Supporting information for:

A novel M_1 PAM VU0486846 exerts efficacy in cognition models without displaying agonist activity or cholinergic toxicity

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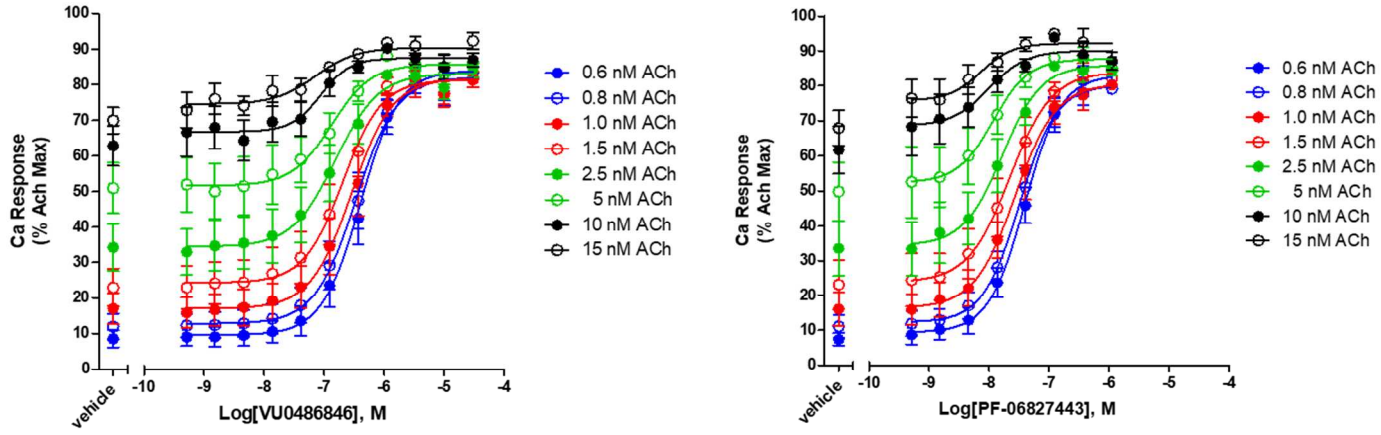

% ACh max

Ach conc.	0.6 nM	0.8 nM	1.0 nM	1.5 nM	2.5 nM	5 nM	10 nM	15 nM
Vehicle	9 ± 2	12 ± 4	17 ± 4	23 ± 5	34 ± 7	51 ± 7	63 ± 6	70 ± 4
VU0486846	84 ± 2	82 ± 2	82 ± 2	82 ± 2	83 ± 2	86 ± 1	88 ± 1	90 ± 1
PF-06827443	83 ± 2	80 ± 2	81 ± 3	83 ± 2	85 ± 2	88 ± 2	90 ± 1	92 ± 1

PAM EC50 (nM)

Ach conc.	0.6 nM	0.8 nM	1.0 nM	1.5 nM	2.5 nM	5 nM	10 nM	15 nM
VU0486846	430 ± 120	360 ± 118	308 ± 110	217 ± 72	161 ± 54	130 ± 22	83 ± 17	68 ± 11
PF-06827443	40 ± 6	33 ± 6	30 ± 8	23 ± 6	16 ± 3	11 ± 1	9 ± 3	7 ± 1

PAM EC50 Fold Shift

Ach conc.	0.6 nM	0.8 nM	1.0 nM	1.5 nM	2.5 nM	5 nM	10 nM	15 nM
VU0486846	1.0	1.2	1.4	2.0	2.7	3.3	5.1	6.3
PF-06827443	1.0	1.2	1.4	1.8	2.5	3.6	4.6	5.7

Supporting Figure 1. M_1 PAM potency comparison of **6** and VU486 (**16**) at varying acetylcholine concentrations in hM_1-CHO cells. With increasing ACh, M_1 PAM potency increases.

				VU0486846			
				100 mg/kg			
		Time (min)	15	30	60	120	180
Autonomic Nervous System							
	Ptosis		-	-	-	-	-
	Exophtalmus		-	-	-	-	-
	Miosis		-	-	-	-	-
	Mydriasis		-	-	-	-	-
	Corneal reflex loss		-	-	-	-	-
	Pinna reflex loss		-	-	+	+	
	Piloerection		-	++	++	++	++
	Respiratory rate		-	-	-	-	-
	Writhing		-	-	-	-	-
	Tail erection		-	-	-	-	-
	Lacrimation		-	-	-	-	-
	Defication		-	-	-	-	-
	Salivation		-	-	-	-	-
	Vasodilatation		-	-	-	-	-
	Skin color		-	-	-	-	-
	Irritability		-	-	-	-	-
			-	-	-	-	-
Somatomotor systems			-	-	-	-	-
	Motor activity		-	-	-	-	-
	Convulsions		-	-	-	-	-
	Arch/roll		-	-	-	-	-
	Tremors		-	-	-	-	-
	Leg weakness		-	-	-	-	-
	Rigid stance		-	-	-	-	-
	Spraddle		-	-	-	-	-
	Placing loss		-	-	-	-	-
	Grasping loss		-	-	-	-	-
	Righting loss		-	-	-	-	-
	Catalepsy		-	-	-	-	-
	Tail pinch		-	-	-	-	-
	Escape loss		-	-	-	-	-
	The mean scores of eight animals per treatment are classified as follows:						
	- no effect (+) 0.01 – 0.25 + 0.251 – 0.50 ++ 0.51 – 1.0						

Supporting Figure 2. A Modified Irwin Toxicology Battery test in mice was used to assess any potential adverse effects of VU0486846 on the CNS. After administration of vehicle or 100 mg/kg VU0486846 (10% Tween 80, i.p., 10 ml/kg) animals were evaluated at 15, 30, 60 120 and 180 min for behavioral and

physiological changes from pretreatment baselines. An observer blinded to the treatment evaluated a series of autonomic nervous system and somatomotor system assessments and scored them on a scale from 0 (normal) to 3 (severe). The mean Modified Irwin Toxicology Battery test scores are classified as follows: - no effect, (+) 0.01 to 0.25, + 0.251 to 0.50, ++ 0.51 to 1.0. Pretreatment of VU0486846 (100 mg/kg, i.p.) did not cause significant observable adverse effects in normal, healthy mice.

				VU0486846			
				56.6 mg/kg			
		Time (min)	15	30	60	120	180
Autonomic Nervous System							
	Ptosis		-	-	-	-	-
	Exophtalmus		-	-	-	-	-
	Miosis		-	-	-	-	-
	Mydriasis		-	-	-	-	-
	Corneal reflex loss		-	-	-	-	-
	Pinna reflex loss		-	-	-	-	-
	Piloerection		-	-	-	-	-
	Respiratory rate		-	-	-	-	-
	Writhing		-	-	-	-	-
	Tail erection		-	-	-	-	-
	Lacrimation		-	-	-	-	-
	Defication		-	-	-	-	-
	Salivation		-	-	-	-	-
	Vasodilatation		-	-	-	-	-
	Skin color		-	-	-	-	-
	Irritability		-	-	+	-	-
			-	-	-	-	-
Somatomotor systems			-	-	-	-	-
	Motor activity		-	-	-	-	-
	Convulsions		-	-	-	-	-
	Arch/roll		-	-	-	-	-
	Tremors		-	-	-	-	-
	Leg weakness		-	-	-	-	-
	Rigid stance		-	-	-	-	-
	Spraddle		-	-	-	-	-
	Placing loss		-	-	-	-	-
	Grasping loss		-	-	-	-	-
	Righting loss		-	-	-	-	-
	Catalepsy		-	-	-	-	-
	Tail pinch		-	-	-	-	-
	Escape loss		-	-	-	-	-
	The mean scores of eight animals per treatment are classified as follows:						
	- no effect	(+) 0.01 – 0.25	+ 0.251 – 0.50		++ 0.51 – 1.0		

Supporting Figure 2. A Modified Irwin Toxicology Battery test in rats was performed to evaluate any potential adverse effects of VU0486846 on the CNS. After administration of vehicle or 56.6 mg/kg VU0486846 (10% Tween 80, i.p., 2 ml/kg), animals were evaluated at 15, 30, 60 120 and 180 min for

behavioral and physiological changes from pretreatment baselines. An observer blinded to the treatment evaluated a series of autonomic nervous system and somatomotor system assessments and scored them on a scale from 0 (normal) to 3 (severe). The mean Modified Irwin Toxicology Battery test scores are classified as follows: - no effect, (+) 0.01 to 0.25, + 0.251 to 0.50, ++ 0.51 to 1.0. Pretreatment of VU0486846 (56.6 mg/kg, i.p.) did not cause significant observable adverse effects in normal, healthy rats.

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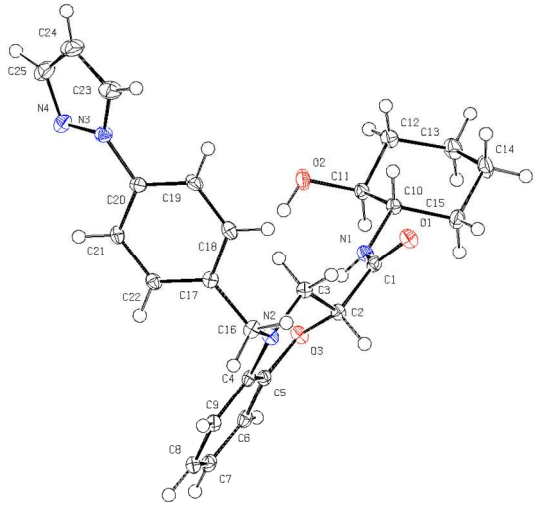

Table 1 Crystal data and structure refinement for Garcia01.

Identification code	Garcia01
Empirical formula	$C_{25}H_{28}N_4O_3$
Formula weight	432.51
Temperature/K	100
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a/Å	5.74144(5)
b/Å	12.67188(16)
c/Å	29.0999(3)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	2117.15(4)
Z	4
ϱ_{calc}g/cm^3	1.357
μ/mm^{-1}	0.731
F(000)	920.0
Crystal size/mm^3	0.431 × 0.196 × 0.045
Radiation	Cu Kα (λ = 1.54184)
2Θ range for data collection/°	7.61 to 147.582
Index ranges	-7 ≤ h ≤ 6, -15 ≤ k ≤ 15, -34 ≤ l ≤ 36
Reflections collected	21610
Independent reflections	4262 [R_{int} = 0.0357, R_{sigma} = 0.0266]
Data/restraints/parameters	4262/0/290
Goodness-of-fit on F^2	1.029
Final R indexes [I>=2σ (I)]	R_1 = 0.0318, wR_2 = 0.0801
Final R indexes [all data]	R_1 = 0.0338, wR_2 = 0.0817
Largest diff. peak/hole / e Å^{-3}	0.15/-0.18
Flack parameter	-0.12(7)

Table 2 Fractional Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$) for Garcia01. U_{eq} is defined as 1/3 of of the trace of the orthogonalised U_{IJ} tensor.

Atom	*x*	*y*	*z*	U(eq)
O1	3009(2)	3934.6(11)	3601.3(5)	18.3(3)
O2	10096(3)	5726.5(12)	3703.9(5)	22.9(3)
O3	8007(2)	2471.3(11)	3264.0(4)	14.8(3)
N1	6848(3)	4055.4(13)	3786.4(5)	14.8(3)
N2	5254(3)	1961.1(13)	2495.3(5)	13.5(3)
N3	5473(3)	5306.2(13)	849.8(6)	16.0(3)
N4	7668(3)	5701.2(14)	794.6(6)	19.4(4)
C1	5036(3)	3618.3(15)	3569.5(6)	13.5(4)
C2	5560(3)	2673.6(15)	3265.9(6)	12.7(4)
C3	4719(3)	2869.5(15)	2777.5(6)	13.6(4)
C4	7247(3)	1385.2(15)	2588.0(6)	11.9(3)
C5	8605(3)	1633.2(15)	2977.7(6)	12.2(4)
C6	10568(3)	1064.2(15)	3093.1(6)	14.1(4)
C7	11304(3)	223.2(16)	2822.2(7)	16.6(4)
C8	9990(3)	-44.3(15)	2438.2(6)	16.0(4)
C9	7988(3)	520.6(15)	2326.7(6)	14.1(3)
C10	6605(3)	5014.3(15)	4062.6(6)	13.3(4)
C11	8986(3)	5527.8(15)	4131.5(6)	13.9(4)
C12	8682(3)	6567.3(16)	4386.7(7)	17.3(4)
C13	7503(4)	6400.3(18)	4851.3(7)	21.2(4)
C14	5171(4)	5835.1(18)	4793.5(7)	23.3(4)
C15	5479(4)	4801.7(16)	4530.1(7)	17.7(4)
C16	3895(3)	1847.2(15)	2075.6(6)	14.3(4)
C17	4332(3)	2720.1(15)	1729.1(6)	12.7(4)
C18	2622(3)	3480.0(16)	1660.5(6)	16.1(4)
C19	2977(3)	4323.9(16)	1364.4(6)	16.4(4)
C20	5081(3)	4404.9(15)	1130.7(6)	14.0(4)
C21	6793(3)	3637.6(16)	1183.1(6)	15.1(4)
C22	6416(3)	2801.2(15)	1483.5(6)	14.3(4)
C23	3826(4)	5945.1(18)	659.7(7)	23.4(4)
C24	4988(5)	6789.8(18)	471.6(7)	27.1(5)
C25	7344(4)	6602.4(18)	566.8(7)	24.4(5)

Table 3 Anisotropic Displacement Parameters ($Å^2 \times 10^3$) for Garcia01. The Anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U_{11}+2hka^*b^*U_{12}+\ldots]$.

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O1	15.9(7)	19.7(7)	19.5(6)	-3.6(5)	0.0(6)	0.5(5)
O2	24.8(8)	21.7(8)	22.4(7)	-5.0(6)	12.5(6)	-4.7(6)
O3	11.6(6)	16.2(7)	16.5(6)	-5.3(5)	-2.5(5)	-0.3(5)
N1	14.6(7)	14.7(8)	15.0(7)	-3.9(6)	-0.4(6)	-1.1(6)
N2	12.8(7)	15.1(8)	12.7(7)	-2.2(6)	-2.3(6)	1.1(6)
N3	18.3(8)	14.8(8)	14.9(7)	1.2(6)	-1.5(6)	1.9(6)
N4	22.4(8)	19.4(9)	16.6(7)	2.4(6)	0.7(6)	-3.2(7)
C1	15.8(9)	14.3(8)	10.4(7)	0.6(6)	0.2(7)	-1.7(7)
C2	9.9(8)	14.1(9)	14.3(9)	-1.9(7)	-0.2(6)	-1.7(7)
C3	13.1(8)	13.8(9)	13.8(8)	-0.8(6)	-1.0(7)	-1.1(7)
C4	9.5(8)	14.4(8)	11.7(8)	2.0(6)	0.6(6)	-2.2(7)
C5	11.4(8)	12.3(8)	12.8(8)	-0.9(6)	1.5(6)	-2.8(7)
C6	10.3(8)	17.9(9)	14.0(8)	3.3(7)	-0.2(6)	-2.6(7)
C7	12.1(8)	18(1)	19.6(9)	4.6(7)	2.5(7)	0.1(7)
C8	16.0(8)	15.5(9)	16.6(9)	-0.2(7)	4.2(7)	0.2(7)
C9	14.1(8)	14.4(9)	13.7(8)	-1.6(7)	0.7(7)	-2.3(7)
C10	13.6(8)	13.7(9)	12.5(8)	-2.5(7)	-0.2(7)	-1.2(7)
C11	12.0(8)	14.1(9)	15.5(8)	-2.0(7)	1.7(7)	-1.4(7)
C12	17.0(9)	16.7(10)	18.0(9)	-4.8(7)	-0.2(7)	-2.9(7)
C13	25.7(10)	22.4(10)	15.5(9)	-7.6(7)	-0.1(8)	-0.9(8)
C14	24.9(10)	26.3(11)	18.8(9)	-7.6(8)	7.8(8)	-1.6(9)
C15	18.7(9)	18.4(10)	16.1(9)	-1.5(7)	3.7(7)	-4.6(8)
C16	13.3(8)	17.4(9)	12.3(8)	0.0(7)	-3.1(7)	-3.1(7)
C17	12.5(8)	15.1(9)	10.4(8)	-1.3(7)	-2.7(6)	-1.4(7)
C18	12.6(8)	19.2(10)	16.6(9)	-2.3(7)	0.3(7)	2.6(7)
C19	15.1(8)	16.1(9)	17.8(8)	-0.5(7)	-0.3(7)	5.7(7)
C20	17.7(9)	12.8(8)	11.4(8)	-0.7(6)	-2.0(7)	2.0(7)
C21	11.1(8)	18.8(10)	15.4(8)	-0.8(7)	2.4(7)	1.0(7)
C22	11.3(8)	16.2(9)	15.4(8)	-0.2(7)	-0.9(7)	2.9(7)
C23	24.3(10)	21.8(11)	24.2(10)	2.9(8)	-6.2(9)	6.1(9)
C24	42.4(13)	18.7(10)	20.2(10)	5.0(8)	-2.2(10)	5.5(10)
C25	37.3(12)	19.5(10)	16.4(9)	3.6(8)	0.4(8)	-4.5(9)

Table 4 Bond Lengths for Garcia01.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
O1	C1	1.235(2)	C6	C7	1.391(3)
O2	C11	1.420(2)	C7	C8	1.391(3)
O3	C2	1.428(2)	C8	C9	1.392(3)
O3	C5	1.393(2)	C10	C11	1.527(2)
N1	C1	1.337(2)	C10	C15	1.530(2)
N1	C10	1.463(2)	C11	C12	1.522(3)
N2	C3	1.447(2)	C12	C13	1.527(3)
N2	C4	1.384(2)	C13	C14	1.528(3)
N2	C16	1.456(2)	C14	C15	1.528(3)
N3	N4	1.365(2)	C16	C17	1.518(2)
N3	C20	1.423(2)	C17	C18	1.390(3)
N3	C23	1.362(3)	C17	C22	1.397(3)
N4	C25	1.333(3)	C18	C19	1.388(3)
C1	C2	1.518(2)	C19	C20	1.390(3)
C2	C3	1.521(2)	C20	C21	1.391(3)
C4	C5	1.412(2)	C21	C22	1.391(3)
C4	C9	1.400(3)	C23	C24	1.375(3)
C5	C6	1.379(3)	C24	C25	1.401(4)

Table 5 Bond Angles for Garcia01.

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
C5	O3	C2	112.44(14)	C8	C9	C4	121.78(17)
C1	N1	C10	121.93(16)	N1	C10	C11	109.91(14)
C3	N2	C16	116.18(15)	N1	C10	C15	112.48(15)
C4	N2	C3	118.97(15)	C11	C10	C15	109.67(15)
C4	N2	C16	123.64(16)	O2	C11	C10	111.22(15)
N4	N3	C20	120.53(16)	O2	C11	C12	108.99(15)
C23	N3	N4	112.03(17)	C12	C11	C10	109.28(15)
C23	N3	C20	126.91(18)	C11	C12	C13	111.28(16)
C25	N4	N3	104.10(17)	C12	C13	C14	110.85(16)
O1	C1	N1	124.32(17)	C15	C14	C13	110.85(17)
O1	C1	C2	119.11(16)	C14	C15	C10	110.12(16)
N1	C1	C2	116.57(16)	N2	C16	C17	113.36(15)
O3	C2	C1	109.80(15)	C18	C17	C16	118.93(16)
O3	C2	C3	109.74(15)	C18	C17	C22	118.72(17)
C1	C2	C3	110.62(15)	C22	C17	C16	122.34(17)
N2	C3	C2	109.45(15)	C19	C18	C17	121.28(18)
N2	C4	C5	119.74(17)	C18	C19	C20	119.21(17)
N2	C4	C9	123.93(16)	C19	C20	N3	118.56(17)
C9	C4	C5	116.27(17)	C19	C20	C21	120.58(17)
O3	C5	C4	120.94(16)	C21	C20	N3	120.82(17)
C6	C5	O3	117.01(16)	C22	C21	C20	119.46(17)
C6	C5	C4	122.05(17)	C21	C22	C17	120.70(17)
C5	C6	C7	120.71(17)	N3	C23	C24	106.7(2)
C8	C7	C6	118.48(18)	C23	C24	C25	104.95(19)
C7	C8	C9	120.67(18)	N4	C25	C24	112.2(2)

Table 6 Hydrogen Atom Coordinates (Å×10^4) and Isotropic Displacement Parameters (Å^2×10^3) for Garcia01.

Atom	*x*	*y*	*z*	U(eq)
H2	10932	5208	3633	34
H1	8228	3758	3762	18
H2A	4735	2042	3392	15
H3A	3018	2996	2778	16
H3B	5494	3503	2650	16
H6	11426	1249	3360	17
H7	12674	−160	2898	20
H8	10462	−618	2250	19
H9	7101	314	2066	17
H10	5593	5520	3890	16
H11	9989	5047	4319	17
H12A	7729	7054	4198	21
H12B	10226	6898	4434	21
H13A	7247	7092	5001	25
H13B	8532	5976	5052	25
H14A	4496	5686	5100	28
H14B	4076	6298	4625	28
H15A	3943	4462	4485	21
H15B	6472	4315	4710	21
H16A	2220	1840	2156	17
H16B	4274	1160	1932	17
H18	1183	3421	1819	19
H19	1796	4840	1322	20
H21	8208	3684	1015	18
H22	7588	2279	1522	17
H23	2191	5830	658	28
H24	4336	7373	312	33
H25	8570	7064	479	29

Experimental

Single crystals of $C_{25}H_{28}N_4O_3$ **[Garcia01]** were **[?]**. A suitable crystal was selected and **[polyimide loop]** on a **SuperNova, Dual, Cu at zero, EosS2** diffractometer. The crystal was kept at 100 K during data collection. Using Olex2 [1], the structure was solved with the ShelXT [2] structure solution program using Intrinsic Phasing and refined with the ShelXL [3] refinement package using Least Squares minimisation.

1. Dolomanov, O.V., Bourhis, L.J., Gildea, R.J, Howard, J.A.K. & Puschmann, H. (2009), J. Appl. Cryst. 42, 339-341.
2. Sheldrick, G.M. (2015). Acta Cryst. A71, 3-8.
3. Sheldrick, G.M. (2015). Acta Cryst. C71, 3-8.

Crystal structure determination of [Garcia01]

Crystal Data for $C_{25}H_{28}N_4O_3$ (M =432.51 g/mol): orthorhombic, space group $P2_12_12_1$ (no. 19), a = 5.74144(5) Å, b = 12.67188(16) Å, c = 29.0999(3) Å, V = 2117.15(4) Å^3, Z = 4, T = 100 K, μ(Cu Kα) = 0.731 mm^{-1}, *Dcalc* = 1.357 g/cm^3, 21610 reflections measured (7.61° ≤ 2Θ ≤ 147.582°), 4262 unique (R_{int} = 0.0357, R_{sigma} = 0.0266) which were used in all calculations. The final R_1 was 0.0318 (I > 2σ(I)) and wR_2 was 0.0817 (all data).

checkCIF (full publication check) running

Checking for embedded fcf data in CIF ...
Found embedded fcf data in CIF. Extracting fcf data from uploaded CIF, please wait ...

checkCIF/PLATON (full publication check)

Structure factors have been supplied for datablock(s) I

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No syntax errors found. CIF dictionary
Please wait while processing .... Interpreting this report

Structure factor report

Datablock: I

Bond precision: C-C = 0.0028 A Wavelength=1.54184

Cell: a=5.74144(5) b=12.67188(16) c=29.0999(3)
alpha=90 beta=90 gamma=90

Temperature: 100 K

	Calculated	Reported
Volume	2117.16(4)	2117.15(4)
Space group	P 21 21 21	P 21 21 21
Hall group	P 2ac 2ab	P 2ac 2ab
Moiety formula	C25 H28 N4 O3	C25 H28 N4 O3
Sum formula	C25 H28 N4 O3	C25 H28 N4 O3
Mr	432.51	432.51
Dx,g cm-3	1.357	1.357
Z	4	4
Mu (mm-1)	0.731	0.731
F000	920.0	920.0
F000'	922.72	
h,k,lmax	7,15,36	7,15,36
Nref	4278[2495]	4262
Tmin,Tmax	0.842,0.968	0.541,1.000
Tmin'	0.730	

Correction method= # Reported T Limits: Tmin=0.541 Tmax=1.000
AbsCorr = GAUSSIAN

Data completeness= 1.71/1.00 Theta(max)= 73.791

R(reflections)= 0.0318(4090) wR2(reflections)= 0.0817(4262)

S = 1.029 Npar= 290

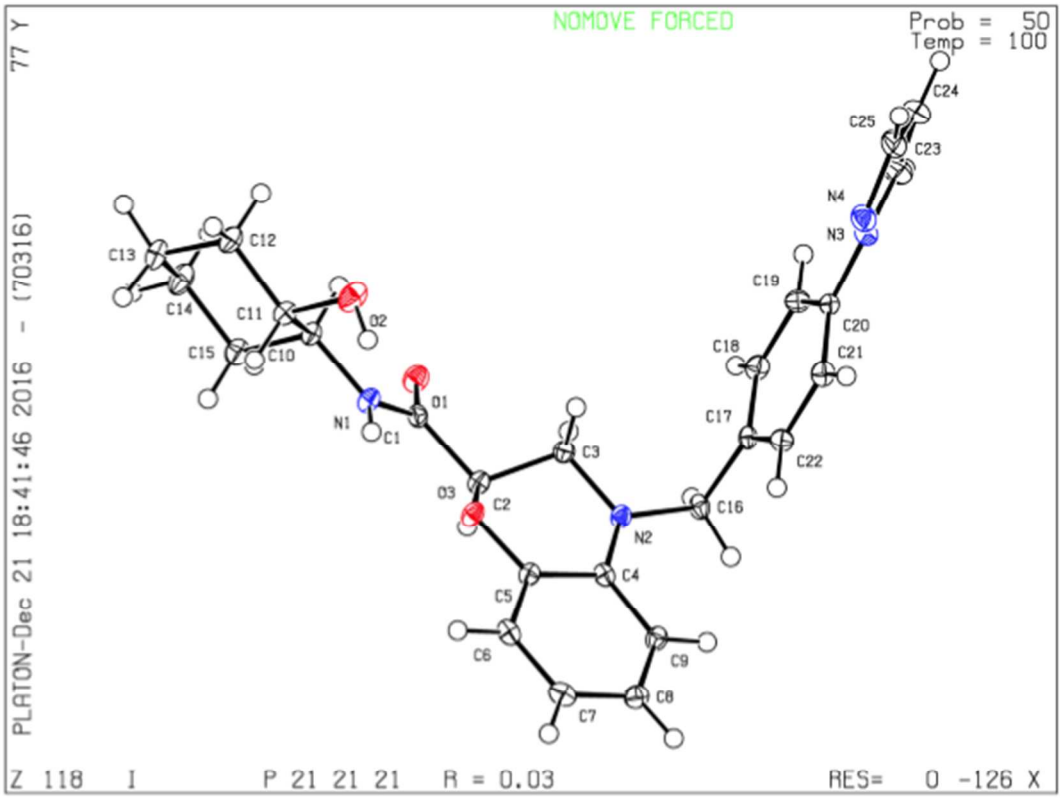
The following ALERTS were generated. Each ALERT has the format
test-name_ALERT_alert-type_alert-level.
Click on the hyperlinks for more details of the test.

Alert level C

PLAT019_ALERT_1_C _diffrn_measured_fraction_theta_full/*_max < 1.0 0.990 Report
PLAT410_ALERT_2_C Short Intra H...H Contact H9 .. H16B .. 1.98 Ang.
PLAT934_ALERT_3_C Number of (Iobs-Icalc)/SigmaW > 10 Outliers .... 1 Check

Alert level G

PLAT007_ALERT_5_G Number of Unrefined Donor-H Atoms ..... 2 Report
PLAT791_ALERT_4_G The Model has Chirality at C2 (Chiral SPGR) R Verify



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Eurofins Lead Profiling Data

A radioligand binding panel of 68 targets (GPCRs, ion channels, transporters, and nuclear hormones) with data reported as % inhibition of radioligand binding at a 10 μM concentration of **16** (VU0486846).

Supporting Table 1: Eurofins Profiling of **16** (VU0486846).

Target/Protein	Species	% Inhibition
Adenosine A_1	Human	3
Adenosine A_{2A}	Human	-2
Adenosine A_3	Human	7
Adrenergic α_{1A}	Rat	12
Adrenergic α_{1B}	Rat	5
Adrenergic α_{1D}	Human	2
Adrenergic α_{2A}	Human	21
Adrenergic β_1	Human	-1
Adrenergic β_2	Human	-1
Androgen (Testosterone)	Human	1
Bradykinin B_1	Human	-9
Bradykinin B_2	Human	16
Calcium Channel L-Type, Benzothiazepine	Rat	7
Calcium Channel L-Type, Dihydropyridine	Rat	5
Calcium Channel N-Type	Rat	-1
Cannabinoid CB_1	Human	-1
Dopamine D_1	Human	-2
Dopamine D_{2S}	Human	-9
Dopamine D_3	Human	2

Dopamine $D_{4.2}$	Human	-2
Endothelin ET_A	Human	0
Endothelin ET_B	Human	2
Epidermal Growth Factor (EGF)	Human	-5
Estrogen ERα	Human	2
$GABA_A$, Flunitrazepam, Central	Rat	4
$GABA_A$, Muscimol, Central	Rat	-4
$GABA_{B1A}$	Human	2
Glucocorticoid	Human	0
Glutamate, Kainate	Rat	2
Glutamate, NMDA, Agonism	Rat	-2
Glutamate, NMDA, Glycine	Rat	-5
Glutamate, NMDA, Phencyclidine	Rat	-3
Histamine H_1	Human	2
Histamine H_2	Human	-8
Histamine H_3	Human	4
Imidazoline I_2, Central	Rat	22
Interleukin IL-1	Mouse	16
Leukotriene, Cysteinyl $CysLT_1$	Human	-2
Melatonin MT_1	Human	2
Muscarinic M_1	Human	12
Muscarinic M_2	Human	-2
Muscarinic M_3	Human	-14
Neuropeptide Y Y_1	Human	-1
Neuropeptide Y Y_2	Human	2

Nicotinic Acetylcholine	Human	-2
Nicotinic Acetylcholine α1, Bungarotoxin	Human	-2
Opiate δ_1 (OP1, DOP)	Human	-3
Opiate κ (OP2, KOP)	Human	5
Opiate μ (OP3, MOP)	Human	-9
Phorbol Ester	Mouse	5
Platelet Activating Factor (PAF)	Human	-4
Potassium Channel [K_{ATP}]	Hamster	3
Potassium Channel hERG	Human	10
Prostanoid EP_4	Human	2
Purinergic P2X	Rabbit	19
Purinergic P2Y	Rat	-9
Rolipram	Rat	2
Serotonin (5-HT_{1A})	Human	-10
Serotonin (5-HT_{2B})	Human	-3
Serotonin (5-HT_3)	Human	-11
Sigma σ_1	Human	2
Sodium Channel, Site 2	Rat	19
Tachykinin NK_1	Human	-1
Thyroid Hormone	Rat	-3
Transporter, Dopamine (DAT)	Human	4
Transporter, GABA	Rat	4
Transporter, Norepinephrine (NET)	Human	0
Transporter, Serotonin (SERT)	Human	0

Supplemental field electrophysiology methods: Recording pipettes were constructed from thin-walled borosilicate capillary glass tubing (I.D.=1.17 mm, O.D. 1.50 mm; Warner Instruments, Hamden, CT), pulled with a horizontal pipette puller (P-97 Sutter Instrument Co., Novata, CA) to a resistance of 1-3MΩ when filled with ACSF. Layer II/III was visualized using an Olympus BX50WI upright microscope (Olympus, Lake Success, NY) microscope according to landmarks illustrated in the Allen mouse brain atlas (Lein et al, 2007) and the recording electrode was laterally placed approximately 200μM away from layer 2/3 into layer V so that the recording and stimulating electrodes were parallel to each other. Input-output curves were generated to determine the stimulus intensity that produced approximately 70% of the maximum fEPSP slope before each experiment, which was then used as the baseline stimulation. Similarly, the recording electrode for ofEPSP was placed in layer V and an input-output curve was generated to produce approximately 70% of the maximal ofEPSP slope. Data were digitized using a Multiclamp 700B, using a sampling rate of 20,000kHz and were filtered at 0.5kHz, with a Digidata 1322A, pClamp 9.2 and Clampex 10.6.2 software (Molecular Devices) running on a Dell PC (Round Rock, TX). Offline data analysis to calculate fEPSP slope or ofEPSP slope was performed using Clampfit 10.2 (Molecular Devices).