

SUPPORTING INFORMATION

Computational Studies Towards the Identification of CB2R-M1R Dual Modulators

Israa H. Isawi^{1}, Rufaida Al-Zoubi¹, Rima Hajjo², Rayan M. Obeidat¹, Islam H. AlKhawaldeh¹, Omar M. Al Kilani¹, Mahmoud J. Alhaj Hasan³, Paula Morales^{4*}*

¹Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, 22110, Jordan

²Department of Pharmacy, Faculty of Pharmacy, Al-Zaytoonah University of Jordan, Amman 11733, Jordan

³Department of Computer Science, Faculty of Computer and Information Technology, Jordan University of Science and Technology, Irbid, 22110, Jordan

⁴Instituto de Química Médica, Consejo Superior de Investigaciones Científicas (IQM-CSIC), Madrid, 28006, Spain

*Corresponding Authors: ihisawi@just.edu.jo; paula.morales@iqm.csic.es

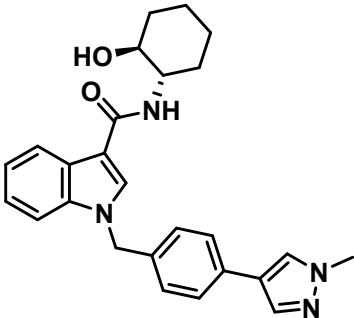
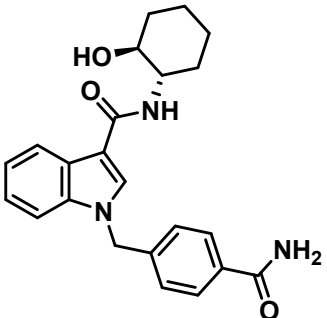
Table S1. *Fingerprint notations along with the open-source software packages used for their calculation.*

Fingerprints Name	Description	Number of Bits	Package	Reference
MACCS[#]	MACCS fingerprint that generates 166-bit MACCS keys	166	CDK	1,2
PUB[#]	PubChem fingerprints that generate 881-bit-long structural key	881	CDK	3
KR[*]	Klekota-Roth fingerprints based on 4860 substructures	4860	CDK	4
GRAPH[*]	Graph fingerprint which does not take bond orders into account	1024	CDK	1,2
SUB[*]	Bit set type fingerprint based on 307 substructures	307	CDK	1,2
HYB[*]	Fingerprint based on hybridization state of atoms	1024	CDK	1,2
MFP2[#]	Morgan connectivity invariants (ECFP-like) with radius = 2	2048	RDKit	5
FeatMFP2[#]	Morgan feature invariants (FCFP-like) with radius = 2	2048	RDKit	5
PAIR[#]	Atom pairs fingerprints	2048	RDKit	6
PAT[#]	SMARTS Pattern fingerprints	2048	RDKit	5
RDKIT[#]	Daylight-like fingerprints	2048	RDKit	5
TORS[#]	Topological torsion fingerprints	2048	RDKit	7
FP2[*]	Indexes linear fragments up to 7 atoms		Pybel	8

Fingerprints generated using KNIME fingerprint nodes

* Fingerprints generated using custom Python scripts

Table S2. Chemical Structures of M1R PAMs Selected to Investigate their Potential Cross-Affinity with CB2R using IFD.

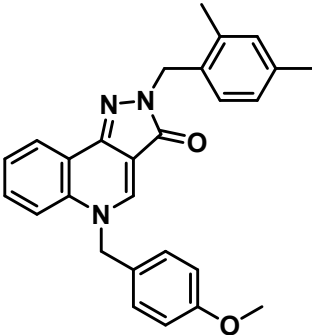
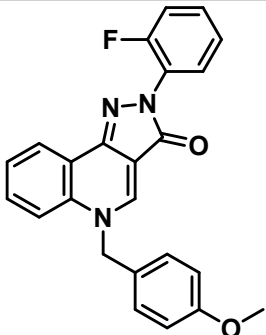
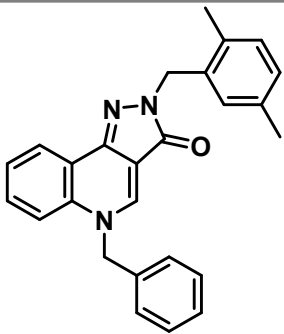
ChEMBL ID	Chemical Structure	IP/EC ₅₀ (nM) Or Activity	SMILES	Reference
(1) CHEMBL4224929		active	<chem>Cn1cc(-c2ccc(Cn3cc(C(=O)N[C@H]4CCCC[C@@H]4O)c4cccc43)cc2)cn1</chem>	9
(2) CHEMBL4227499		active	<chem>NC(=O)c1ccc(Cn2cc(C(=O)N[C@H]3CCCC[C@@H]3O)c3cccc32)cc1</chem>	9

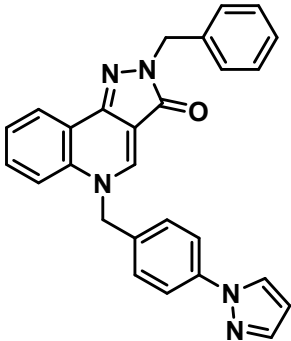
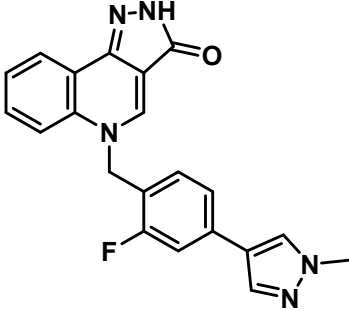
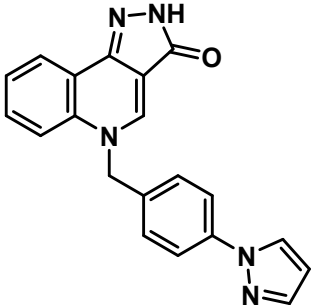
(3) CHEMBL4227561		active	<chem>Cn1cc(-c2ccc(Cn3nc(C(=O)N[C@H]4CCCC[C@@H]4O)c4cccc43)cc2)cn1</chem>	9
(4) CHEMBL3745855		EC ₅₀ = 248	<chem>O=C(N[C@H]1CC[C@@H]1(CO)CC1)c1cn(Cn3cccn3)cc2c2ccnc12</chem>	10
(5) CHEMBL3745995		EC ₅₀ =478	<chem>N#Cc1ccc(Cn2cc(C(=O)N[C@H]3CCCC[C@@H]3O)c3ncccc32)c1</chem>	10

(6) CHEMBL3746904		EC ₅₀ =185	<chem>O=C(N[C@H]1CCCC[C@@H]1O)c1cn(Cc2ccc3ncccc3c2)c2cccnc2</chem> 12	10
(7) CHEMBL4759834		EC ₅₀ =149	<chem>O=C(N[C@@H]1CCCC[C@@H]1O)C[C@H]1O)c1cc(Cc2ccc(cc3ccc(F)cn3)cc2)c2ccn2n1</chem>	11
(8) CHEMBL4783540		EC ₅₀ =182	<chem>Cn1cc(c2ccc(Cc3cc(C(=O)NC4CCOCC4)nn4cccc34)cc2)cn1</chem>	11

(9) CHEMBL4745001		EC ₅₀ =405	<chem>CC(C)(O)CNC(=O)c1cc(Cc2ccc(c3ccncc3)cc2)c2cccn2n1</chem>	11
(10) (CID 53307538)		EC ₅₀ =63	<chem>O=C(N[C@H]1CCCCC1O)C2=CC=C(C=C2)Cc3ccc(Cl)nc32)c2ccccc2n1</chem>	12
(11) (CID 53307539)		EC ₅₀ =102	<chem>Cc1ccc(Cc2cc(C(=O)N[C@H]3CCCC[C@H]3O)nc3ccccc23)cn1</chem>	12

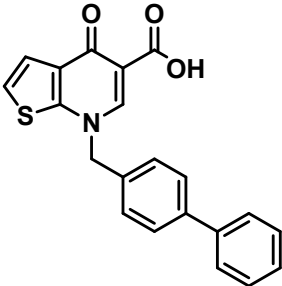
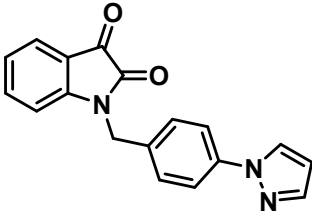
(12) CHEMBL3360928		EC ₅₀ =1000	<chem>O=c1c2cn(Cc3ccccc3)c3ccccc3c-2nn1Cc1ccccc1F</chem>	13
(13) CHEMBL3360931		EC ₅₀ =776.25	<chem>Cc1ccccc1Cn1nc2c3ccc3n(Cc3ccccc3)cc-2c1=O</chem>	13
(14) CHEMBL3360938		EC ₅₀ =656	<chem>Cc1ccc(Cn2nc3c4ccccc4n(Cc4ccccc4F)cc-3c2=O)c(C)c1</chem>	13

(15) CHEMBL3360947		EC ₅₀ =812.83 <chem>COc1ccc(Cn2cc3c(=O)n(Cc4ccc(C)cc4C)nc3ccccc32)cc1</chem>	13
(16) CHEMBL4218803		EC ₅₀ =410 <chem>COc1ccc(Cn2cc3c(=O)n(-c4ccccc4F)nc3ccccc32)cc1</chem>	14
(17) CHEMBL1556989		EC ₅₀ =473 <chem>Cc1ccc(C)c(Cn2nc3c4ccccc4n(Cc4ccccc4)cc3c2=O)c1</chem>	13

(18) CHEMBL3360959		EC ₅₀ >10	<chem>O=c1c2cn(Cc3ccc(-n4cccn4)cc3)c3ccccc3c-2nn1Cc1cccc1</chem>	13
(19) CHEMBL3360954		EC ₅₀ =0.6	<chem>Cn1cc(-c2ccc(Cn3cc4c(=O)[nH]nc4cc3cc(F)c5cnn5)cc2)cc1</chem>	13
(20) CHEMBL3360955		EC ₅₀ =1.2	<chem>O=c1[nH]nc2c3ccccc3n(Cc3ccc(n4cccn4)cc3)cc1-2</chem>	13

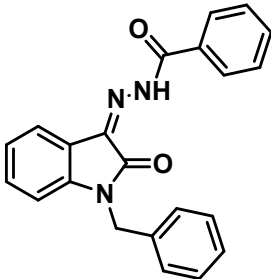
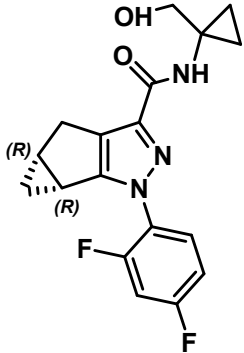
(21) CHEMBL2203584		IP=381	<chem>CO[C@H]1CCCC[C@H]1NC(=O)c1cc(CN2CCC(C#N)(c3cccn3)CC2)c2cccn2c1=O</chem>	15
(22) CHEMBL2204291*		IP=253	<chem>N#CC1(c2cccn2)CCN(Cc2cc(C(=O)NC3CCCC3)c(=O)n3ccccc23)CC1</chem>	15
(23) CHEMBL2204292		IP=304	<chem>N#CC1(c2cccn2)CCN(Cc2cc(C(=O)NC3CCCCC3)c(=O)n3cccc23)CC1</chem>	15

(24) CHEMBL2204294*		IP=338	<chem>N#CC1(c2cccn2)CCN(Cc2cc(C(=O)NC3C(COCC3)c(=O)n3ccccc23)CC1</chem>	15
(25) CHEMBL2204297		IP=190	<chem>C[C@H]1CCCC[C@@H]1NC(=O)c1cc(CN2CCC(C#N)(c3cccn3)CC2)c2cccn2c1=O</chem>	15
(26) CHEMBL2204298		IP=205	<chem>N#CC1(c2cccn2)CCN(Cc2cc(C(=O)NC3C(C(F)(F)F)C3)c(=O)n3ccccc23)CC1</chem>	15

(27) CHEMBL1090104		IP=370	<chem>O=C(O)c1cn(Cc2ccc(-c3ccccc3)cc2)c2sccc2c1=O</chem>	16
(28) CHEMBL3360956		EC ₅₀ =10	<chem>O=C1C(=O)N(Cc2ccc(n3cccn3)cc2)c2ccccc21</chem>	13

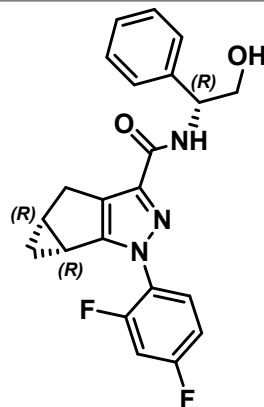
* Compounds have affinity on P-glycoprotein (P-gp) efflux transporters

Table S3. Chemical Structures of CB2R Agonist Compounds Selected to Investigate their Potential Cross-Affinity with M1R using IFD.

ChEMBL ID	Chemical Structure	EC ₅₀ (nM)	SMILES	Reference
(29) ChEMBL456535		63.7	<chem>O=C(N/N=C1\C(=O)N(Cc2ccccc2)c2ccccc21)c1ccccc1</chem>	17
(30) ChEMBL3354953		13.6	<chem>O=C(NC1(CO)CC1)c1nn(-c2ccc(F)cc2F)c2c1C[C@@H]1C[C@H]21</chem>	18

(30') CHEMBL3354964	Chemical structure of (30'): A bicyclic system consisting of a cyclopropane ring fused to a five-membered ring containing a nitrogen atom. The nitrogen is substituted with a 4,6-difluorophenyl group. The five-membered ring is also substituted with a carbonyl group linked to a cyclopropylmethyl group. Stereochemistry is indicated with (S) labels.	24.8	<chem>O=C(NC1(CO)CC1)c1nn(-c2ccc(F)cc2F)c2c1C[C@@H]1C[C@H]21</chem>	18
(31) CHEMBL3354954	Chemical structure of (31): A bicyclic system consisting of a cyclobutane ring fused to a five-membered ring containing a nitrogen atom. The nitrogen is substituted with a 4,6-difluorophenyl group. The five-membered ring is also substituted with a carbonyl group linked to a cyclobutylmethyl group. Stereochemistry is indicated with (R) labels.	1.2	<chem>O=C(NC1(CO)CCC1)c1nn(-c2ccc(F)cc2F)c2c1C[C@H]1C[C@@H]21</chem>	18
(32) CHEMBL3354955	Chemical structure of (32): A bicyclic system consisting of a cyclopentane ring fused to a five-membered ring containing a nitrogen atom. The nitrogen is substituted with a 4,6-difluorophenyl group. The five-membered ring is also substituted with a carbonyl group linked to a cyclopentylmethyl group. Stereochemistry is indicated with (R) labels.	0.7	<chem>O=C(NC1(CO)CCCC1)c1nn(-c2ccc(F)cc2F)c2c1C[C@H]1C[C@@H]21</chem>	18

(33)
CHEMBL3354957

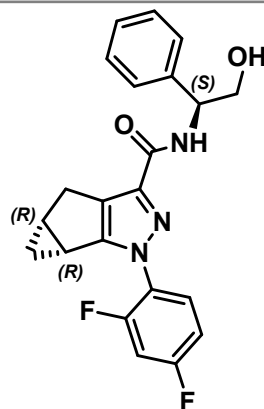


5.4

O=C(N[C@@H](CO)c1ccccc1)c1nn(-c2ccc(F)cc2F)c2c1C[C@H]1C[C@@H]21

18

(33')
CHEMBL3354956

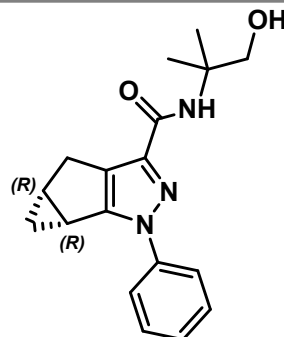


38.8

O=C(N[C@H](CO)c1ccccc1)c1nn(-c2ccc(F)cc2F)c2c1C[C@H]1C[C@@H]21

18

(34)
CHEMBL3354967

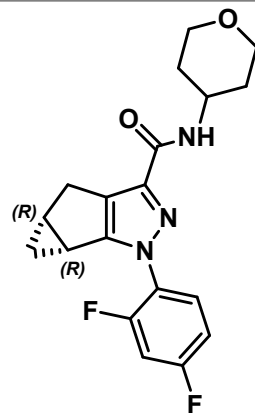


100.9

CC(C)(CO)NC(=O)c1nn(-c2ccccc2)c2c1C[C@H]1C[C@@H]21

18

(35)
CHEMBL3354958

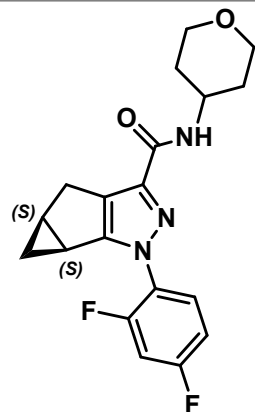


15.2

O=C(NC1CCOCC1)c
1nn(-
c2ccc(F)cc2F)c2c1C[C
@H]1C[C@@H]21

18

(35')
CHEMBL3354965

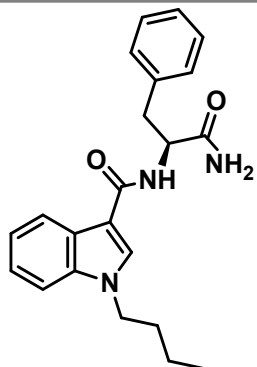


38.5

O=C(NC1CCOCC1)c
1nn(-
c2ccc(F)cc2F)c2c1C[C
@@H]1C[C@H]21

18

(36)
ChEMBL5199876

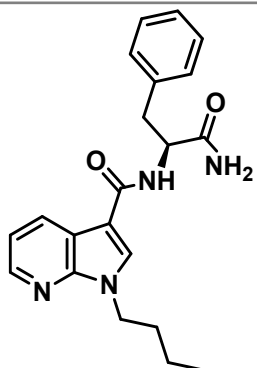


40

CCCCn1cc(C(=O)N[C@@H](Cc2ccccc2)C(N)=O)c2ccccc21

19

(37)
ChEMBL5209051

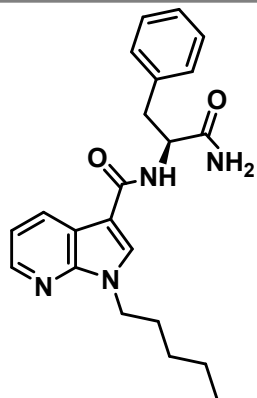


316.23

CCCCn1cc(C(=O)N[C@@H](Cc2ccccc2)C(N)=O)c2ccnc21

19

(38)
ChEMBL5178204

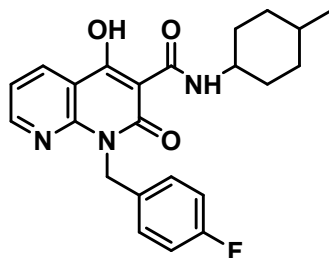


381

CCCCCn1cc(C(=O)N[C@@H](Cc2ccccc2)C(N)=O)c2ccnc21

19

(39)
ChEMBL3422790

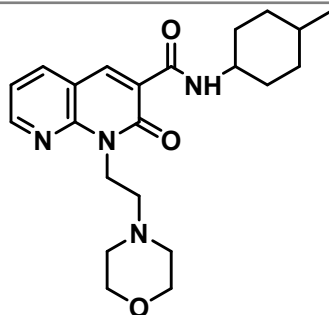


3.162

CC1CCC(NC(=O)c2c(O)c3ccnc3n(Cc3ccc(F)cc3)c2=O)CC1

20

(40)
ChEMBL466223

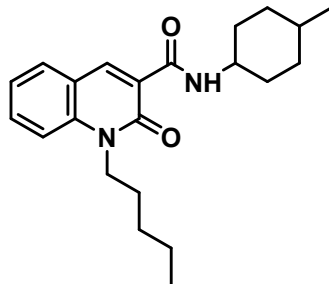


28

CC1CCC(NC(=O)c2c3ccnc3n(CCN3CCOCC3)c2=O)CC1

21

(41)
ChEMBL3422788

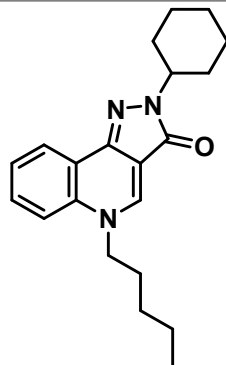


1.995

CCCCCn1c(=O)c(C(=O)NC2CCC(C)CC2)c2ccccc21

20

(42)
ChEMBL3421689

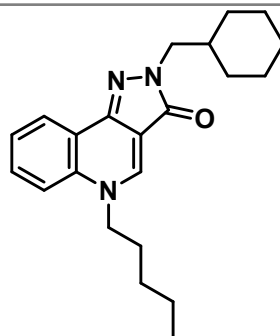


204

CCCCCn1cc2c(=O)n(
C3CCCCC3)nc-
2c2cccc21

22

(43)
ChEMBL 3421697

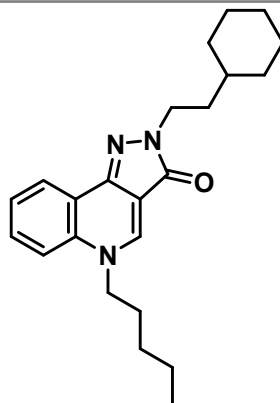


14.3

CCCCCn1cc2c(=O)n(
CC3CCCCC3)nc-
2c2cccc21

22

(44)
ChEMBL3421698

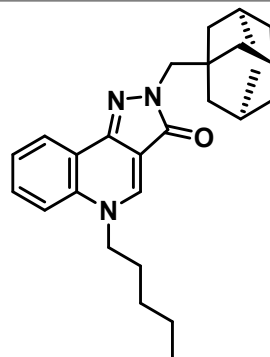


5.4

CCCCCn1cc2c(=O)n(
CCC3CCCCC3)nc-
2c2cccc21

22

(45)
CHEMBL3421699

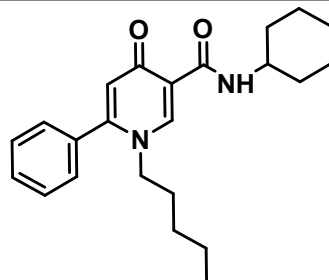


64

CCCCCn1cc2c(=O)n(
CC34CC5CC(CC(C5)
C3)C4)nc-2c2ccccc21

22

(46)
CHEMBL1641947

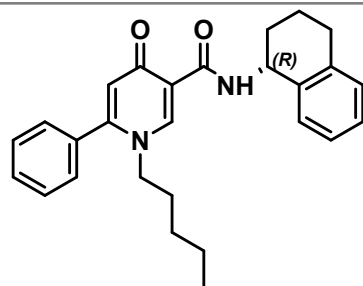


1.9

CCCCCn1cc(C(=O)N
C2CCCCC2)c(=O)cc1
-c1ccccc1

23

(47)
CHEMBL1641948

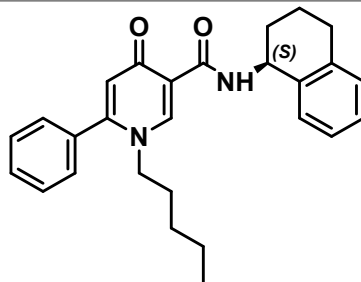


1.9

CCCCCn1cc(C(=O)N
[C@@H]2CCc3ccccc
32)c(=O)cc1-c1ccccc1

23

(48)
ChEMBL1641949

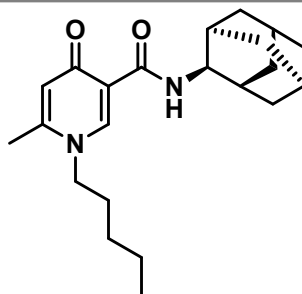


60

CCCCCn1cc(C(=O)N
[C@H]2CCCC3CCCCC3
2)c(=O)cc1-c1ccccc1

23

(49)
ChEMBL2170545

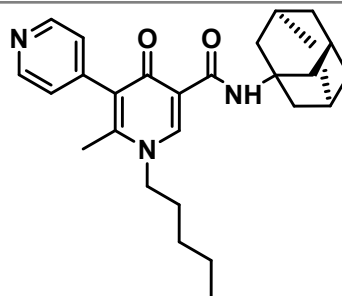


38.4

CCCCCn1cc(C(=O)N
[C@H]2[C@@H]3C[C@H]4C[C@@H](C3)C[C@@H]2C4)c(=O)cc1
C

24

(50)
ChEMBL2170529*



114

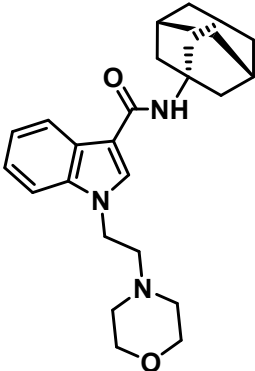
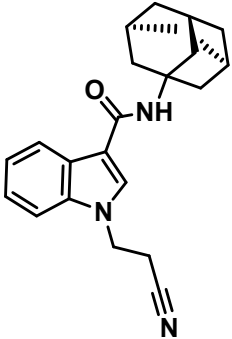
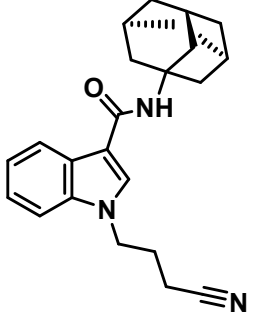
CCCCCn1cc(C(=O)N
[C@]23C[C@H]4C[C@H](C[C@H](C4)C2)
C3)c(=O)c(-
c2ccncc2)c1C

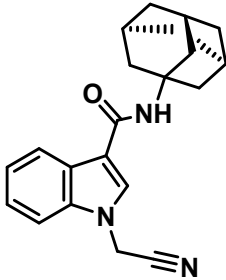
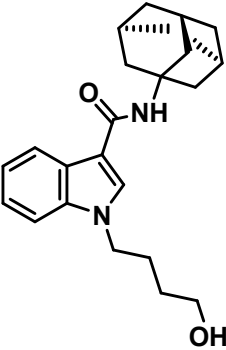
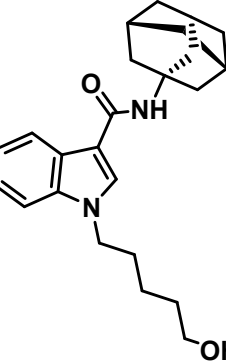
24

(51) ChEMBL2170549		452	<chem>CCCCCn1cc(C(=O)N[C@H]2[C@@H]3C[C@H]4C[C@@H](C3)C[C@@H]2C4)c(=O)cc1C(C)(C)C</chem>	24
(52) ChEMBL2170544		84.2	<chem>CCCCCn1cc(C(=O)N[C@@H](C)[C@]23C[C@H]4C[C@H](C[C@H](C4)C2)C3)c(=O)cc1C</chem>	24
(53) ChEMBL1642940		6.5	<chem>CCCCCn1cc(C(=O)N[C@@H](C)[C@]23C[C@H]4C[C@H](C[C@H](C4)C2)C3)c(=O)cc1-c1ccccc1</chem>	23
(54) ChEMBL1641942		10.9	<chem>CCCCCn1cc(C(=O)N[C@]23C[C@H]4C[C@H](C[C@H](C4)C2)c(=O)cc1-c1ccccc1</chem>	23

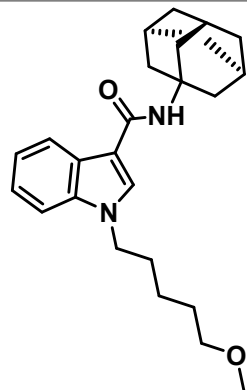
(55) ChEMBL2170546		53.1	<chem>CCCCCn1cc(C(=O)N[C@H]2C[C@H]4[C@H](C[C@H](C4)C2)C3)C(=O)cc1C</chem>	24
(56) ChEMBL16414938		7.8	<chem>CCCCCn1cc(C(=O)N[C@@H]2CCCCC2)C(=O)cc1C(C)(C)C</chem>	23
(57) ChEMBL2152800		17.2	<chem>CCn1cc(C(=O)N[C@@H]2C(C)(C)[C@H]3CC[C@]2(C)C3)C(=O)c2ccc(F)cc21</chem>	25
(58) ChEMBL264521		24.8	<chem>CCCCCn1cc(C(=O)N[C@H]2[C@@H]3C[C@H]4[C@@H](C3)C[C@@H]2C4)C(=O)c2ccccc21</chem>	26

(59) CHEMBL372544		14.1	<chem>CCCCCn1cc(C(=O)N[C@@H]2C[C@H]4C[C@@](C)(C)[C@@](C)(C4)C2)C3c(=O)c2cccc21</chem>	26
(60) CHEMBL380610		157.4	<chem>CCCCCn1cc(C(=O)N[C@H]2CCCC3CCCCC32)c(=O)c2cccc21</chem>	26
(61) CHEMBL4079817		420	<chem>O=C(NC1CCCCC1)c1cc2cccc2n1CCCCC1O</chem>	27
(62) CHEMBL1828807		6.31	<chem>COCCn1cc(C(=O)Nc2cccc3cccc23)c2cccc21</chem>	28

(63) CHEMBL4073323		59	<chem>O=C(N[C@]12C[C@H]3C[C@H](C3)C1)C2)c1cn(CCN2CCOCC2)c2cccc12</chem>	27
(64) CHEMBL4062303		180	<chem>N#CCCN1cc(C(=O)N[C@]23C[C@H]4C[C@H](C4)C2)C3)c2cccc21</chem>	27
(65) CHEMBL4087526		31	<chem>N#CCCCN1cc(C(=O)N[C@]23C[C@H]4C[C@H](C4)C2)C3)c2cccc21</chem>	27

(66) CHEMBL4090311		410	<chem>N#CCn1cc(C(=O)N[C@]23C[C@H]4C[C@H](C[C@H](C4)C2)C3)c2ccccc21</chem>	27
(67) CHEMBL4066155		160	<chem>O=C(N[C@]12C[C@H]3C[C@H](C[C@H](C3)C1)C2)c1cn(CCCCO)c2ccccc12</chem>	27
(68) CHEMBL4079650		62	<chem>O=C(N[C@]12C[C@H]3C[C@H](C[C@H](C3)C1)C2)c1cn(CCCCO)c2ccccc12</chem>	27

(69)
CHEMBL4103902

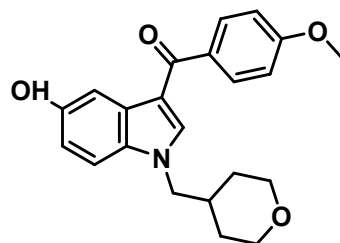


82

COCCCCCN1CC(C(=O)N[C@]23C[C@H]4C[C@H](C[C@H](C4)C2)C3)C2CCCCC21

27

(70)
CHEMBL4284943

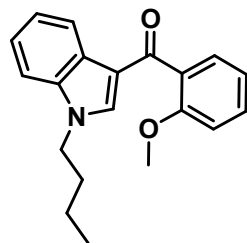


4.4

COc1ccc(C(=O)c2cn(CCC3CCOCC3)c3ccc(O)cc23)cc1

29

(71)
CHEMBL4089341

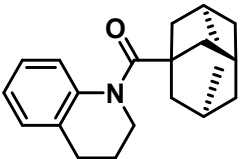
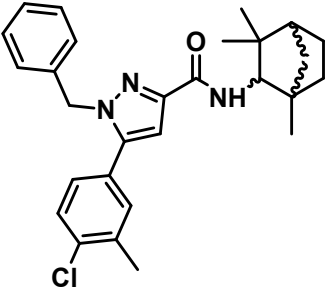
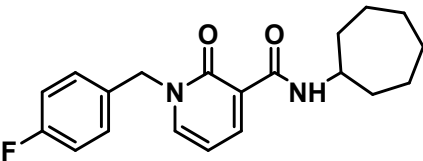
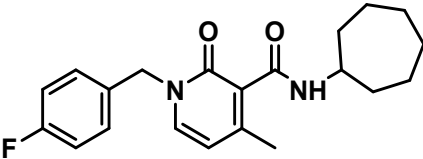


4.5

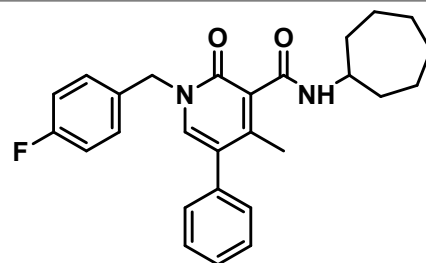
CCCCN1CC(C(=O)c2c(O)ccccc2OC)C2CCCCC21

30

(72) ChEMBL3934189		291.8	<chem>O=C(N[C@H]1CCCC[C@@H]1O)c1cccc(C2CCCCC2)n1</chem>	31
(73) ChEMBL3928538		157.6	<chem>O=C(N[C@@H]1CCCC[C@H]1CO)c1cccc(C2CCCCC2)n1</chem>	31
(74) ChEMBL2338178		23	<chem>O=C(N1CCc2ccccc21)[C@]12C[C@H]3C[C@H](C[C@H](C3)C1)C2</chem>	32
(75) ChEMBL2338181		16	<chem>CC1(C)CN(C(=O)[C@]23C[C@H]4C[C@H](C[C@H](C4)C2)C3)c2ccccc21</chem>	32

(76) CHEMBL246952		72	<chem>O=C(N1CCCCc2ccccc21)[C@]12C[C@H]3C[C@H](C[C@H](C3)C1)C2</chem>	32
(77) CHEMBL381689		1.8	<chem>Cc1ccc(Cn2nc(C(=O)N[C@H]3C(C)(C)[C@H]4CC[C@@]3(C)C4)cc2-c2ccc(Cl)c(C)c2)cc1</chem>	23
(78) CHEMBL2022698		6.31	<chem>O=C(NC1CCCCC1)c1cccn(Cc2ccc(F)cc2)c1=O</chem>	33
(79) CHEMBL4175577		1.9	<chem>Cc1ccn(Cc2ccc(F)cc2)c(=O)c1C(=O)NC1CCCCC1</chem>	34

(80)
ChEMBL4167303



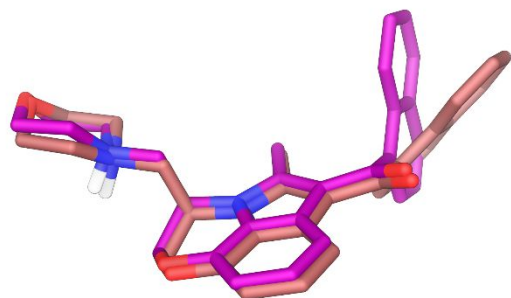
12

Cc1c(-
c2ccccc2)cn(Cc2ccc(F)
cc2)c(=O)c1C(=O)NC
1CCCCC1

34

*Compounds have affinity on P-glycoprotein (P-gp) efflux transporters

A. WIN 55212-2



B. CP55,940

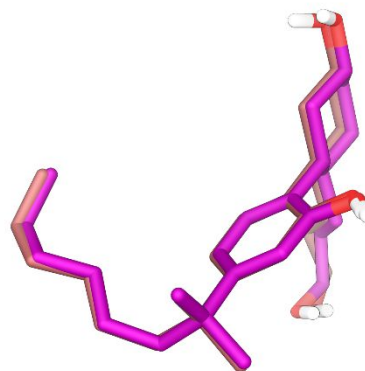


Figure S1. Superimposition of Docked and Co-Crystallized Poses of CB2R Agonists. **A.** IFD pose of WIN 55,212-2 (magenta) overlaid with its co-crystallized pose (dark rose) from PDB ID: 6PT0, with an RMSD of 1.030 Å. **B.** IFD pose of CP 55,940 (magenta) overlaid with its co-crystallized pose (dark rose) from PDB ID: 8GUR, with an RMSD of 0.402 Å.

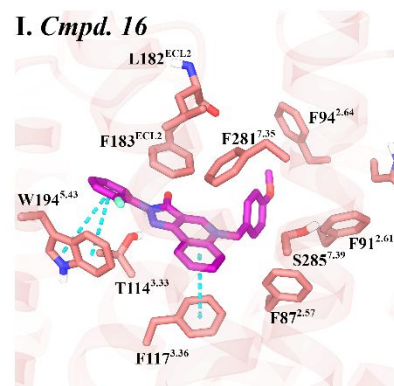
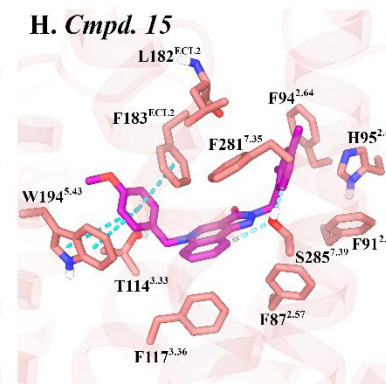
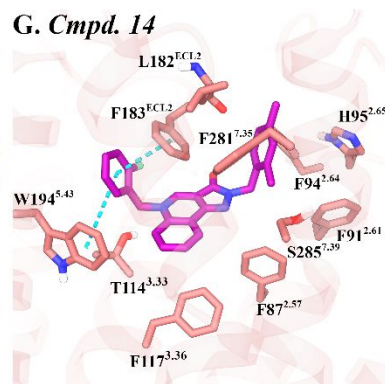
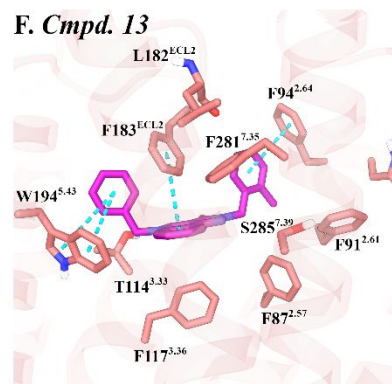
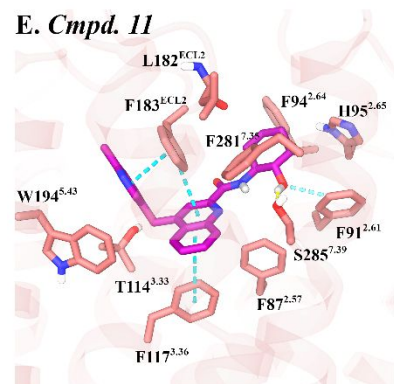
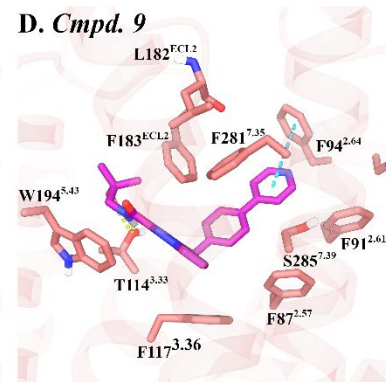
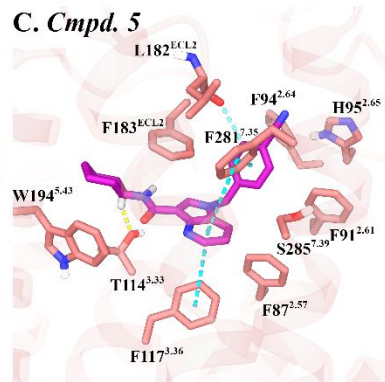
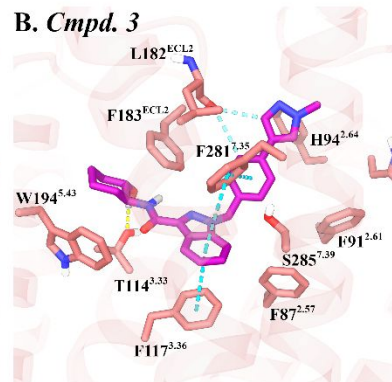
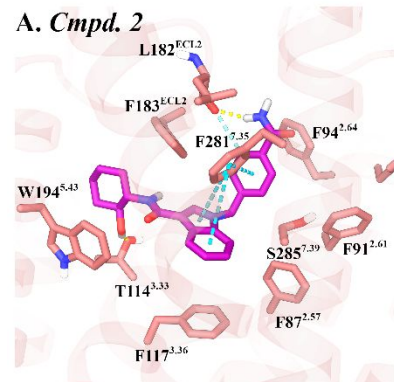


Figure S2. Key Interactions Between the M1R PAMs Docked Within the CB2R Orthosteric Binding Pocket. Key interacting residues for **A.** Compound 2, **B.** Compound 3, **C.** Compound 5, **D.** Compound 9, **E.** Compound 11, **F.** Compound 13, **G.** Compound 14, **H.** Compound 15, and **I.** Compound 16, all docked into the orthosteric site of CB2R (PDB ID: 8GUR or 6PT0). CB2R is shown as rose ribbons and key ligand-binding residues are displayed as dark rose sticks. Ligands are represented as magenta sticks within their respective binding sites. Hydrogen bonds are indicated with yellow dashed lines, π - π stacking interactions with cyan dashed lines, aromatic hydrogen bonds with light cyan dashed lines, and π -cation interactions with green dashed lines.

Figure S3. Key Interactions Between the CB2R Agonists Docked Within the M1R Allosteric Binding Pocket. Key interacting residues for **A.** Compound 31, **B.** Compound 32, **C.** Compound 36, **D.** Compound 38, **E.** Compound 40, **F.** Compound 41, **G.** Compound 43, **H.** Compound 47, **I.** Compound 48, **J.** Compound 62, and **K.** Compound 71, all docked into the allosteric site of M1R in the presence of the orthosteric agonist Iperoxo (IXO) (PDB ID: 6OIJ). M1R is shown as sky-blue ribbons and key ligand-binding residues are displayed as dark sky-blue sticks. Ligands are represented as magenta sticks within their respective binding sites. Hydrogen bonds are indicated with yellow dashed lines, π - π stacking interactions with cyan dashed lines, aromatic hydrogen bonds with light cyan dashed lines, and π -cation interactions with green dashed lines.

References

- (1) Steinbeck, C.; Han, Y.; Kuhn, S.; Horlacher, O.; Luttmann, E.; Willighagen, E. The chemistry development kit (CDK): an open-source Java library for chemo- and bioinformatics. *J. Chem. Inf. Comput. Sci.* **2003**, 43 (2), 493–500. <https://doi.org/10.1021/ci025584y>.
- (2) Steinbeck, C.; Hoppe, C.; Kuhn, S.; Floris, M.; Guha, R.; Willighagen, E. Recent developments of the chemistry development kit (CDK) – an open-source Java library for chemo- and bioinformatics. *Curr. Pharm. Des.* **2006**, 12 (17), 2111–2120. <https://doi.org/10.2174/138161206777585274>.
- (3) PubChem Substructure Fingerprint. National Center for Biotechnology Information (NCBI), PubChem Project: Bethesda, MD, 2009. https://ftp.ncbi.nlm.nih.gov/pubchem/specifications/pubchem_fingerprints.pdf (accessed September 21, 2024).
- (4) Klekota, J.; Roth, F. P. Chemical substructures that enrich for biological activity. *Bioinformatics* **2008**, 24 (21), 2518–2525. <https://doi.org/10.1093/bioinformatics/btn479>.
- (5) Landrum, G. RDKit: Open-source cheminformatics. <https://www.rdkit.org/> (accessed September 21, 2024).
- (6) Carhart, R. E.; Smith, D. H.; Venkataraghavan, R. Atom pairs as molecular features in structure-activity studies: definition and applications. *J. Chem. Inf. Comput. Sci.* **1985**, 25 (2), 64–73. <https://doi.org/10.1021/ci00046a002>.
- (7) Nilakantan, R.; Bauman, N.; Dixon, J. S.; Venkataraghavan, R. Topological torsion: a new molecular descriptor for SAR applications. Comparison with other descriptors. *J. Chem. Inf. Comput. Sci.* **1987**, 27 (2), 82–85. <https://doi.org/10.1021/ci00054a008>.
- (8) O'Boyle, N. M.; Morley, C.; Hutchison, G. R. Pybel: a Python wrapper for the Open Babel cheminformatics toolkit. *Chem. Cent. J.* **2008**, 2 (1), 5. <https://doi.org/10.1186/1752-153X-2-5>.

- (9) Dallagnol, J. C. C.; Khajehali, E.; van der Westhuizen, E. T.; Jörg, M.; Valant, C.; Gonçalves, A. G.; Capuano, B.; Christopoulos, A.; Scammells, P. J. Synthesis and pharmacological evaluation of heterocyclic carboxamides: positive allosteric modulators of the M1 muscarinic acetylcholine receptor with weak agonist activity and diverse modulatory profiles. *J. Med. Chem.* **2018**, 61 (7), 2875–2894. <https://doi.org/10.1021/acs.jmedchem.7b01812>.
- (10) Davoren, J. E.; O’Neil, S. V.; Anderson, D. P.; Brodney, M. A.; Chenard, L.; Dlugolenski, K.; Edgerton, J. R.; Green, M.; Garnsey, M.; Grimwood, S.; Harris, A. R.; Kauffman, G. W.; Lachapelle, E.; Lazzaro, J. T.; Lee, C. W.; Lotarski, S. M.; Nason, D. M.; Obach, R. S.; Reinhart, V.; Salomon-Ferrer, R.; Steyn, S. J.; Webb, D.; Yan, J.; Zhang, L. Design and optimization of selective azaindole amide M1 positive allosteric modulators. *Bioorg. Med. Chem. Lett.* **2016**, 26 (2), 650–655. <https://doi.org/10.1016/j.bmcl.2015.11.053>.
- (11) Blass, B. E. Pyrrolo-pyridazine derivatives as muscarinic M1 receptor positive allosteric modulators. *ACS Med. Chem. Lett.* **2021**, 12 (5), 677–678. <https://doi.org/10.1021/acsmedchemlett.1c00118>.
- (12) Kuduk, S. D.; Schlegel, K. A.; Yang, Z. Q. **Merck Sharp & Dohme LLC, assignee.** *Quinoline amide M1 receptor positive allosteric modulators*. U.S. Patent **9,199,939**, December 1, 2015; Merck Sharp & Dohme LLC, Assignee.
- (13) Han, C.; Chatterjee, A.; Noetzel, M. J.; Panarese, J. D.; Smith, E.; Chase, P.; Hodder, P.; Niswender, C.; Conn, P. J.; Lindsley, C. W.; Stauffer, S. R. Discovery and SAR of muscarinic receptor subtype 1 (M1) allosteric activators from a molecular libraries high throughput screen. Part 1: 2,5-dibenzyl-2H-pyrazolo[4,3-c]quinolin-3(5H)-ones as positive allosteric modulators. *Bioorg. Med. Chem. Lett.* **2015**, 25 (2), 384–388. <https://doi.org/10.1016/j.bmcl.2014.11.011>.
- (14) Beshore, D. C.; Di Marco, C. N.; Chang, R. K.; Greshock, T. J.; Ma, L.; Wittmann, M.; Seager, M. A.; Koeplinger, K. A.; Thompson, C. D.; Fuerst, J.; Hartman, G. D.; Bilodeau, M. T.; Ray, W. J.; Kuduk, S. D. MK-7622: a first-in-class M1 positive allosteric modulator development candidate. *ACS Med. Chem. Lett.* **2018**, 9 (7), 652–656. <https://doi.org/10.1021/acsmedchemlett.8b00095>.

- (15) Kuduk, S. D.; Chang, R. K.; Greshock, T. J.; Ray, W. J.; Ma, L.; Wittmann, M.; Seager, M. A.; Koeplinger, K. A.; Thompson, C. D.; Hartman, G. D.; Bilodeau, M. T. Identification of amides as carboxylic acid surrogates for quinolizidinone-based M1 positive allosteric modulators. *ACS Med. Chem. Lett.* **2012**, 3 (12), 1070–1074. <https://doi.org/10.1021/ml300280g>.
- (16) Kuduk, S. D.; Di Marco, C. N.; Chang, R. K.; Ray, W. J.; Ma, L.; Wittmann, M.; Seager, M. A.; Koeplinger, K. A.; Thompson, C. D.; Hartman, G. D.; Bilodeau, M. T. Heterocyclic fused pyridone carboxylic acid M1 positive allosteric modulators. *Bioorg. Med. Chem. Lett.* **2010**, 20 (8), 2533–2537. <https://doi.org/10.1016/j.bmcl.2010.02.096>.
- (17) Diaz, P.; Xu, J.; Astruc-Diaz, F.; Pan, H. M.; Brown, D. L.; Naguib, M. Design and synthesis of a novel series of N-alkyl isatin acylhydrazone derivatives that act as selective cannabinoid receptor 2 agonists for the treatment of neuropathic pain. *J. Med. Chem.* **2008**, 51 (16), 4932–4947. <https://doi.org/10.1021/jm8002203>.
- (18) Han, S.; Thoresen, L.; Zhu, X.; Narayanan, S.; Jung, J. K.; Strah-Pleyne, S.; Decaire, M.; Choi, K.; Xiong, Y.; Yue, D.; Semple, G.; Thatte, J.; Solomon, M.; Fu, L.; Whelan, K.; Al-Shamma, H.; Gatlin, J.; Chen, R.; Dang, H.; Pride, C.; Gaidarov, I.; Unett, D. J.; Behan, D. P.; Sadeque, A.; Usmani, K. A.; Chen, C.; Edwards, J.; Morgan, M.; Jones, R. M. Discovery of 1a,2,5,5a-tetrahydro-1H-2,3-diaza-cyclopropa[a]pentalen-4-carboxamides as potent and selective CB2 receptor agonists. *Bioorg. Med. Chem. Lett.* **2015**, 25 (2), 322–326. <https://doi.org/10.1016/j.bmcl.2014.11.040>.
- (19) Sparkes, E.; Cairns, E. A.; Kevin, R. C.; Lai, F.; Grafinger, K. E.; Chen, S.; Deventer, M. H.; Ellison, R.; Boyd, R.; Martin, L. J.; McGregor, I. S.; Gerona, R. R.; Auwärter, V.; Glass, M.; Stove, C.; Banister, S. D. Structure–activity relationships of valine, tert-leucine, and phenylalanine amino acid-derived synthetic cannabinoid receptor agonists related to ADB-BUTINACA, APP-BUTINACA, and ADB-P7AICA. *RSC Med. Chem.* **2022**, 13 (2), 156–174. <https://doi.org/10.1039/D1MD00242B>.
- (20) Manera, C.; Malfitano, A. M.; Parkkari, T.; Lucchesi, V.; Carpi, S.; Fogli, S.; Bertini, S.; Laezza, C.; Ligresti, A.; Saccomanni, G.; Savinainen, J. R.; Ciaglia, E.; Pisanti, S.; Gazzerri, P.; Di Marzo, V.; Nieri, P.; Macchia, M.; Bifulco, M. New quinolone- and 1,8-naphthyridine-3-carboxamides as selective CB2 receptor agonists with anticancer and immunomodulatory activity. *Eur. J. Med. Chem.* **2015**, 97, 10–18. <https://doi.org/10.1016/j.ejmech.2015.04.034>.

- (21) Lucchesi, V.; Hurst, D. P.; Shore, D. M.; Bertini, S.; Ehrmann, B. M.; Allarà, M.; Lawrence, L.; Ligresti, A.; Minutolo, F.; Saccomanni, G.; Sharir, H.; Macchia, M.; Di Marzo, V.; Abood, M. E.; Reggio, P. H.; Manera, C. CB2-selective cannabinoid receptor ligands: synthesis, pharmacological evaluation, and molecular modeling investigation of 1,8-naphthyridin-2(1H)-one-3-carboxamides. *J. Med. Chem.* **2014**, 57 (21), 8777–8791. <https://doi.org/10.1021/jm500807e>.
- (22) El Bakali, J.; Muccioli, G. G.; Body-Malapel, M.; Djouina, M.; Klupsch, F.; Ghinet, A.; Barczyk, A.; Renault, N.; Chavatte, P.; Desreumaux, P.; Lambert, D. M.; Millet, R. Conformational restriction leading to a selective CB2 cannabinoid receptor agonist orally active against colitis. *ACS Med. Chem. Lett.* **2015**, 6 (2), 198–203. <https://doi.org/10.1021/ml500439x>.
- (23) El Bakali, J.; Muccioli, G. G.; Renault, N.; Pradal, D.; Body-Malapel, M.; Djouina, M.; Hamtiaux, L.; Andrzejak, V.; Desreumaux, P.; Chavatte, P.; Lambert, D. M.; Millet, R. 4-Oxo-1,4-dihydropyridines as selective CB2 cannabinoid receptor ligands: structural insights into the design of a novel inverse agonist series. *J. Med. Chem.* **2010**, 53 (22), 7918–7931. <https://doi.org/10.1021/jm100286k>.
- (24) El Bakali, J.; Gilleron, P.; Body-Malapel, M.; Mansouri, R.; Muccioli, G. G.; Djouina, M.; Barczyk, A.; Klupsch, F.; Andrzejak, V.; Lipka, E.; Furman, C.; Lambert, D. M.; Chavatte, P.; Desreumaux, P.; Millet, R. 4-Oxo-1,4-dihydropyridines as selective CB2 cannabinoid receptor ligands. Part 2: discovery of new agonists endowed with protective effect against experimental colitis. *J. Med. Chem.* **2012**, 55 (20), 8948–8952. <https://doi.org/10.1021/jm3008568>.
- (25) Pasquini, S.; De Rosa, M.; Ligresti, A.; Mugnaini, C.; Brizzi, A.; Caradonna, N. P.; Cascio, M. G.; Bolognini, D.; Pertwee, R. G.; Di Marzo, V.; Corelli, F. Investigations on the 4-quinolone-3-carboxylic acid motif. 6. Synthesis and pharmacological evaluation of 7-substituted quinolone-3-carboxamide derivatives as high affinity ligands for cannabinoid receptors. *Eur. J. Med. Chem.* **2012**, 58, 30–43. <https://doi.org/10.1016/j.ejmech.2012.09.035>.
- (26) Stern, E.; Muccioli, G. G.; Millet, R.; Goossens, J. F.; Farce, A.; Chavatte, P.; Poupaert, J. H.; Lambert, D. M.; Depreux, P.; Hénichart, J. P. Novel 4-oxo-1,4-dihydroquinoline-3-carboxamide derivatives as new CB2 cannabinoid receptor agonists:

synthesis, pharmacological properties and molecular modeling. *J. Med. Chem.* **2006**, 49 (1), 70–79.

<https://doi.org/10.1021/jm050467q>.

- (27) Shi, Y.; Duan, Y. H.; Ji, Y. Y.; Wang, Z. L.; Wu, Y. R.; Gunosewoyo, H.; Xie, X. Y.; Chen, J. Z.; Yang, F.; Li, J.; Tang, J.; Xie, X.; Yu, L. F. Amidoalkylindoles as potent and selective cannabinoid type 2 receptor agonists with *in vivo* efficacy in a mouse model of multiple sclerosis. *J. Med. Chem.* **2017**, 60 (16), 7067–7083. <https://doi.org/10.1021/acs.jmedchem.7b00724>.
- (28) Blaazer, A. R.; Lange, J. H. M.; van der Neut, M. A. W.; Mulder, A.; den Boon, F. S.; Werkman, T. R.; Kruse, C. G.; Wadman, W. J. Novel indole and azaindole (pyrrolopyridine) cannabinoid (CB) receptor agonists: design, synthesis, structure–activity relationships, physicochemical properties and biological activity. *Eur. J. Med. Chem.* **2011**, 46 (10), 5086–5098. <https://doi.org/10.1016/j.ejmech.2011.08.021>.
- (29) Cooper, A. G.; MacDonald, C.; Glass, M.; Hook, S.; Tyndall, J. D. A.; Vernall, A. J. Alkyl indole-based cannabinoid type 2 receptor tools: exploration of linker and fluorophore attachment. *Eur. J. Med. Chem.* **2018**, 145, 770–789. <https://doi.org/10.1016/j.ejmech.2017.11.076>.
- (30) Spinelli, F.; Capparelli, E.; Abate, C.; Colabufo, N. A.; Contino, M. Perspectives of cannabinoid type 2 receptor (CB2R) ligands in neurodegenerative disorders: structure–affinity relationship (SAfiR) and structure–activity relationship (SAR) studies. *J. Med. Chem.* **2017**, 60 (24), 9913–9931. <https://doi.org/10.1021/acs.jmedchem.7b00155>.
- (31) Bissantz, C.; Grether, U.; Hebeisen, P.; Kimbara, A.; Liu, Q.; Nettekoven, M.; Prunotto, M.; Röver, S.; Rogers-Evans, M.; Schulz-Gasch, T.; Ullmer, C. *Pyridine derivatives as agonists of the CB2 receptor*. U.S. Patent **9,321,727**, April 26, 2016.
- (32) Nettekoven, M.; Fingerle, J.; Grether, U.; Grüner, S.; Kimbara, A.; Püllmann, B.; Rogers-Evans, M.; Röver, S.; Schuler, F.; Schulz-Gasch, T.; Ullmer, C. Highly potent and selective cannabinoid receptor 2 agonists: initial hit optimization of an adamantyl hit series identified from high-throughput screening. *Bioorg. Med. Chem. Lett.* **2013**, 23 (5), 1177–1181. <https://doi.org/10.1016/j.bmcl.2013.01.044>.

- (33) Lucchesi, V.; Parkkari, T.; Savinainen, J. R.; Malfitano, A. M.; Allarà, M.; Bertini, S.; Castelli, F.; Del Carlo, S.; Laezza, C.; Ligresti, A.; Saccomanni, G.; Bifulco, M.; Di Marzo, V.; Macchia, M.; Manera, C. 1,2-dihydro-2-oxopyridine-3-carboxamides: the C-5 substituent is responsible for functionality switch at CB2 cannabinoid receptor. *Eur. J. Med. Chem.* **2014**, *74*, 524–532. <https://doi.org/10.1016/j.ejmech.2013.10.070>.
- (34) Chicca, A.; Arena, C.; Bertini, S.; Gado, F.; Ciaglia, E.; Abate, M.; Digiacomo, M.; Lapillo, M.; Poli, G.; Bifulco, M.; Macchia, M.; Tuccinardi, T.; Gertsch, J.; Manera, C. Polypharmacological profile of 1,2-dihydro-2-oxo-pyridine-3-carboxamides in the endocannabinoid system. *Eur. J. Med. Chem.* **2018**, *154*, 155–171. <https://doi.org/10.1016/j.ejmech.2018.05.019>.