Supporting information

Identification of Novel α4β2-Nicotinic Acetylcholine Receptor (nAChR) Agonists Based on an Isoxazole Ether Scaffold that Demonstrate Antidepressant-like Activity

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Broad Screening at Other Neurotransmitter Receptors and Transporters

Table 1. Primary binding competition efficacies (%) of compound **43** at various neurotransmitter receptors and transporters[a]

Target	Serotonergic Receptors								
	5-HT_{1A}	5-HT_{1B}	5-HT_{1D}	5-HT_{1E}	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}	5-HT_3	5-HT_{5A}
Inhibition	16.4	5.8	1.3	1.3	3.5	9.8	1.2	0.6	4.8

Target	Serotonergic Receptors		GABA	Dopaminergic Receptors					BZPR[c]
	5-HT_6	5-HT_7	$GABA_A$	D_1	D_2	D_3	D_4	D_5	
Inhibition	-4.9[b]	14.3	30.1	14.6	14.7	-0.8	13.1	10.8	44.7

Target	Adrenergic Receptors								
	α_{1A}	α_{1B}	α_{1D}	α_{2A}	α_{2B}	α_{2C}	β_1	β_2	β_3
Inhibition	-8.4	-0.3	-0.5	15.5	31.1	30.2	6.5	-1.3	21.9

Target	Histaminergic Receptors			Muscarinic Receptors					
	H_1	H_2	H_3	H_4	M_1	M_2	M_3	M_4	M_5
Inhibition	-9.7	20.1	14.7	-2.5	-3.7	4.2	-9.5	-9.4	-2.3

Target	Opioid Receptors[d]			Transporters[e]			Sigma Receptors	
	DOR	KOR	MOR	DAT	NET	SERT	σ 1	σ 2
Inhibition	16.2	4.5	21.8	48.5	5.2	-6.1	19.7	24.6

[a] The default concentration for primary binding experiments is 10 μM (n = 4). For details see Experimental Section. [b] Negative inhibition represents a stimulation of binding. [c] BZPR: Benzodiazepine Receptors (rat brain site). [d] DOR: Delta Opioid Receptor; KOR: Kappa Opioid Receptor; MOR: Mu Opioid Receptor. [e] DAT: Dopamine Transporter; NET: Norepinephrine Transporter; SERT: Serotonin Transporter.

Preliminary *in vitro* ADME-Tox Profile

Table 2. Effect of compound **43** on *in vitro* CYP activity in human liver microsomes [a]

Inhibitor Test Article	Conc μM	1A2 (Phenacetin)		2B6 (Bupropion)		2C9 (Diclofenac)		2C19 (Mephenytoin)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Furafylline	10	42.9	1.4	98.0	5.8	101.8	3.2	93.5	1.0
ThioTEPA	10	85.6	2.0	34.4	1.1	93.2	9.5	97.9	3.1
Sulfaphenazole	3	90.2	1.1	93.6	2.2	13.2	0.7	92.5	8.8
Tranylcypromine	10	74.1	2.9	20.3	1.3	76.2	5.0	71.5	2.6
Quinidine	2	105.3	4.6	100.9	3.5	93.2	4.4	95.5	0.3
Ketoconazole	5	71.1	1.5	36.2	0.5	68.4	1.2	71.1	4.6
Compound **43**	1	96.1	2.3	96.3	2.1	90.6	4.5	99.8	0.6
Compound **43**	10	101.4	10.4	103.0	8.0	100.3	8.8	104.6	8.4

Inhibitor Test Article	Conc μM	2D6 (Bufuralol)		3A4 (Testosterone)		3A4 (Midazolam)	
		Mean	SD	Mean	SD	Mean	SD
Furafylline	10	95.1	6.3	98.8	2.9	101.9	2.2
ThioTEPA	10	86.5	1.6	88.6	3.7	89.7	1.3
Sulfaphenazole	3	84.2	2.0	101.7	1.8	100.8	3.7
Tranylcypromine	10	77.7	8.7	93.4	1.5	97.4	2.3

Quinidine	2	22.3	1.0	97.6	3.1	98.1	2.5
Ketoconazole	5	71.1	0.3	12	0.1	2.4	0.2
Compound **43**	1	89.9	1.0	100.8	3.8	101.4	2.0
Compound **43**	10	102.3	8.7	100.8	3.8	103.3	1.9

[a] Values represent mean of triplicate determinations ± SD

Table 3. *In vitro* metabolic stability of compound **43** and midazolam using human and mouse liver microsomes

Species	Time, min	Mean % Remaining vs T = 0 min[a,b]		
		1 μM compound **43**	10 μM compound **43**	10 μM Midazolam
HUMAN	15	79.4	80.4	42.9
	30	62.3	74.2	26.6
	60	53.4	67.2	9.5
HI[c] HUMAN	60	99.3	102.6	100.0
MOUSE	15	94.0	90.6	20.2
	30	86.6	82.6	3.0
	60	67.5	73.7	0.0
HI[c] MOUSE	60	102.1	98.9	108.0

[a] % remaining at T=0 is 100%
[b] Samples were assayed in duplicate.
[c] HI = Heat Inactivated

In vitro binding studies, broad-range screening, and hERG inhibition were carried out by the National Institute of Mental Health's Psychoactive Drug Screening Program, contract # HHSN-271-2008-00025-C (NIMH PDSP). For experimental details please refer to the PDSP web site http://pdsp.med.unc.edu/