Electronic Supplementary Material (ESI) for RSC Chemical Biology.

Supporting File 1

Containing Supplementary Figures and Tables for:

Inhibition of SC4MOL and HSD17B7 shifts cellular sterol composition and promotes oligodendrocyte formation

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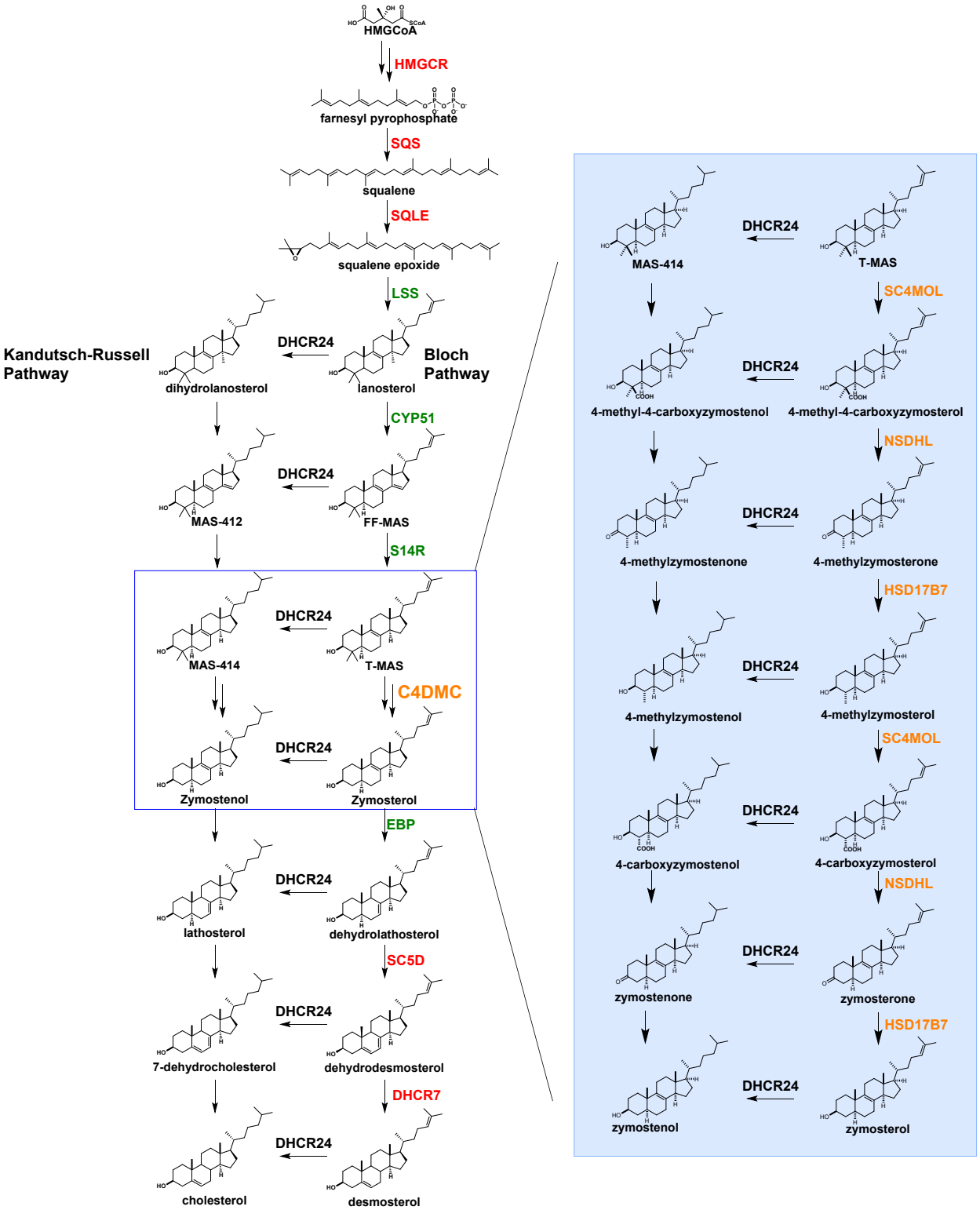


Fig. S1. Complete cholesterol biosynthesis pathway with sterol structures. Lanosterol synthase (LSS) provides the first sterol, lanosterol, within the cholesterol biosynthesis pathway. Processing of lanosterol to cholesterol can proceed via the Bloch and/or Kandutsch-Russell pathways, which share the same enzymes and their substrates vary by the presence or absence of C24 double bond, respectively. Inset shows individual enzymatic steps of SC4MOL, NSDHL,

and HSD17B7, the enzymes that comprise the C4-demethylation complex. Green indicates enzyme targets whose inhibition promotes oligodendrocyte formation. Red indicates enzyme targets whose inhibition does not promote oligodendrocyte formation. Orange indicates enzymes within the C4-demethylation complex.

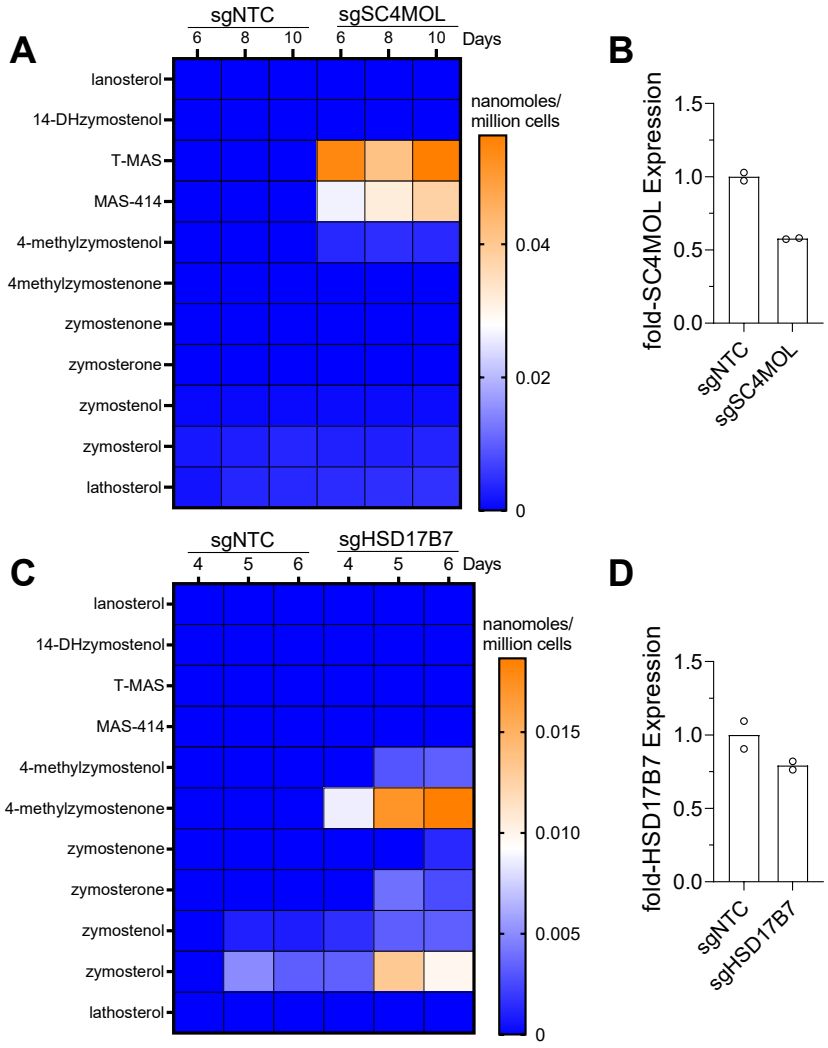


Fig. S2. Cholesterol biosynthesis sterol accumulation and qPCR characterization of OPC-expressing Cas9 cells CRISPR-targeted SC4MOL and HSD17B7. A, Heatmap representing GC-MS-based quantitation of cholesterol biosynthesis sterols in Cas9 OPCs targeted with NTC or SC4MOL. n = 2 wells per condition. **B,** Fold-change in gene expression of SC4MOL measured by RT-qPCR in Cas9-expressing OPCs transfected with sgRNA targeting SC4MOL. Cells were cultured for 4 days following electroporation and then plated in differentiation media for 2 days. n = 2 wells per condition. **C,** Heatmap representing GC-MS-based quantitation of cholesterol biosynthesis sterols in Cas9 OPCs targeted with NTC or HSD17B7. n = 2 wells per condition. **D,** Fold-change in gene expression of HSD17B7 measured by RT-qPCR in Cas9-expressing OPCs transfected with sgRNA targeting HSD17B7. Cells were cultured for 2 days following electroporation and then plated in differentiation media for 2 days and then analyzed.

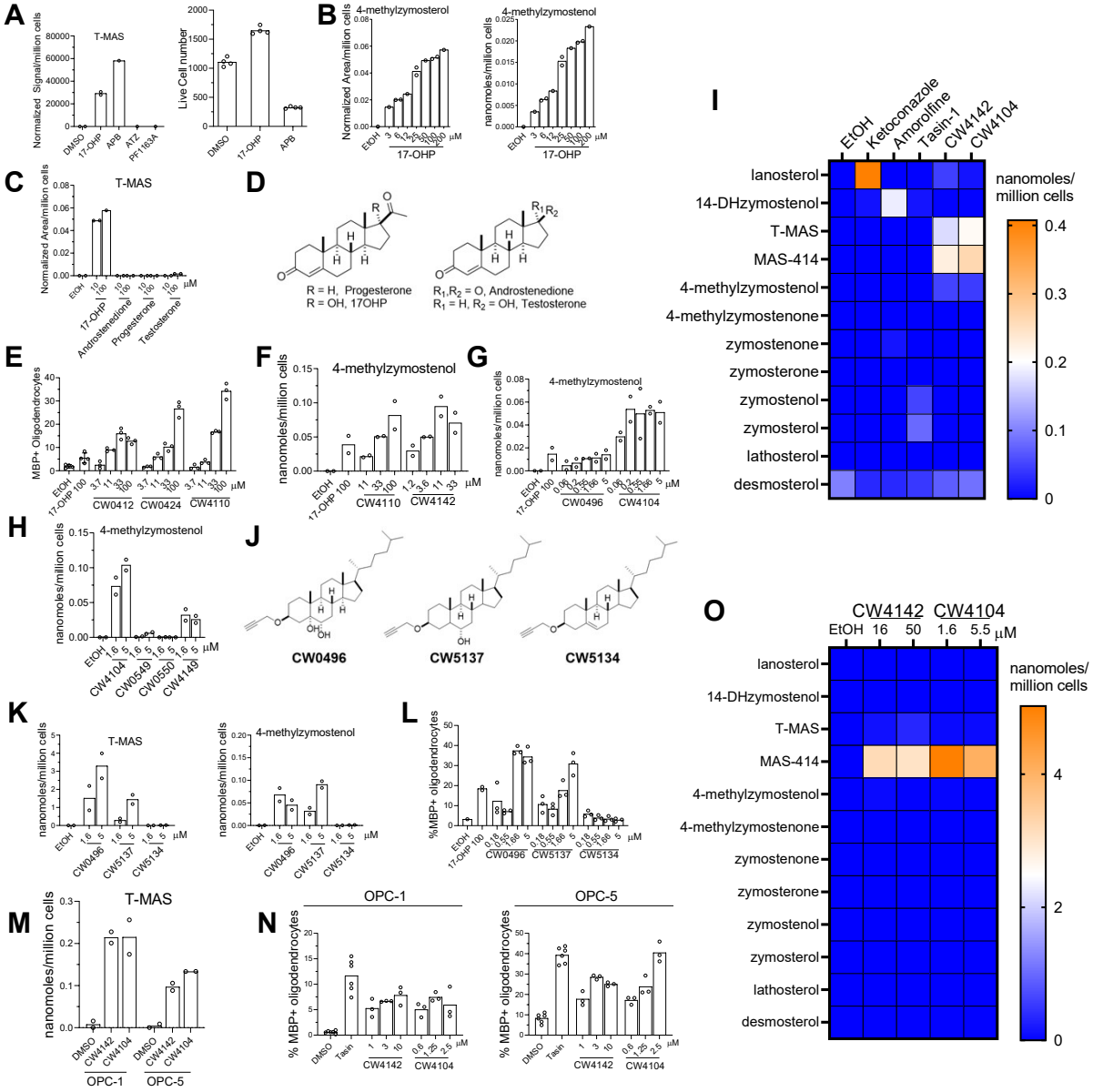


Fig S3. Characterization and optimization of SC4MOL inhibitors. A, GC-MS-based quantitation of the SC4MOL substrate T-MAS in OPCs treated with previously found SC4MOL inhibitors at 100 μM. n = 1 wells per condition. Live cell number from OPCs following treatment of 17-OHP or APB at 100 μM. n = 4 wells per condition. **B,** GC-MS-based quantitation of the SC4MOL substrates 4-methylzymosterol and 4-methylzymostenol in OPCs treated with17-OHP. n = 1 or 2 wells per condition**. C,** GC-MS-based quantitation of the SC4MOL substrate T-MAS in OPCs treated with different steroids. n = 2 wells per condition. **D,** Structures of 17-OHP analogs. **E,** Percentage of MBP+ oligodendrocytes generated from OPCs following treatment with CW0412, CW0424, or CW4110. n = 3 wells per condition. **F,** GC-MS-based quantitation of the SC4MOL substrate 4-methylzymostenol in OPCs treated with CW4110 or CW4142. n = 2 wells per condition. **G,** GC-MS-based quantitation of the SC4MOL substrate 4-methylzymostenol in

OPCs treated CW0496 or CW4104. n = 2 wells per condition. **H,** GC-MS-based quantitation of the SC4MOL substrate 4-methylzymostenol in OPCs treated with CW4104, CW059, CW0550, or CW4149. n = 2 wells per condition. **I,** Heatmap representing GC-MS-based quantitation of cholesterol biosynthesis sterols in OPCs treated with CW4142 (10 μM), CW4104 (2 μM), CYP51 inhibitor Ketoconazole (2.5 μM), Sterol 14-reductase inhibitor Amorolfine (300 nM), EBP inhibitor Tasin-1 (100 nM). n = 2 wells per condition **J,** Structures of CW0496 analogs. **K,** GC-MS-based quantitation of the SC4MOL substrates T-MAS and 4-methylzymostenol in OPCs treated with CW0496, CW5137, or CW5134. n = 2 wells per condition. **L,** Percentage of MBP+ oligodendrocytes generated from OPCs following treatment with CW0496, CW5137, or CW5134. n = 3 wells per condition. **M,** GC-MS-based quantitation of SC4MOL substrate T-MAS in OPC-1 or OPC-5 cells treated with CW4142 (10 uM) or CW4104 (2.5 uM). n = 2 wells per condition. **N,** Percentage of MBP+ oligodendrocytes generated from OPC-1 or OPC-5 cells following treatment with CW4142 or CW4104. n = 3 wells per condition. **O,** Heatmap representing GC-MS-based quantitation of cholesterol biosynthesis sterols in GBM528 cells treated with CW4142 or CW4104. n = 2 wells per condition. **A, B, C, , E, F, G, H, I, K, L, M, N, O** are representative of two independent biological experiments. Error bars indicate ± Standard Deviation.

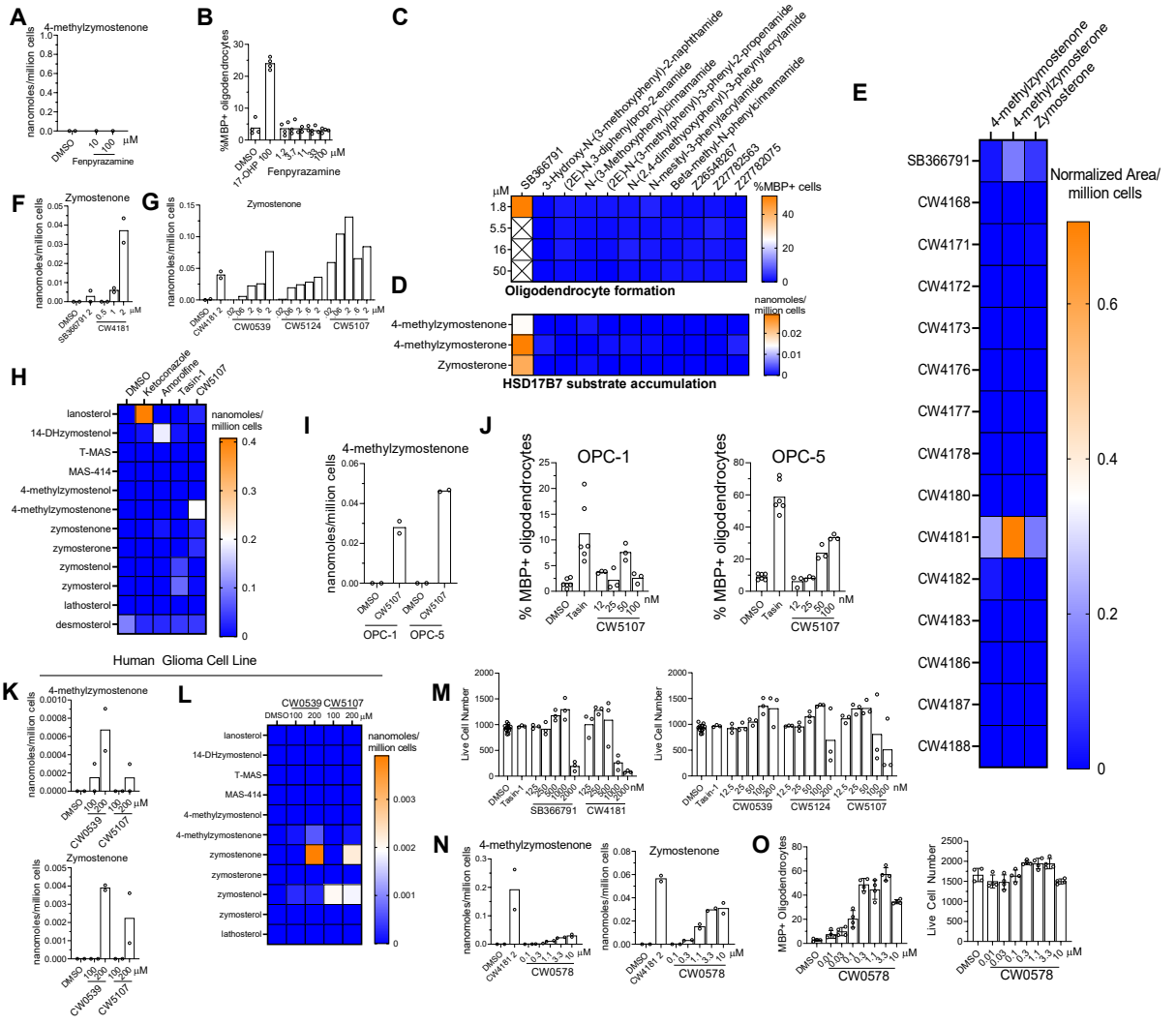
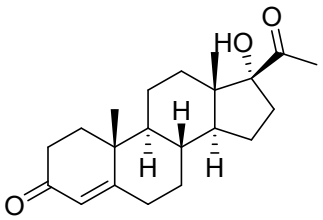
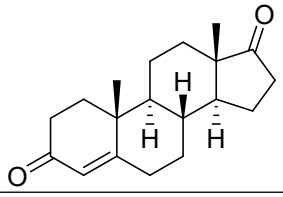
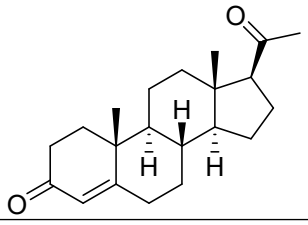
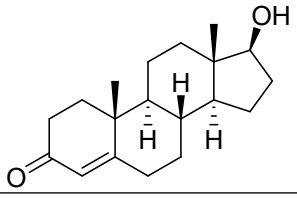
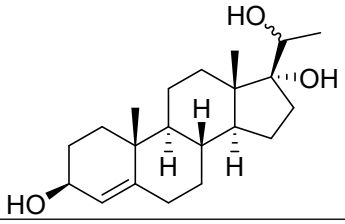
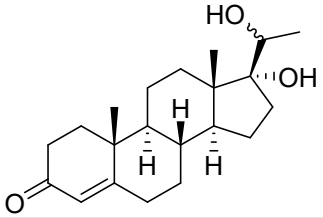
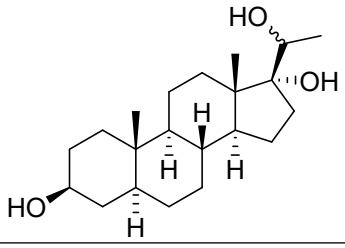
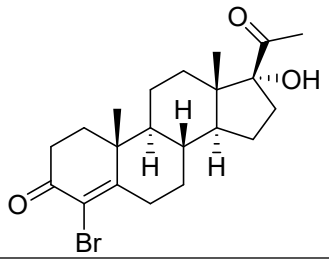
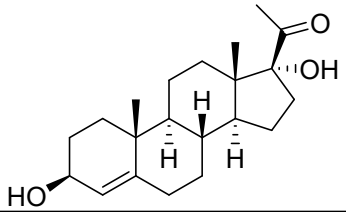
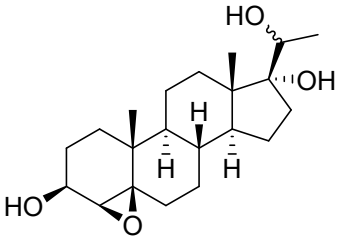
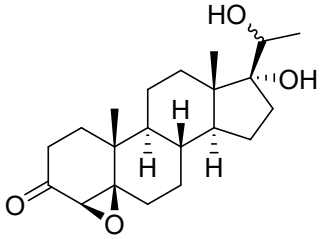
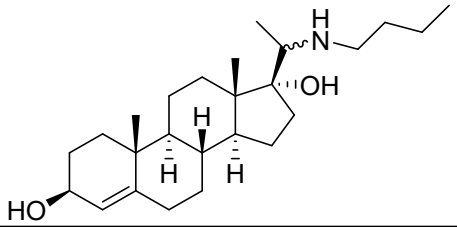
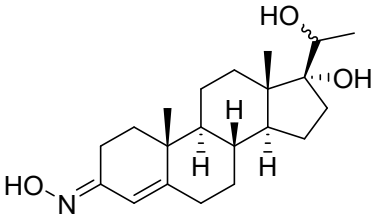
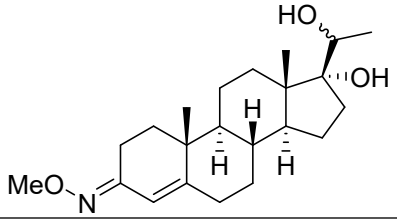


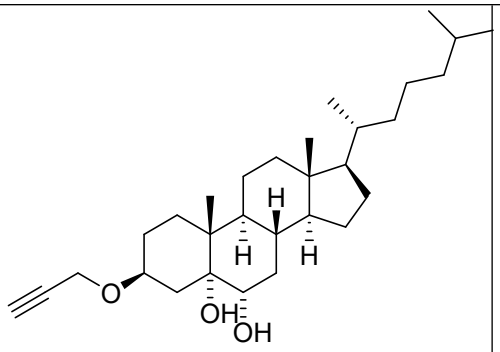
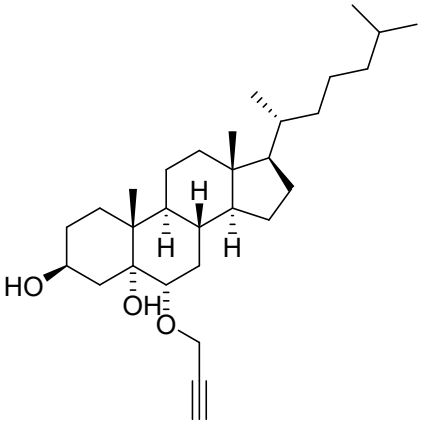
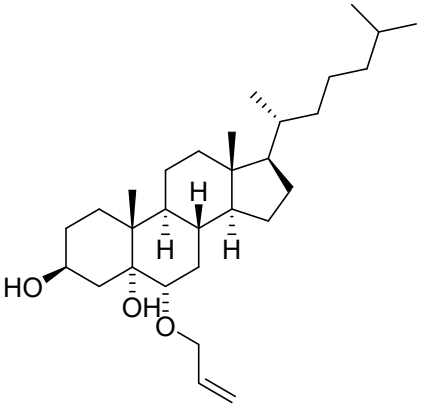
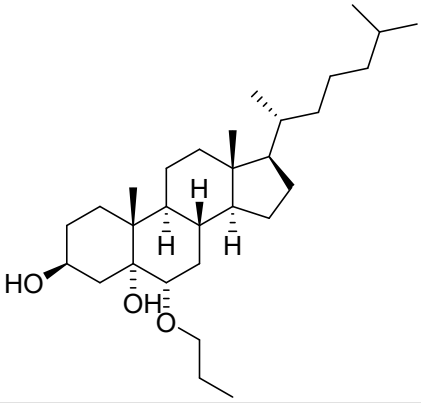
Fig. S4. Characterization and development of HSD17B7 inhibitors, A, GC-MS-based quantitation of the HSD17B7 substrate 4-methylzymostenone in OPCs treated with fenpyrazamine, a previously identified HSD17B7 inhibitor. **B,** Percentage of MBP+ oligodendrocytes generated from OPCS following treatment of fenpyrazamine. n = 4 wells per condition. **C,** Heatmap representing average percentage of MBP+ oligodendrocytes generated following treatment of 10 commercial analogs of SB3667891. Square marked with an X indicates not tested. n = 2 wells per condition. **D,** Heatmap representing the accumulation of HSD17B7 substrates from GC-MS-based quantification following treatment of OPCs with 10 commercial analogs of SB366791. All compounds tested at 5 μM. n = 2 for SB366791, n = 1 for commercial analogs. **E,** Heatmap representing the accumulation of HSD17B7 substrates from GC-MS-based quantification following treatment of OPCs with 14 structural analogs of SB366791. All compounds tested at 2 μM. n = 1. **F,** GC-MS-based quantitation of the HSD17B7 substrate zymostenone in OPCs treated with SB366791 or CW4181. n = 2 wells per condition. **G,** GC-MS-based quantitation of the HSD17B7 substrate zymostenone in OPCs treated with CW0539, CW5124, or CW5107. n = 1 wells per condition. **H,** Heatmap representing GC-MS-based quantitation of cholesterol biosynthesis sterols in OPCs treated with CW5107 (500 nM), CYP51

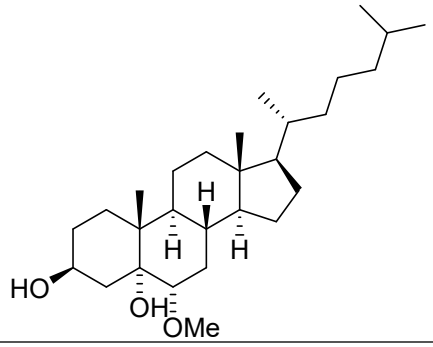
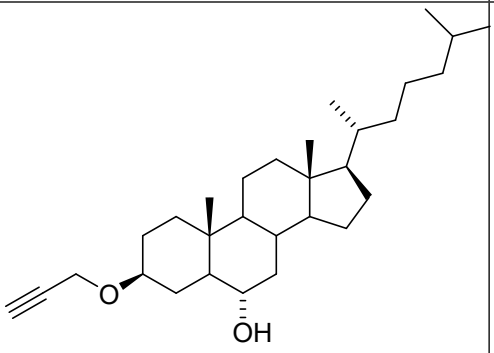
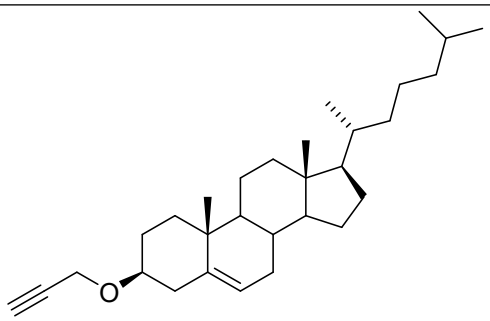
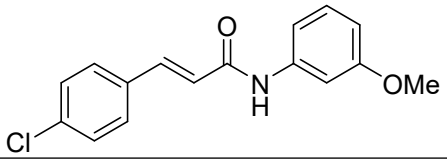
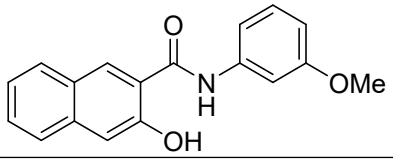
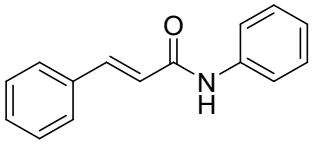
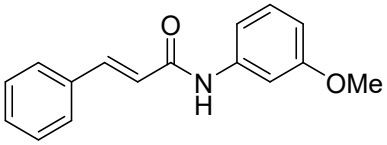
inhibitor Ketoconazole (2.5 μM), Sterol 14-reductase inhibitor Amorolfine (300 nM), EBP inhibitor Tasin-1 (100 nM). n = 2 wells per condition. **I,** GC-MS-based quantitation of HSD17B7 substrate 4-methylzymostenone in OPC-1 or OPC-5 cells treated with CW5107 (200 nM). n = 2 wells per condition. **J,** Percentage of MBP+ oligodendrocytes generated from OPC-1 and OPC-5 cells following treatment with CW5107. n = 3 wells per condition. **K,** GC-MS-based quantitation of the HSD17B7 substrate 4-methylzymostenone and zymostenone in human GBM528 treated with CW0539 or CW5107. n = 2 wells per condition. **L,** Heatmap representing GC-MS-based quantitation of cholesterol biosynthesis sterols in GBM528 cells treated with CW5107 or CW0539. n = 2 wells per condition. **M,** Live cell number from OPCs following treatment of top HSD17B7 inhibitors **N,** GC-MS-based quantitation of the HSD17B7 substrate 4-methylzymostenone and zymostenone in OPCs treated with CW0578. n = 2 wells per condition. **O,** Percentage of MBP+ oligodendrocytes and live cell number generated from OPCs following treatment of CW0578. n = 4 wells per condition. **A, B, F, G, H, I, J, K, L, M, N, O** are representative of two independent biological experiments. Error bars indicate ± Standard Deviation.

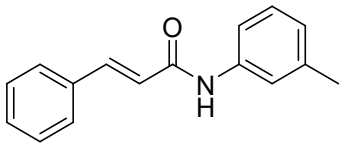
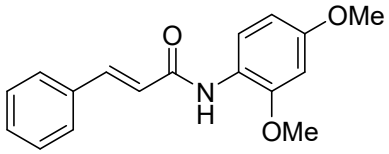
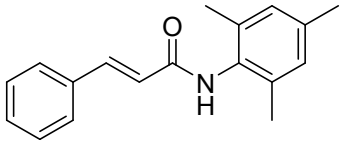
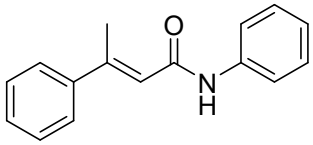
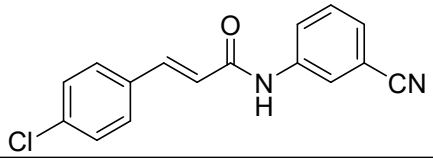
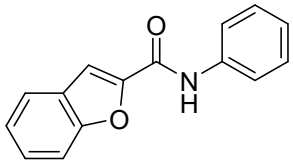
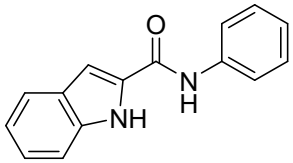
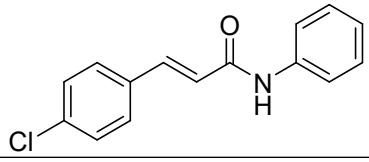
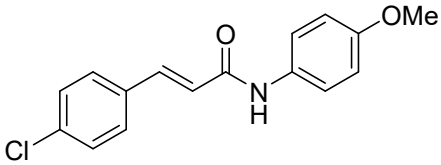
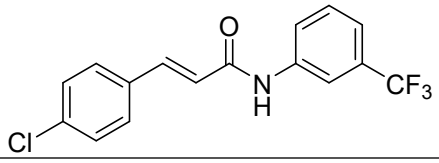
Table S1 Chemical structures of small molecules.

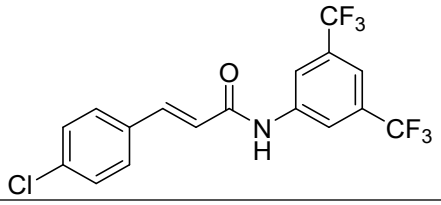
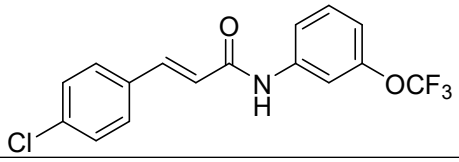
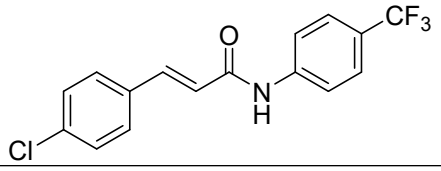
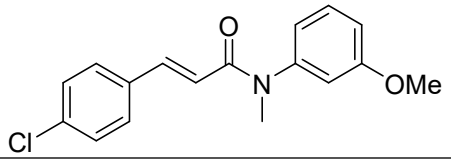
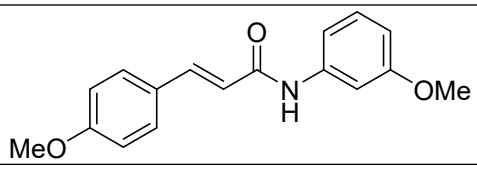
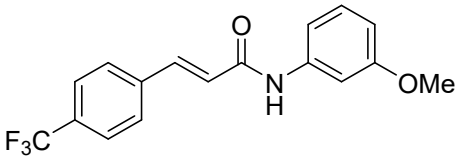
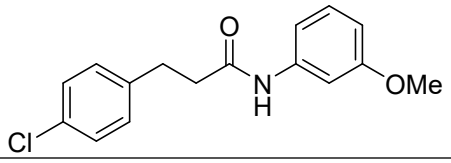
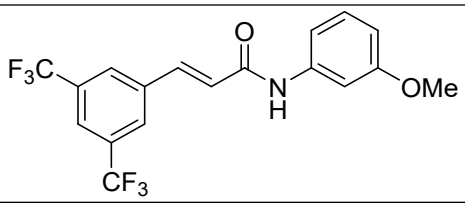
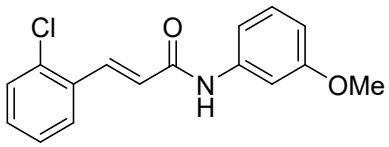
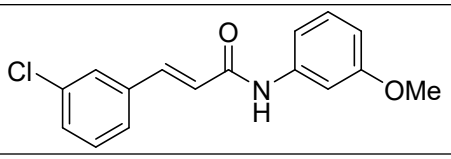
Entry	Number	Structure	Derivative	Intended Target
1	17-OHP		17-OHP	SC4MOL
2	Androstenedione		17-OHP	SC4MOL
3	Progesterone		17-OHP	SC4MOL
4	Testosterone		17-OHP	SC4MOL
5	CW0412		17-OHP	SC4MOL
6	CW0424		17-OHP	SC4MOL
7	CW0464		17-OHP	SC4MOL

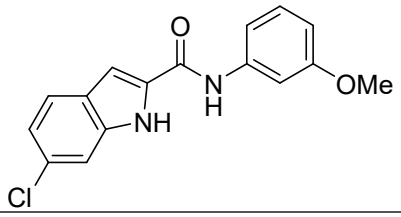
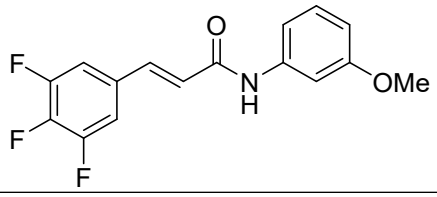
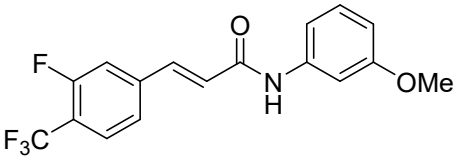
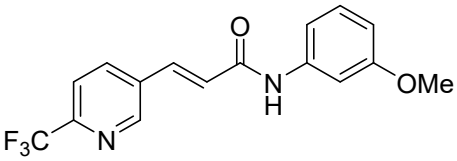
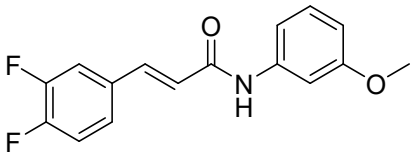
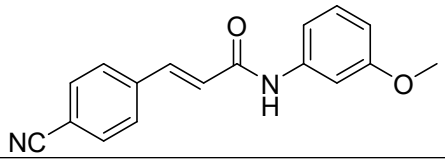
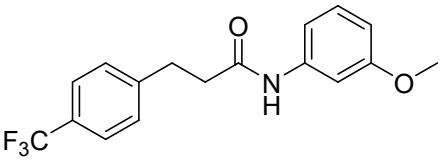
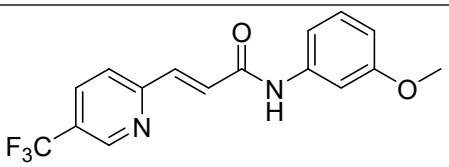
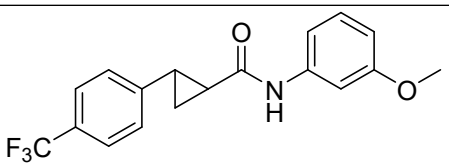
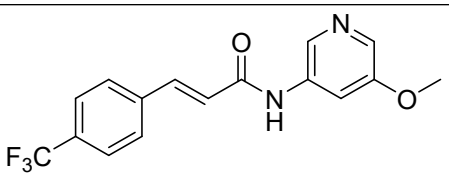
8	CW0475		17-OHP	SC4MOL
9	CW0480		17-OHP	SC4MOL
10	CW0485		17-OHP	SC4MOL
11	CW0487		17-OHP	SC4MOL
12	CW4105		17-OHP	SC4MOL
13	CW4110		17-OHP	SC4MOL
14	CW4142		17-OHP	SC4MOL

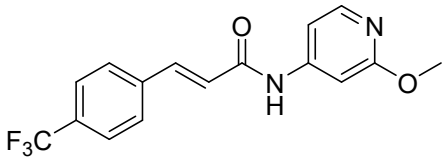
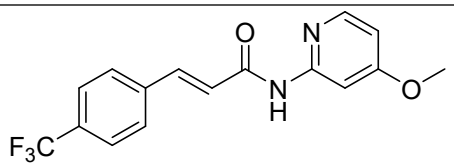
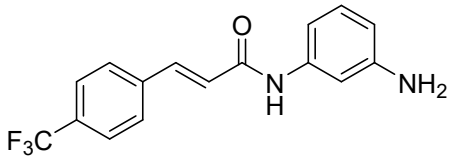
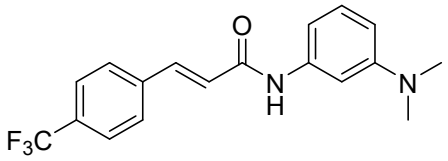
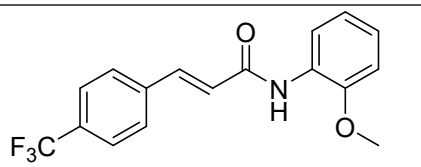
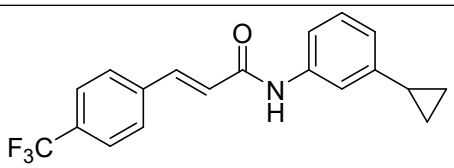
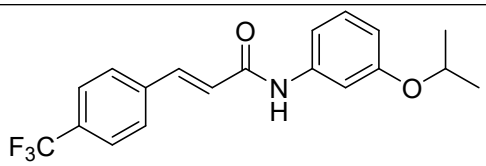
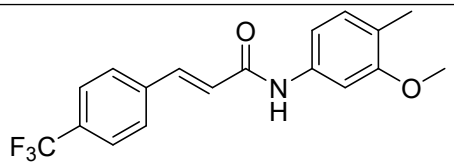
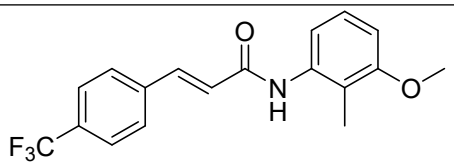
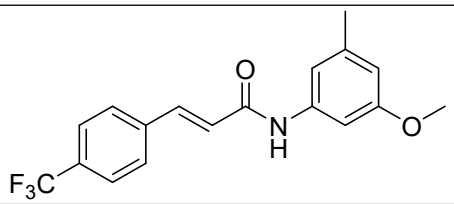
15	CW0496		Alkynyl Sterol	SC4MOL
16	CW4104		Alkynyl Sterol	SC4MOL
17	CW0549		Alkynyl Sterol	SC4MOL
18	CW0550		Alkynyl Sterol	SC4MOL

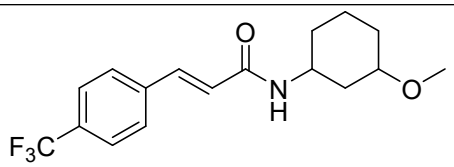
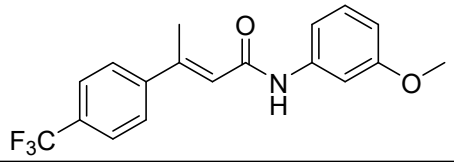
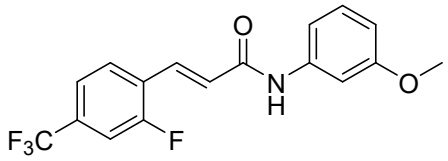
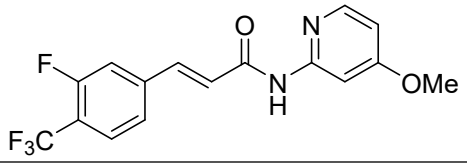
19	CW4149		Alkynyl Sterol	SC4MOL
20	CW5137		Alkynyl Sterol	SC4MOL
21	CW5134		Alkynyl Sterol	SC4MOL
22	SB366791		SB366791	HSD17B7
23	3-Hydroxy-N-(3-methoxyphenyl)-2-naphthamide		SB366791 Commercial	HSD17B7
24	(2E)-N,3-diphenylprop-2-enamide		SB366791 Commercial	HSD17B7
25	N-(3-Methoxyphenyl)cinnamamide		SB366791 Commercial	HSD17B7

26	(2E)-N-(3-methylphenyl)-3-phenyl-2-propenamide		SB366791 Commercial	HSD17B7
27	N-(2,4-dimethyoxyphenyl)-3-pheynylacrylamide		SB366791 Commercial	HSD17B7
28	N-mesityl-3-phenylacrylamide		SB366791 Commercial	HSD17B7
29	Beta-methyl-N-phenylcinnamamide		SB366791 Commercial	HSD17B7
30	Z26548267		SB366791 Commercial	HSD17B7
31	Z27782563		SB366791 Commercial	HSD17B7
32	Z27782075		SB366791 Commercial	HSD17B7
33	CW4168		SB366791 Synthetic	HSD17B7
34	CW4171		SB366791 Synthetic	HSD17B7
35	CW4172		SB366791 Synthetic	HSD17B7

36	CW4173		SB366791 Synthetic	HSD17B7
37	CW4176		SB366791 Synthetic	HSD17B7
38	CW4177		SB366791 Synthetic	HSD17B7
39	CW4178		SB366791 Synthetic	HSD17B7
40	CW4180		SB366791 Synthetic	HSD17B7
41	CW4181		SB366791 Synthetic	HSD17B7
42	CW4182		SB366791 Synthetic	HSD17B7
43	CW4183		SB366791 Synthetic	HSD17B7
44	CW4186		SB366791 Synthetic	HSD17B7
45	CW4187		SB366791 Synthetic	HSD17B7

46	CW4188		SB366791 Synthetic	HSD17B7
47	CW0538		SB366791 Synthetic	HSD17B7
48	CW0539		SB366791 Synthetic	HSD17B7
49	CW0540		SB366791 Synthetic	HSD17B7
50	CW0558		SB366791 Synthetic	HSD17B7
51	CW0559		SB366791 Synthetic	HSD17B7
52	CW0560		SB366791 Synthetic	HSD17B7
53	CW0564		SB366791 Synthetic	HSD17B7
54	CW0565		SB366791 Synthetic	HSD17B7
55	CW0567		SB366791 Synthetic	HSD17B7

56	CW0568		SB366791 Synthetic	HSD17B7
57	CW5124		SB366791 Synthetic	HSD17B7
58	CW0570		SB366791 Synthetic	HSD17B7
59	CW0572		SB366791 Synthetic	HSD17B7
60	CW0573		SB366791 Synthetic	HSD17B7
61	CW0574		SB366791 Synthetic	HSD17B7
62	CW0575		SB366791 Synthetic	HSD17B7
63	CW0578		SB366791 Synthetic	HSD17B7
64	CW0579		SB366791 Synthetic	HSD17B7
65	CW0580		SB366791 Synthetic	HSD17B7

66	CW0581		SB366791 Synthetic	HSD17B7
67	CW0583		SB366791 Synthetic	HSD17B7
68	CW0584		SB366791 Synthetic	HSD17B7
69	CW5107		SB366791 Synthetic	HSD17B7