

Supplemental Information

NIR-mbc94, a Fluorescent Ligand

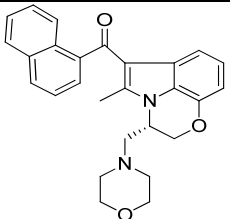
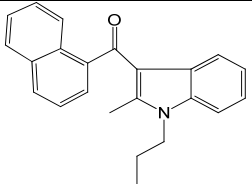
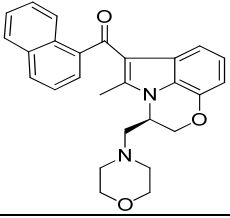
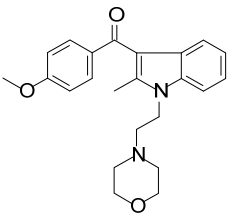
that Binds to Endogenous CB₂ Receptors

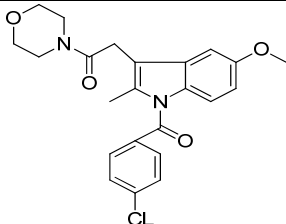
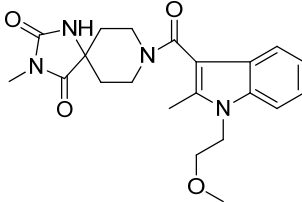
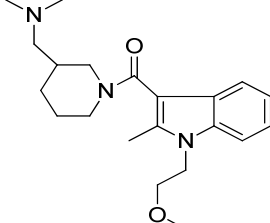
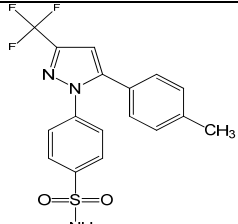
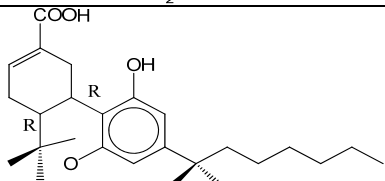
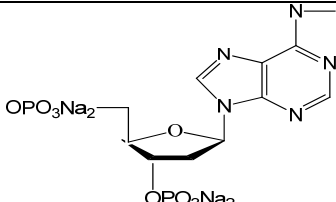
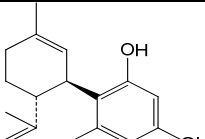
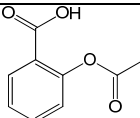
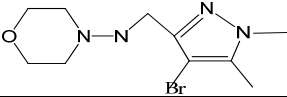
and Is Amenable to High-Throughput Screening

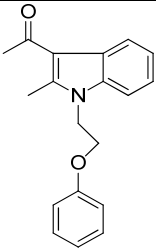
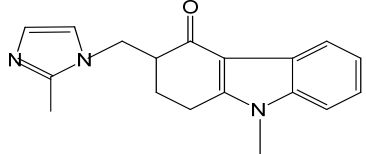
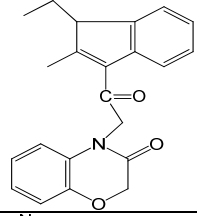
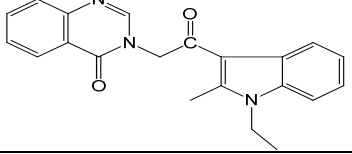
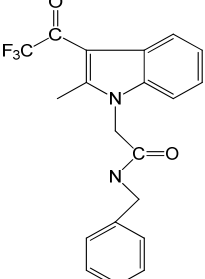
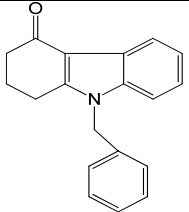
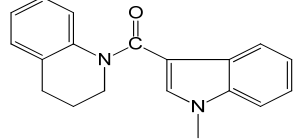
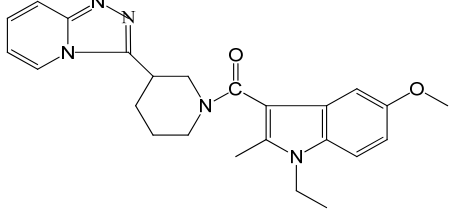
Michelle Sexton, Grace Woodruff, Eric A. Horne, Yi Hsing Lin, Giulio G. Muccioli, Mingfeng Bai, Eric Stern, Darryl J. Bornhop, and Nephi Stella

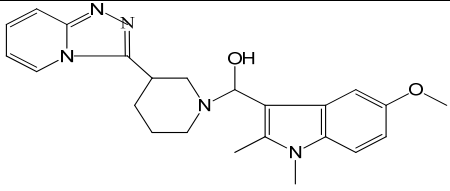
Supplementary Table 1, related to Figure 2: Screening compounds against NIR-mbc94 binding in intact cells

Each compound was incubated at 1 μ M with CB₂ mid cells and fluorescence emitted by NIR-mbc-94 binding quantified as described in the Materials and Methods section. Two positive controls (*i.e.* WIN55,212-2 and JWH-015) outcompeted mbc-94 binding by 23-26% percent (*i.e.* 77 and 74% of total binding), respectively. Thus, these and any other compound that outcompeted mbc-94 binding by \geq 25% were highlighted in bold.

Systematic IUPAC name	Compound name	NIR-mbc94 binding (% of total)	Chemical Structure
(R)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6yl]-1-naphthalenylmethanone	WIN 55,212-2	77 \pm 7	
(2-Methyl-1-propyl-1H-indol-3-yl)-1-naphthalenylmethanone	JWH 015	74 \pm 7	
[(3S)-2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone monomethanesulfonate	Win 55,212-3	110 \pm 57	
4-Methoxyphenyl(2-methyl-1-(2-(4-morpholinyl)ethyl)-1H-indol-3-yl)methanone	Pravadoline	119 \pm 48	

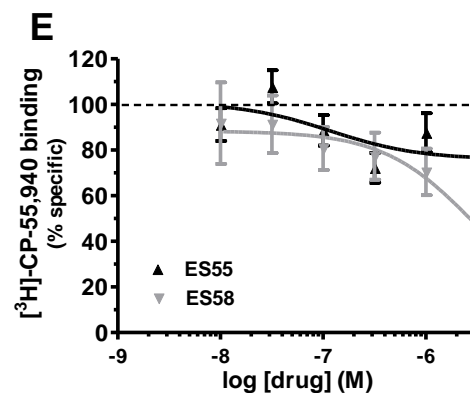
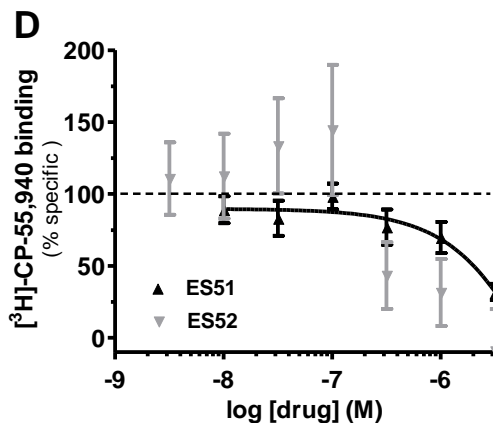
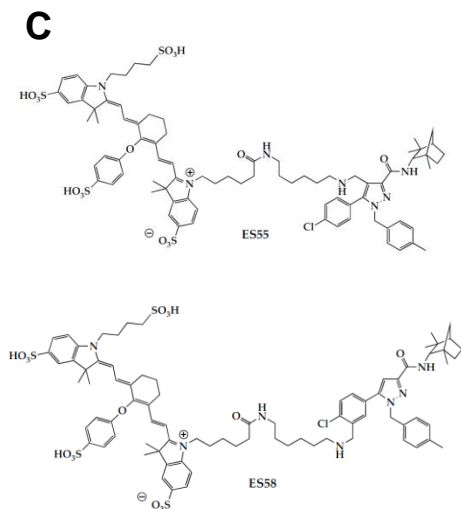
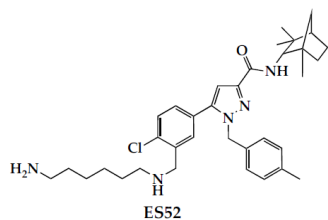
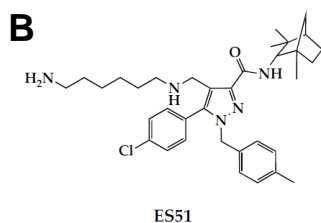
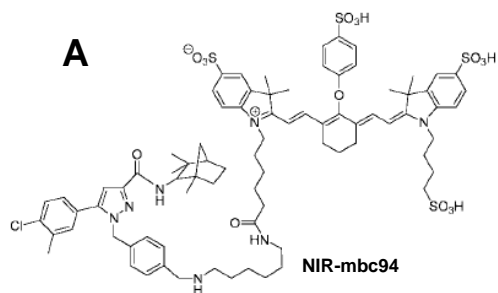
1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid, morpholineamide	Indomethacin morpholinylamide	111 ± 33	
1,3,8-Triazaspiro[4.5]decane-2,4-dione, 8-[[1-(2-methoxyethyl)-2-methyl-1H-indol-3-yl]carbonyl]-3-methyl-	SYN 22867422	86 ± 9	
(3-((dimethylamino)methyl)piperidin-1-yl)(1-(2-methoxyethyl)-1H-indol-3-yl)methanone	SYN 19993370	70 ± 2	
4-[5-(4-methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide	SC 58215	107 ± 27	
1',1'-dimethylheptyl-Δ ⁸ -tetrahydrocannabinol-11-oic acid	Ajulemic Acid	107 ± 21	
N ⁶ -methyl 2Ċ-deoxyadenosine 3Ċ,5Ċ-bisphosphate	MRS 2179	79 ± 6	
5-methyl-4-[(1R,6R)-3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-1,3-benzenediol	O-1602	84 ± 4	
2-acetoxybenzoic acid	Aspirin	85 ± 12	
4-bromo-1,5-dimethyl-N-4-morpholinyl-1H-pyrazole-3-carboxamide		105 ± 24	

1--[2-methyl-1-(2-phenoxyethyl)-1H-indol-3-yl]ethanone		84 ± 8	
9-methyl-3-[(2-methyl-1H-imidazol-1-yl)methyl]-1,2,3,9-tetrahydro-4H-carbazol-4-one hydrochloride hydrate		77 ± 5	
4-[2-(1-ethyl-2-methyl-1H-indol-3-yl)-2-oxoethyl]-2H-1,4-benzoxazin-3(4H)-one		77 ± 6	
3-[2-(1-ethyl-2-methyl-1H-indol-3-yl)-2-oxoethyl]-4(3H)-quinazolinone		80 ± 11	
N-benzyl-2-[2-methyl-3-(trifluoroacetyl)-1H-indol-1-yl]acetamide		70 ± 7	
4-Carbazol-4-one, 1,2,3,9-tetrahydro-9-(phenylmethyl)-		79 ± 6	
(3,4-Dihydro-2H-quinolin-1-yl)-(1-methyl-1H-indol-3-yl)-methanone		85 ± 22	
(1-Ethyl-5-methoxy-2-methyl-1H-indol-3-yl)-(3-[1,2,4]triazolo[4,3-a]pyridin-3-yl)piperidin-1-yl)-methanone		67 ± 9	

<p>(5-Methoxy-1,2-dimethyl-1H-indol-3-yl)- (3-[1,2,4]triazolo[4,3-a]pyridin-3-yl- piperidin-1-yl)-methanone</p>		<p>61 ± 7</p>	
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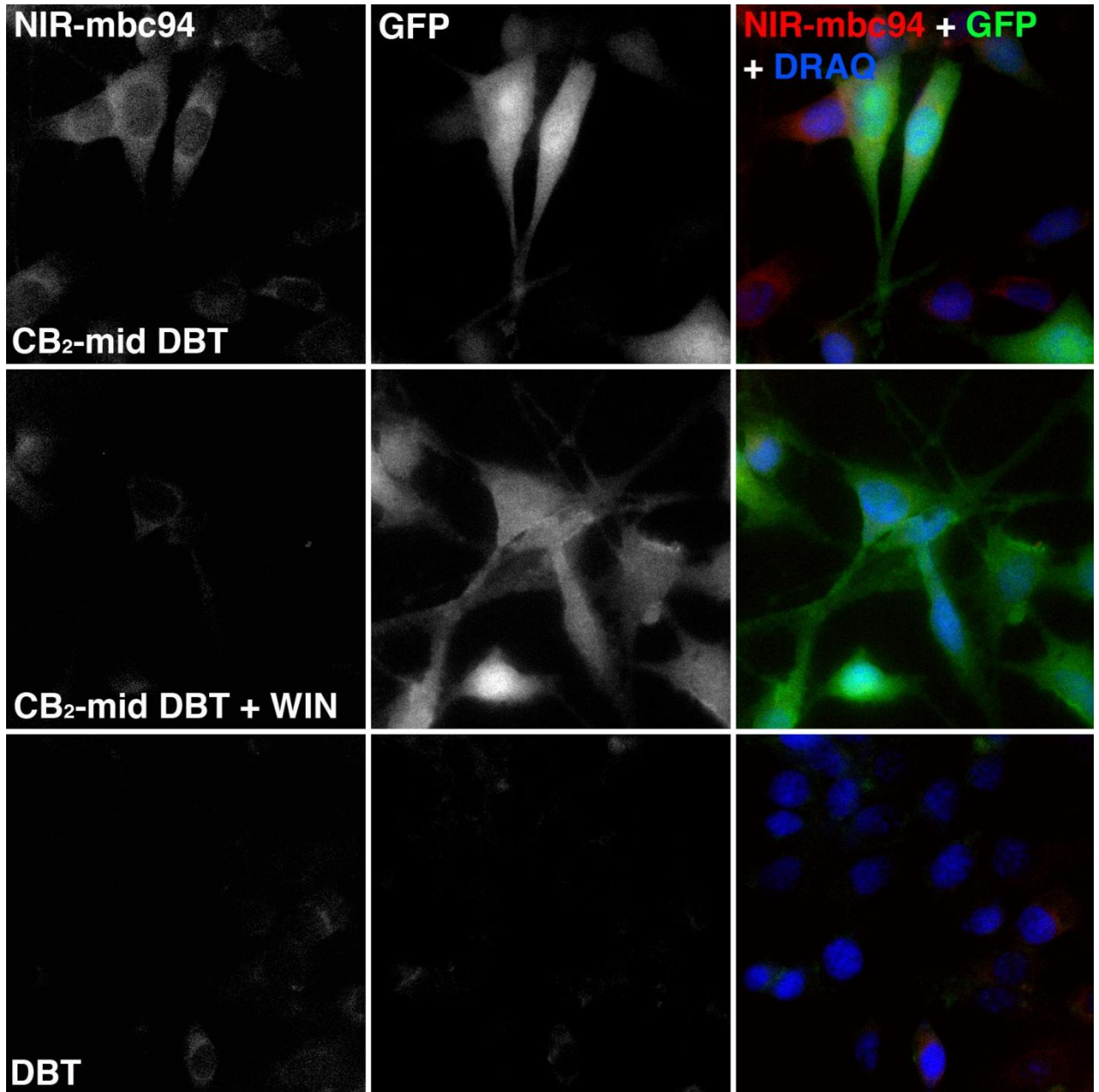
Supplementary Figure 1: SR144528 analogs and their binding to CB₂ receptors

This figure provides structural information on how and where the chemistry was modified. Also included are the binding data of four novel compounds, ES51, ES52, ES55 and ES58, all of which are referred to in the text. A) Chemical structure of NIR-mbc94. B) The addition of linker arms and (C) conjugation of the NIR-emitting dye to the R1 and R2 moieties (see Figure 1A) of SR144528 obliterated binding to CB₂ receptors (D,E), as determined by radioligand binding using [³H]-CP-55,940 and homogenates prepared from DBT cells that heterologously express mouse CB₂ receptors. Each data point represents the mean ± s.e.m. from three experiments, each performed in triplicate (using homogenates prepared from independent cell cultures).



Supplementary Figure 2: Visualization of CB₂ receptors

This figure details the microscopy work provided in Figure 2B. Here we separated the images out to provide microscopic details. Intact DBT cells heterologously-expressing mouse CB₂ receptors were incubated with NIR-mbc94 (CB₂-mid). These cells are co-transfected with GFP and co-stained with DRAQ5 to visualize their cytosol and nuclei, respectively. Here, two points should be emphasized. First, to identify specific staining, all images were gated to the staining of non-transfected DBT cells (set to zero). 2) Co-incubation of NIR-mbc94 with the CB₂ agonist WIN-55,212-2 (WIN; 150 nM) outcompeted the binding of this MI agent to CB₂-mid cells.



Supplementary Figure 3: qPCR result showing increase in CB₂ receptor mRNA in primary microglia.

This figure shows the changes in mRNA for CB₂ receptor expressed by primary microglia stimulated with different cytokines for 72 hours. This result is related to Figure 3B. Note that IL-4 treatment of primary microglia cultures induced a 5-fold change in CB₂ receptor mRNA, while other cytokines did not affect the expression of this message.

