM). CYB210010 was a partial agonist at human α_{2A} receptors with an EC_{50} of 171 nM (clonidine: EC_{50}=0.4 nM).
- CYB210010 induces a robust and enduring head twitch response (HTR) in the mouse when administered either SC or PO.
- Repeated administration of a 3 mg/kg dose of CYB210010 resulted in a blunted HTR that returned to a normal response level after 48-hr. Repeat administration of a 0.3 mg/kg dose did not result in any reduction in the low level HTR response.
- Acute administration of a HTR-inducing dose of CYB210010 (3 mg/kg) resulted in changes in the mRNA levels of several proteins (BDNF, Homer1, EGR2) in the frontal cortex, but not in the hippocampus.
- Administration of a minimally active HTR dose (0.3 mg/kg) of CYB210010 induced changes in the mRNA levels of Homer1 and EGR2 in the frontal cortex.
- CYB210010 was orally bioavailable in the mouse, rat and dog with rapid distribution into the CSF and brain (clinical signs were evident within 5-10 min)
- Ongoing studies are focused on identifying potential therapeutic indications for CYB210010 and other potent agonists in this 2C-X series.