

Supporting Information

The Use of Structure-Based Design to Discover a Potent, Selective, In Vivo Active Phosphodiesterase 10A Inhibitor Lead Series for the Treatment of Schizophrenia

Christopher J. Helal,* Zhijun Kang, Xinjun Hou, Jayvardhan Pandit, Thomas A. Chappie, John M. Humphrey, Eric S. Marr, Kimberly F. Fennell, Lois K. Chenard, Carol Fox, Christopher J. Schmidt, Robert D. Williams, Douglas S. Chapin, Judy Siuciak, Lorraine Lebel, Frank Menniti, Julia Cianfroga, Kari R. Fonseca, Frederick R. Nelson, Rebecca O'Connor, Mary MacDougall, Laura McDowell, and Spiros Liras

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X-ray Crystal Structure Experimental Conditions

Rat PDE10A protein expression, purification and co-crystallization with compounds **1** and **2** was carried out as described previously.¹ All other inhibitor complex crystals were obtained by soaking these crystals for ~12 hours in a 1 mM (or saturated) solution of the inhibitor in crystallization buffer.

PDE10 Assay Data with Standard Error

PDE10 assay is that as reported in Summary Table of PDE10 IC₅₀ data. Each value is the geometric mean of at least 3 separate determinations with Standard Error of the Mean (SEM) reported.

	PDE10 IC₅₀ (nM)	Standard Error of the Mean
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*Corresponding author. Tel: (860) 715-5064; fax: (860) 686-5291; e-mail address: chris.j.helal@pfizer.com; MS 8220-4326, Eastern Point Road, Groton, CT 06340

		(SEM) (nM)
3	146	14
7	1040	144
12	1665	449
13	247	46
14	2338	205
16	12	0.7
19	10	4
20	809	145
24	17	2
25	14	1
26	5	1
27	12	3

PDE Selectivity Data

% Inhibition at 1 uM for **16**, **24**, **26**, and **27** vs. representative PDE families.

Compound 16				
1A	2A	3A	4D	5A
29	16	44	17	14
6A	7A	8A	9A	11A
NA	0	0	0	8

Compound 24				
1A	2A	3A	4D	5A
9	18	51	4	34
6A	7A	8A	9A	11A
NA	0	0	1	17

Compound 26				
1A	2A	3A	4D	5A

91	41	43	20	85
6A	7A	8A	9A	11A
NA	2	3	0	59

Compound 27				
1A	2A	3A	4D	5A
5	13	20	9	15
6A	7A	8A	9A	11A
NA	9	0	0	12

The effect of compounds **25**, **26**, and **27** on striatal cGMP levels in mice.

Doses of **25**, **26**, and **27** or vehicle (28.16 mls 23 % β -cyclodextrin/1.5 mls DMSO/0.034 mL 2N HCl) were administered subcutaneously to male CD-1 mice (Table B1). After the indicated time, animals were sacrificed by focused microwave irradiation, after which striatal tissue was microdissected and cGMP levels determined as previously described.² The mean and standard deviation are shown (n = 3 animals/group; 2 samples analyzed/animal). **25**, **26**, and **27** caused a statistically significant increase in striatal cGMP relative to the levels observed in the vehicle – treated group.

Compound	Dose (mg/kg)	Time of sacrifice post drug dosing (min)	cGMP (pmoles/mg, tissue) (SD)	Vehicle cGMP (pmols/mg, tissue) (SD)	% Increase cGMP relative to control
25	5.6	60	0.173 (0.013)	0.129 (0.017)	34
25	32	60	0.475 (0.172)	0.129 (0.017)	268
26	5.6	60	0.182 (0.038)	0.100 (0.014)	82
27	32	15	0.42 (0.134)	0.183 (0.001)	130
27	32	30	0.328 (0.080)	0.183 (0.001)	79

The effect of compound **26** on Conditioned Avoidance Responding in wild type and PDE10A knockout mice

Testing was performed in rats as previously described (Siuciak et al., 2006)¹ using commercially available shuttle boxes (Coulbourn, Allentown, PA, USA). The Plexiglas shuttle boxes were divided by a guillotine door into two sides, and enclosed in sound-attenuating chambers. The shuttle boxes were fitted with metal grid floors equipped with scrambled/constant current shockers. Training consisted of repeated 30 pairings of multiple conditioned stimuli of duration (activation of house lights, cue lights, and the opening of the guillotine door), followed 5 sec

later by a 0.6 mA shock. The shock was terminated when the animal crossed to the other side of the shuttle box, or after 10 sec. Thirty trials were completed per session, and the number of avoidances (animal crossed before receiving shock, maximum=30), escapes (animal crossed after receiving shock, maximum=30), escape failures (animal failed to cross, maximum=30), latency to avoid (maximum=5 sec), latency to escape (maximum = 10 sec), and adaptation crossovers (number of crossovers during a five minute period before the onset of trials, dark chamber) were recorded by the computer program. The intertrial interval was 30 seconds in duration, during which time the guillotine door remained closed. Training was continued until a shock avoidance criterion of 80% avoidances was achieved. Compounds were administered 30 minutes prior to test sessions. Animals received vehicle treatment one day each week and statistical analyses comparing mean 3 differences in performance under drug to performance under vehicle were tested using paired t-tests with the criterion for significance set at $p < 0.05$.

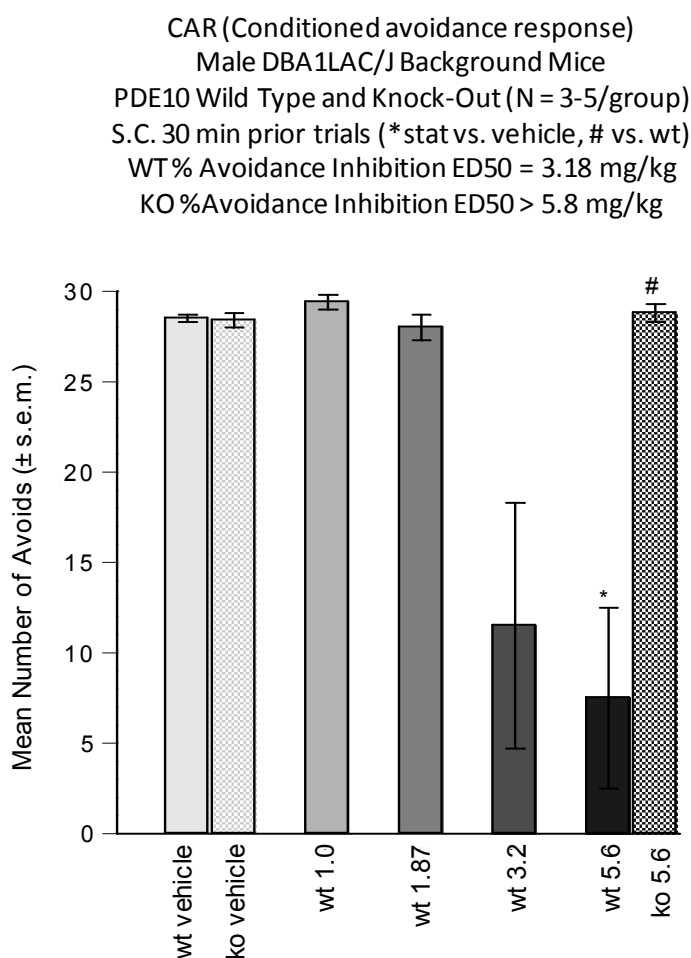


Figure 7. Conditioned avoidance responding study with compound 26

¹ Pandit, J. Crystal Structure of 3', 5'-CyclicNucleotide Phosphodiesterase (PDE10A) and Uses Thereof; Pfizer, Inc.: US 2005/0202550, 2005; pp 72.

² Siuciak, J. A.; Chapin, D. S.; Harms, J. F.; Lebel, L. A.; McCarthy, S. A.; Chambers, L.; Shrikhande, A.; Wong, S.; Menniti, F. S.; Schmidt, C. J. Inhibition of the Striatum-Enriched Phosphodiesterase PDE10A: A Novel Approach to the Treatment of Psychosis. *Neuropharmacology* **2006**, 51, 386-396.