SUPPORTING INFORMATION

A peripherally restricted purine antagonist of type 1 cannabinoid (CB1) receptor blocks alcoholic steatosis in mice

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Contents:

S1. Homology model development and docking of AM6538 and AMBER16 free energy (MMGBSA) scoring of residue/ligand interactions

The MMGBSA docked poses of **AM6538** in the 5TGZ chimeric hCB1 crystal structure are depicted in space filling (CPK) representation in **Figure S1A**. **Figure S1B** shows a closeup view of the crystal structure pose (brown) overlapped with the lowest MMGBSA pose (cyan). The lowest MMGBSA scored pose replicates the crystal structure motif within a small RMSD demonstrating the validity of our approach. A simplified LIGPLOT representation of the lowest free-energy poses of **AM6538** as well as **2** is shown in **Figure S1C**. In **Figure S1C**, a large portion of **AM6538** interacts with hydrophobic residues in the deeper regions of the orthosteric binding site. As shown in **Table S1,** AMBER16 free energy (MMGBSA) scoring of residue/ligand interactions reflect what we see visually, that a great deal of the binding free energy is due to the buried orthosteric site hydrophobic interactions for the ligand extending to the top of the piperidine group. When **2** was docked and free-energy scored, the chloro-aromatic rings overlapped the phenyl rings in **AM6538** in its lowest MMGBSA pose while the purine ring of **2** overlapped the pyrazole ring in **AM6538** allowing the piperidine rings to coincide. The rightmost panel in **Figure S1C** shows, in LIGPLOT that many of the interactions are similar between **AM6538** and **2** except the 4-position piperidine amide in **2** extends out and makes a hydrogen bonding contact with Ser 123 of helix 1.

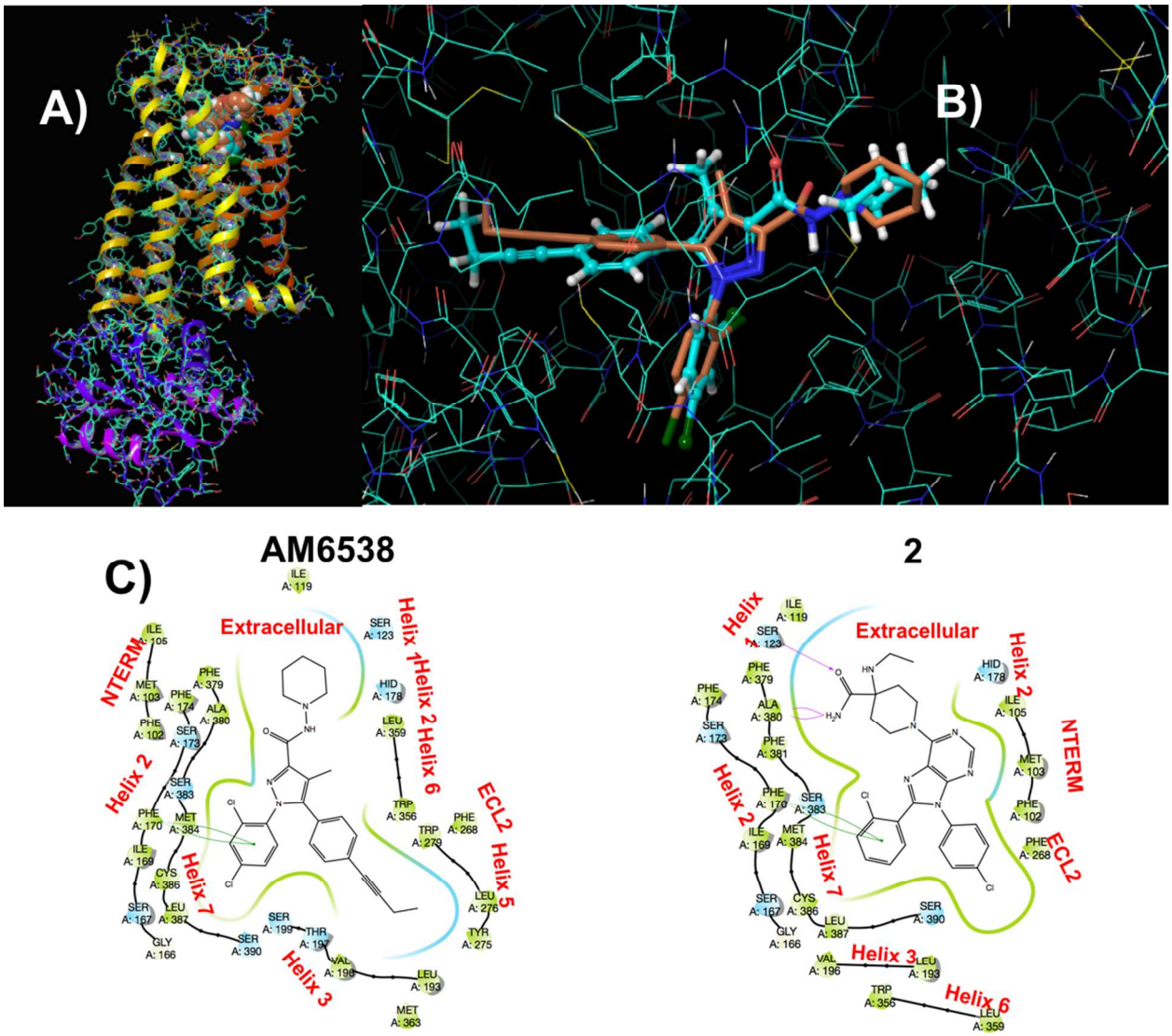


Figure S1. Evaluation of poses for AM6538 (crystallographic ligand) and 2 in hCB1 (PDB-ID 5TGZ). (A) and **(B)** depict **AM6538** in the lowest MMGBSA docked pose (cyan carbons) compared to **AM6538** in the published crystallographic structure (brown carbons). **(C)** depicts the residue/helical contacts for **AM6538** (crystallographic) and lowest MMGBSA pose for **2**.

TABLE S1: PAIRWISE MMGBSA INTERACTION ENERGIES OF BINDING SITE RESIDUES WITH THE CB1 ANTAGONISTS

MMGBSA INTERACTION ENERGETIC DECOMPOSITION (KCAL/MOL)

ANTAGONIST/ RESIDUE*	MET 103	ILE 105	GLN 116	SER 123	GLY 166	PHE 170	HIS 178	VAL 196	PHE 268	TRP 356	LEU 359	PHE 379	ALA 380	SER 383	MET 384	CYS 386	LEU 387
Otenabant	-6.89	-2.58	-0.04	-1.12	-0.95	-4.59	-1.76	-3.74	-2.29	-1.66	-1.92	-1.94	-2.10	-4.48	-3.34	-1.27	-1.90
Rimonabant	-4.50	-2.40	-0.03	-0.63	-1.40	-4.21	-1.13	-4.50	-2.00	-2.16	-1.91	-1.67	-1.85	-4.85	-2.23	-1.36	-2.70
SAR1-6	-6.07	-2.23	-0.02	-0.60	-1.01	-4.19	-0.77	-4.03	-1.90	-1.80	-1.81	-1.48	-1.50	-4.85	-2.14	-1.56	-2.18
SAR1-7	-5.37	-2.68	-0.25	-0.65	-1.10	-4.33	-1.83	-4.48	-2.49	-2.07	-1.90	-2.13	-1.90	-5.19	-2.01	-1.40	-1.84
SAR1-8	-5.75	-2.48	-0.42	-1.16	-1.09	-4.95	-2.28	-4.13	-2.03	-1.69	-1.83	-1.81	-1.84	-5.15	-1.89	-1.80	-2.09
SAR1-9	-5.71	-2.52	-0.20	-1.37	-1.12	-4.24	-1.77	-4.82	-2.07	-2.12	-1.86	-1.66	-1.02	-4.61	-1.93	-1.25	-2.18
SAR1-10	-5.83	-2.36	-0.20	-1.26	-1.10	-4.90	-2.22	-4.19	-2.12	-1.67	-1.84	-1.82	-1.71	-5.47	-1.85	-1.77	-2.04
SAR1-11	-5.07	-3.17	-0.18	-0.89	-1.19	-4.77	-1.21	-4.34	-2.46	-1.57	-2.02	-2.08	-2.53	-5.81	-2.21	-1.66	-1.81
SAR1-12	-7.53	-2.50	-0.22	-2.50	-0.96	-4.59	-1.63	-3.65	-2.39	-1.56	-1.92	-2.03	-1.28	-4.29	-2.47	-1.50	-1.82
SAR1-13	-6.77	-2.42	-1.13	-0.79	-0.97	-4.44	-2.21	-3.85	-2.37	-1.64	-1.91	-1.96	-1.16	-4.28	-2.26	-1.41	-1.92
SAR1-14	-6.35	-2.22	-0.20	-0.99	-0.97	-4.56	-1.38	-4.24	-2.43	-1.89	-1.90	-1.93	-1.08	-4.14	-1.99	-1.32	-2.04
SAR1-15	-5.50	-2.40	-0.44	-1.15	-1.04	-4.90	-2.38	-4.06	-1.90	-1.74	-1.82	-1.69	-2.02	-5.53	-2.05	-1.82	-2.10
SAR1-16	-5.28	-2.37	-0.54	-1.12	-1.00	-4.53	-2.39	-4.43	-2.47	-2.14	-1.91	-2.01	-1.38	-5.98	-1.82	-1.38	-1.95
SAR1-17	-6.56	-2.44	-0.85	-1.03	-0.94	-4.26	-2.40	-3.77	-2.38	-1.53	-1.92	-1.97	-1.14	-4.36	-2.38	-1.49	-1.85
SAR1-18	-5.42	-2.43	-0.86	-1.18	-1.05	-4.86	-2.51	-3.97	-1.84	-1.73	-1.81	-1.76	-2.09	-5.40	-2.15	-1.86	-2.20
SAR2-19	-4.91	-2.47	-0.40	-3.15	-1.00	-4.80	-1.78	-4.26	-1.88	-1.86	-1.88	-1.71	-1.91	-5.67	-2.54	-1.75	-2.21
SAR2-20	-6.10	-2.26	-0.53	-1.66	-1.01	-5.23	-1.60	-3.72	-2.05	-1.54	-1.73	-1.59	-1.80	-4.36	-2.31	-1.50	-1.99
SAR2-21	-6.27	-2.26	-0.74	-1.96	-1.01	-5.01	-1.71	-3.48	-1.92	-1.47	-1.72	-1.56	-1.40	-4.17	-2.11	-1.52	-1.94
SAR2-22	-5.84	-2.31	-0.30	-1.42	-0.99	-5.17	-1.13	-3.75	-2.00	-1.69	-1.76	-1.52	-1.79	-4.44	-2.57	-1.54	-2.06
SAR2-23	-6.48	-2.21	-0.08	-0.85	-1.00	-4.63	-1.12	-3.95	-2.07	-1.82	-1.87	-1.61	-2.64	-4.57	-3.03	-1.56	-2.10
SAR2-24	-4.45	-2.61	-0.10	-0.91	-1.22	-4.61	-0.78	-4.54	-2.13	-1.93	-1.69	-1.96	-3.01	-5.36	-2.65	-1.54	-1.56
SAR2-25	-6.06	-2.53	-0.08	-1.44	-1.07	-4.58	-1.38	-4.04	-1.83	-1.97	-1.88	-1.48	-1.83	-4.68	-3.23	-1.74	-2.23
SAR2-26	-6.02	-2.50	-0.09	-1.34	-1.06	-4.38	-1.31	-4.17	-1.92	-2.16	-1.94	-1.61	-2.30	-4.66	-3.01	-1.56	-2.21
SAR2-27	-5.54	-2.25	-0.45	-1.55	-1.05	-5.04	-1.42	-3.79	-2.17	-1.57	-1.86	-1.81	-2.13	-4.71	-2.22	-1.52	-1.97
SAR2-28	-5.03	-2.82	-0.10	-1.41	-1.18	-4.82	-1.23	-4.38	-2.22	-1.76	-1.94	-2.05	-2.93	-4.56	-2.85	-1.68	-1.79
SAR2-29	-6.08	-2.09	-0.07	-1.24	-0.96	-4.31	-1.82	-3.69	-2.32	-1.78	-1.75	-1.47	-1.90	-4.35	-3.89	-1.25	-1.99
SAR2-30	-5.58	-2.77	-0.09	-1.49	-1.16	-4.68	-0.88	-4.31	-2.23	-1.70	-1.91	-2.02	-2.91	-4.46	-2.69	-1.69	-1.86
SAR2-31	-6.08	-2.40	-0.65	-0.85	-1.07	-4.24	-2.29	-4.34	-2.23	-1.86	-1.82	-1.98	-1.61	-4.43	-2.23	-1.43	-1.98
SAR2-32	-6.35	-2.52	-0.06	-1.23	-1.01	-4.52	-1.08	-4.05	-1.93	-1.84	-1.82	-1.56	-2.18	-4.61	-2.80	-1.60	-2.15

SAR2-33	-6.36	-2.56	-0.07	-1.19	-1.02	-4.59	-1.15	-4.02	-1.93	-1.85	-1.83	-1.56	-2.39	-4.62	-2.67	-1.61	-2.09
SAR2-34	-6.25	-2.62	-0.07	-1.10	-1.02	-4.58	-1.25	-3.89	-1.86	-1.91	-1.84	-1.51	-2.40	-4.50	-2.79	-1.64	-2.13
SAR2-35	-6.31	-2.63	-0.08	-1.32	-0.98	-4.52	-1.35	-3.94	-1.99	-1.79	-1.79	-1.47	-2.29	-4.29	-2.79	-1.49	-2.11
SAR2-36	-6.37	-2.68	-0.07	-1.33	-0.99	-4.46	-1.21	-3.86	-1.90	-1.82	-1.80	-1.49	-2.73	-4.48	-2.82	-1.58	-2.19
SAR2-37	-4.71	-2.24	-1.20	-2.08	-1.08	-5.34	-1.47	-4.11	-1.94	-1.74	-1.87	-1.77	-1.88	-5.28	-2.63	-1.85	-2.09
SAR2-38	-6.47	-2.90	-0.07	-1.21	-1.04	-4.46	-1.50	-3.37	-1.80	-1.72	-1.70	-1.29	-1.21	-4.62	-3.83	-1.46	-2.00
SAR2-39	-6.30	-2.64	-0.07	-1.41	-0.98	-4.35	-1.53	-3.41	-1.96	-1.66	-1.84	-1.40	-1.32	-4.59	-3.83	-1.31	-1.90
AVERAGE	-5.89	-2.49	-0.30	-1.29	-1.05	-4.63	-1.58	-4.04	-2.10	-1.79	-1.85	-1.73	-1.92	-4.77	-2.56	-1.55	-2.03
STANDARD DEVIATION	0.68	0.22	0.32	0.50	0.09	0.30	0.49	0.33	0.21	0.18	0.07	0.23	0.55	0.49	0.56	0.17	0.19
*Note: residue numbers correspond to corresponding sequence number in hCB1 without ICL3 ins																	

S2. Repeat dosing pharmacokinetic study of 25

In an effort to understand whether repeat dosing of **25** leads to accumulation of this compound in brain and other tissues, a 7-day repeat dosing PK study was performed. Female C57BL6 mice were procured from Jackson Laboratories at 8 weeks of age. Dose of **25** was formulated in 0.5% sodium carboxymethylcellulose with 1% NMP and 0.3% Tween 80 at 10 mg/kg. Animals were dosed twice daily by oral gavage at 1.25 mg/kg. On day 7, animals were given a final dose of **25** and three animals per group were sacrificed at various time-points. Adjusted concentrations of the parent compound between the single dose (SD) PK study reported in the main manuscript and repeat dose (RD) PK study were compared in **Table S2**. Pharmacokinetic analyses were performed as has been described in our previous publications using Phoenix WinNonlin (Certara). No significant differences were noted between the two studies indicating lack of brain accumulation of **25** upon repeat dosing for 7 days.

Table S2. Comparative single-dose (SD) and repeat dose (RD) PK analysis

Time post-dose (hr)	Plasma SD (ng/ml)	Plasma RD (ng/ml)	Brain SD (ng/g)	Brain RD (ng/g)
0.5	507	371	17	18
1	139	133	10	8
2	34	24	7	4
4	24	10	5	2
8	23	6	5	0
24	2	2	2	0

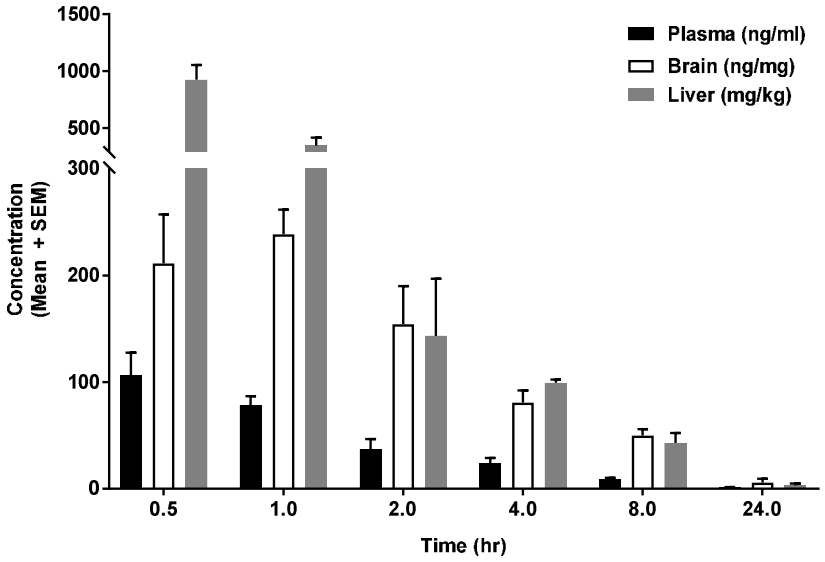
S3. Brain concentration of 25 after 2 weeks of dosing in alcoholic steatosis study

Concentration of **25** was measured from the brains of animals used in the alcohol efficacy study reported in the main manuscript. Approximately 16 hr after the last dose animals were sacrificed and their brains were collected and processed as described for PK studies. Quantification of **25** was performed using LC-MS. Brain concentration of **25** in 4 random animals was 0.42 ng/g $\pm$ 0.19 ng/g at sacrifice. Thus, 2-week exposure to **25** did not lead to increased accumulation of the compound in mouse brains.

S4. Pharmacokinetic assessment of compound 1 (rimonabant)

Female C57BL6 mice were administered rimonabant by oral gavage at a concentration of 5 mg/kg essentially as described for other PK studies. Animals were sacrificed at various time-points and tissue samples were analyzed by LC-MS. Pharmacokinetic analyses were performed using Phoenix WinNonlin (Certara). Data indicate rimonabant has high brain exposure upon oral administration, a feature that is absent from **25**.

Figure S4. PK analyses of rimonabant



S5. Compound strings

#	***Smiles***
6	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(C)CC4)=C3N=C2C5=CC=CC=C5Cl
7	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(C5CCCCC5)CC4)=C3N=C2C6=CC=CC=C6Cl
8	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(C5=CC=CC=C5)CC4)=C3N=C2C6=CC=CC=C6Cl
9	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(C5=CC=CC=N5)CC4)=C3N=C2C6=CC=CC=C6Cl
10	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(C5=CC=NC=C5)CC4)=C3N=C2C6=CC=CC=C6Cl
11	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(C5=CC=CC=C5C#N)CC4)=C3N=C2C6=CC=CC=C6Cl
12	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(C5=CC=CC=C5OC)CC4)=C3N=C2C6=CC=CC=C6Cl
13	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(C5=CC=CC(OC)=C5)CC4)=C3N=C2C6=CC=CC=C6Cl
14	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(C5=CC=CC(O)=C5)CC4)=C3N=C2C6=CC=CC=C6Cl
15	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(C5=CC=C(F)C=C5)CC4)=C3N=C2C6=CC=CC=C6Cl
16	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(C5=CC=C(C#N)C=C5)CC4)=C3N=C2C6=CC=CC=C6Cl
17	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(C5=CC=C(OC)C=C5)CC4)=C3N=C2C6=CC=CC=C6Cl
18	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(C5=CC=C(O)C=C5)CC4)=C3N=C2C6=CC=CC=C6Cl
19	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(CC5=CC=CC=C5)CC4)=C3N=C2C6=CC=CC=C6Cl
20	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(C(C5=CC=CC=C5)C)CC4)=C3N=C2C6=CC=CC=C6Cl
21	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(C(C#N)C5=CC=CC=C5)CC4)=C3N=C2C6=CC=CC=C6Cl
22	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(CC5=CC=NC=C5)CC4)=C3N=C2C6=CC=CC=C6Cl
23	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(C(C)C5=CC=NC=C5)CC4)=C3N=C2C6=CC=CC=C6Cl
24	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(CC5=C(F)C=CC=C5)CC4)=C3N=C2C6=CC=CC=C6Cl
25	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(CC5=C(Cl)C=CC=C5)CC4)=C3N=C2C6=CC=CC=C6Cl
26	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(CC5=C(Cl)C=NC=C5)CC4)=C3N=C2C6=CC=CC=C6Cl
27	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(C(OC)=O)CC4)=C3N=C2C5=CC=CC=C5Cl
28	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(CC5=C(C(F)(F)F)C=CC=C5)CC4)=C3N=C2C6=CC=CC=C6Cl
29	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(CC5=C(OC(F)(F)F)C=CC=C5)CC4)=C3N=C2C6=CC=CC=C6Cl
30	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(CC5=CC=CC=C5C#N)CC4)=C3N=C2C6=CC=CC=C6Cl
31	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(CC5=CC=CC(Cl)=C5)CC4)=C3N=C2C6=CC=CC=C6Cl
32	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(CC5=CC=C(F)C=C5)CC4)=C3N=C2C6=CC=CC=C6Cl
33	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(CC5=CC=C(Cl)C=C5)CC4)=C3N=C2C6=CC=CC=C6Cl
34	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(CC5=CC=C(C)C=C5)CC4)=C3N=C2C6=CC=CC=C6Cl
35	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(CC5=CC=C(OC)C=C5)CC4)=C3N=C2C6=CC=CC=C6Cl
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38	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(CCC5=CC=CC=C5)CC4)=C3N=C2C6=CC=CC=C6Cl
39	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(CCC5=CC=NC=C5)CC4)=C3N=C2C6=CC=CC=C6Cl
40	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(C5=CC=CC=C5F)CC4)=C3N=C2C6=CC=CC=C6Cl
41	ClC(C=C1)=CC=C1N2C3=NC=NC(N4CCN(C5=CC=CC=C5Cl)CC4)=C3N=C2C6=CC=CC=C6Cl