SUPPLEMENTAL MATERIAL

Pharmacological characterization of the imipridone anti-cancer drug ONC201 reveals a negative allosteric mechanism of action at the D_2 dopamine receptor

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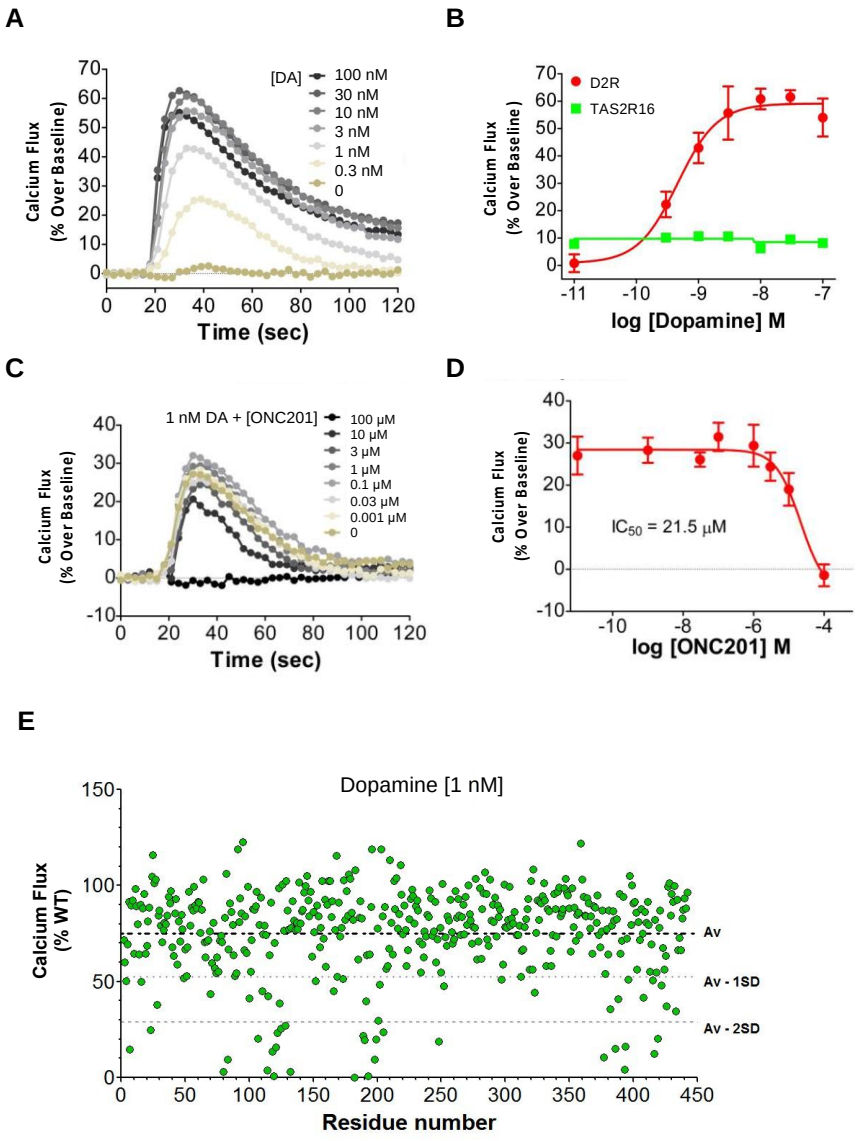

Supplemental Figure S1. Scanning mutagenesis to determine critical residues for D2R-mediated Ca^{2+} flux. HEK293T cells were transiently transfected with the wild-type (WT) D2R or D2R mutants and a chimeric $G\alpha_{16}$ subunit. After 22 hr, Ca^{2+} flux experiments were performed as described in the Materials and Methods. **(A)** Ca^{2+} flux was measured over time

after the addition of the indicated concentrations of dopamine. **(B)** The peak responses from the D2R experiment in panel **A** were plotted as a function of the dopamine concentration yielding an EC_{50} of 0.45 nM. Ca^{2+} flux was also measured in response to dopamine in cells transfected with the bitter taste receptor TAS2R16. No significant response was observed. **(C)** Ca^{2+} flux was measured over time after the addition of 1 nM dopamine plus the indicated concentrations of ONC201. **(D)** Peak responses from the experiment in panel **C** were plotted as a function of the ONC201 concentration yielding an IC_{50} of 21.5 μM. (**E**) The D2R alanine-scan mutant library (with wild-type alanines changed to serines) comprised 442 clones, covering residues 2 - 443 of the human D2R long isoform. Ca^{2+} flux was measured in response to 1 nM dopamine for each of the 442 D2R clones and expressed as a percentage of the wild-type D2R response. The data represent average values from three experiments. Mutant receptors were considered to be deficient in Ca^{2+} flux if they demonstrated flux values less than two standard deviations below the average Ca^{2+} flux value (AV – 2SD) for the entire library. These mutant receptors are listed in Supplemental Table 1.

Supplemental Table 1. GPCR abbreviations used in Figure 1B.

Abbreviation	Receptor Name
ADRA1B	α_{1B} Adrenergic receptor
ADRA2A	α_{2A} Adrenergic receptor
ADRA2B	α_{2B} Adrenergic receptor
ADRA2C	α_{2C} Adrenergic receptor
ADRB2	β_2 Adrenergic receptor
V1AR	Arginine Vasopressin receptor 1A
V1BR	Arginine Vasopressin receptor 1B
CALCRL-RAMP3	Calcitonin receptor-like receptor activity modifying protein 3
CCR1	C-C Chemokine receptor type 1
CCR3	C-C Chemokine receptor type 3
CCR4	C-C Chemokine receptor type 4
CCR5	C-C Chemokine receptor type 5
CCR8	C-C Chemokine receptor type 8
M3R	Muscarinic acetylcholine receptor 3
CB1R	Cannabinoid receptor 1
CB2R	Cannabinoid receptor 2
CX3CR1	CX3C chemokine receptor 1
CXCR4	C-X-C chemokine receptor type 4
DRD1	D1 dopamine receptor
DRD2	D2 dopamine receptor
DRD3	D3 dopamine receptor
DRD4	D4 dopamine receptor
DRD5	D5 dopamine receptor
EDG4	Lysophosphatidic acid receptor 2
EDG7	Lysophosphatidic acid receptor 7
HR1	Histamine H1 receptor
HR2	Histamine H2 receptor
HR3	Histamine H3 receptor
5-HT1AR	5-hydroxytryptamine receptor 1A
5-HT1BR	5-hydroxytryptamine receptor 1B
5-HT1ER	5-hydroxytryptamine receptor 1E
5-HT1FR	5-hydroxytryptamine receptor 1F
5-HT2AR	5-hydroxytryptamine receptor 2A
5-HT2CR	5-hydroxytryptamine receptor 2C
5-HT5AR	5-hydroxytryptamine receptor 5A
MC5R	Melanocortin receptor 5
MCHR1	Melanin-concentrating hormone receptor 1
KOR	Kappa opioid receptor
MOR	Mu opioid receptor

SSTR1	Somatostatin receptor type 1
SSTR3	Somatostatin receptor type 3
NK2R	Tachykinin receptor/Substance-K receptor 2
TRHR	Thyrotropin-releasing hormone receptor
TSHR(L)	Thyrotropin receptor

Supplemental Table 2. D2R mutants with highly reduced dopamine-stimulated Ca^{2+} flux.

D2R Mutation*	Ca^{2+} Flux (% WT)
S7A	15
N23A	25
D80A	3
V83A	10
C107A	20
D114A	4
C118A	14
T119A	1
S121A	16
I122A	23
L125A	25
R132A	3
C182A	0
I184A	0
F189A	22
V190A	20
S193A	1
S197A	0
F198A	10
Y199A	20
T205A	24
E248A	19
I377A	11
W386A	15
H393A	4
I394A	16
Y416A	12
S419A	20

*Residues were identified from the analysis shown in **Supplemental Fig. 1E**. D2R mutants were considered to be deficient in Ca^{2+} flux if they demonstrated flux values less than 2 standard deviations below the average Ca^{2+} flux value (AV – 2SD) for the entire library. Data represent the % Ca^{2+} flux seen in response to 1 nM dopamine for the wild-type (WT) D2R.

Supplemental Data File 1: D2R_ONC201_inactive.pdb

Supplemental Data File 2: D2R_ONC_active_pose1.pdb

Supplemental Data File 3: D2R_ONC_active_pose2.pdb