Bioorganic & Medicinal Chemistry Letters 21 (2011) 5625–5629

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Structure selectivity relationship studies of 17-cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(4′-pyridyl)carboxamido]morphinan derivatives toward the development of the mu opioid receptor antagonists

Yunyun Yuan [a], Orgil Elbegdorj [a], Jianyang Chen [a], Shashidhar K. Akubathini [a], Irina O. Beletskaya [b], Dana E. Selley [b], Yan Zhang [a,*]

[a] Department of Medicinal Chemistry, Virginia Commonwealth University, Biotech I, 800 E. Leigh Street, Richmond, VA 23298, United States
[b] Department of Pharmacology and Toxicology, Virginia Commonwealth University, 410 North 12th Street, Richmond, VA 23298, United States

ARTICLE INFO

Article history:
Received 25 April 2011
Revised 7 June 2011
Accepted 10 June 2011
Available online 18 July 2011

Keywords:
Mu opioid receptor
Antagonist
NAP
Structure selectivity relationship

ABSTRACT

Mu opioid receptor antagonists have been applied to target a variety of diseases clinically. The current study is designed to explore the structure selectivity relationship (SSR) of 17-cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(4′-pyridyl)carboxamido]morphinan (NAP), a lead compound identified as a selective mu opioid receptor antagonist based on the previous study. Among a series of NAP derivatives synthesized, compounds **6** (NMP) and **9** (NGP) maintained comparable binding affinity, selectivity and efficacy to the lead compound. Particularly, the mu opioid receptor selectivity over kappa opioid receptor of NGP was considerably enhanced compared to that of NAP. Overall, the preliminary SSR supported our original hypothesis that an alternate 'address' domain may exist in the mu opioid receptor, which favors the ligands carrying a hydrogen bond acceptor and an aromatic system to selectively recognize the mu opioid receptor.

Published by Elsevier Ltd.

Opioid receptor selective antagonists not only serve as important pharmacological probes in opioid receptor structural characterization and functional studies of opioid agonists, but also exhibit a variety of biological activities to treat different disorders and diseases. For example, nalorphine (Fig. 1), the first important mu opioid receptor (MOR) antagonist, was developed as opiate antidote in 1954.[1] It was also combined with morphine for pain management to abolish respiratory depression. Since nalorphine also possesses evident kappa opioid receptor (KOR) agonism, it was eventually replaced by naloxone,[2] the first neutral opioid antagonist. Naloxone has a higher affinity for MOR and KOR over δ opioid receptor (DOR) (K_i value ratios, $\delta/\mu \approx 96$, $\delta/\kappa \approx 69$).[3] However, due to its extremely short duration of action, naloxone is primarily used to treat opiate overdose. Later on, naltrexone[4] was identified and showed greater potency and longer duration of action than naloxone.[5] Those features of naltrexone are advantageous in its application to treat opiate dependence where a long-term opioid receptor blockade is preferred. Naltrexone is now being used as an adjunct therapy in opiate addiction management and for treating alcohol dependence as well.[6–13] Apart from the above utilities, β-funaltrexamine (β-FNA), an irreversible MOR antagonist, showed dose-dependent inhibition of cytokine-induced nitric-oxide synthase (iNOS) expression, which provides insights to treat and prevent brain pathologies associated with neuroinflammation.[14] CTAP, one of the most highly selective, reversible and peptidic MOR antagonists, was also found to block interleukin-6 (IL-6) fever.[15] KOR antagonists 5′-guanidinonaltrindole (GNTI), nor-binaltorphimine (norBNI), and (3*R*)-7-hydroxy-*N*-((1*S*)-1-{[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide (JDTic) were demonstrated to have antidepressant and anxiolytic activity.[16–21] It was also suggested that kappa antagonists may find a place to treat alcoholism,[22–24] cocaine[25–28] and nicotine[29] addictions. As a selective DOR antagonist, naltrindole (NTI) was reported to be able to attenuate the discriminative stimulus properties of cocaine,[30] reduce both alcohol and saccharin intake in rats bred for alcohol preference.[31]

Because the addiction/abuse liability of many opiates and alcoholism primarily involves MOR agonism,[32] naltrexone represents a potentially viable strategy to treat alcohol and opiate addiction. However, naltrexone is subjected to significant first pass metabolism.[33] Moreover, compared with the high selectivity of GNTI for KOR (K_i value ratios, $\mu/\kappa \approx 120$, $\delta/\kappa \approx 250$)[34] and NTI for DOR ($K_i$ value ratios, $\mu/\delta \approx 152$, $\kappa/\delta \approx 276$),[35] naltrexone only has a moderate selectivity for MOR over KOR (K_i value ratios, $\delta/\mu \approx 450$, $\kappa/\mu \approx 20$).[36] Another commercial available ligand cyprodime[37] carries a moderate selectivity for the MOR over the DOR and KOR (K_i value ratios are $\kappa/\mu \approx 45$, $\delta/\mu \approx 40$) while it has much lower affinity for the MOR than naloxone and naltrexone, which

* Corresponding author. Tel.: +1 804 828 0021; fax: +1 804 828 7625.
E-mail address: yzhang2@vcu.edu (Y. Zhang).

0960-894X/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2011.06.135

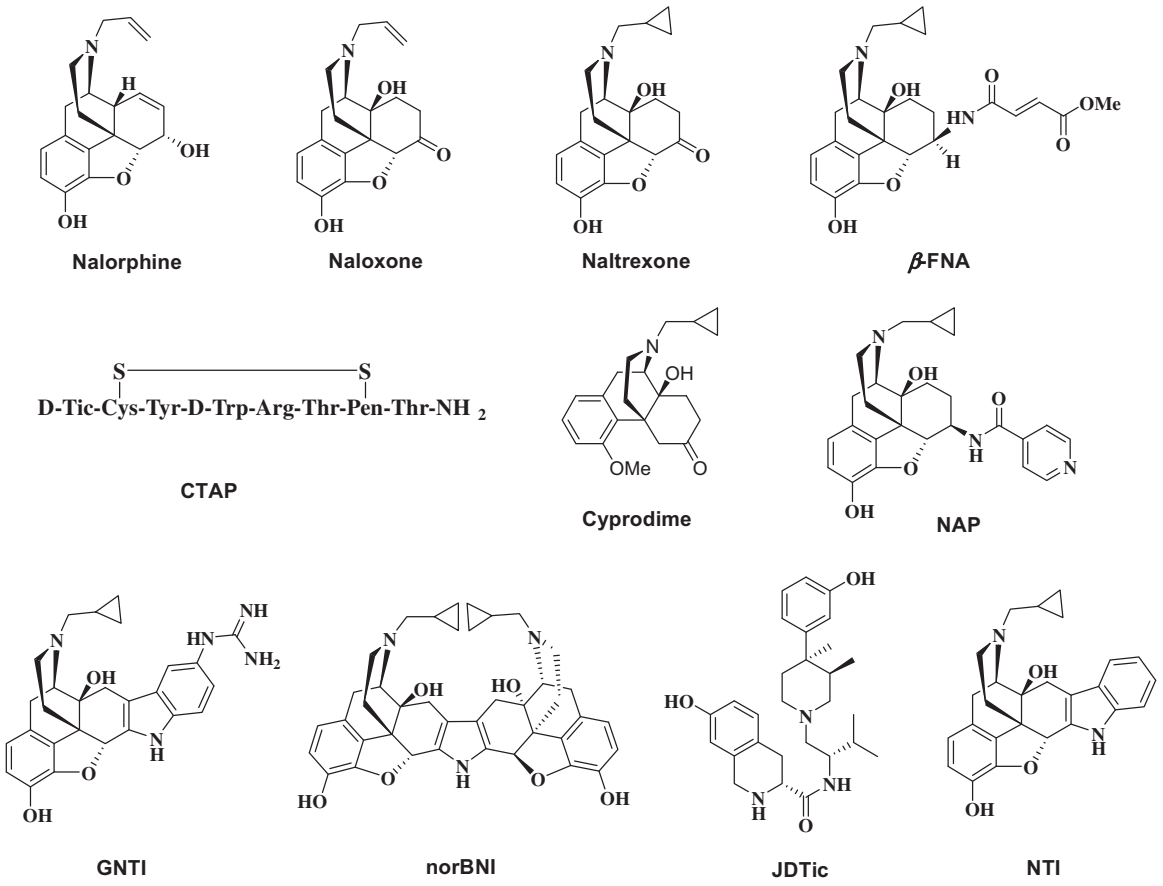


Figure 1. Examples of opioid receptor modulators.

generally limits its application.[38] Hence, the need to develop small molecule MOR antagonists with enhanced selectivity for alcohol and opiate addiction treatment still remains.

Based on our previous studies, a derivative of naltrexone, 17-cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[(4′-pyridyl)carboxamido]morphinan (NAP, Fig. 2) was identified as a potent and selective MOR antagonist.[36] Moreover, it was suggested that an alternate 'address' domain may be present in MOR, which may recognize a ligand (e.g., NAP) bearing an aromatic system and a hydrogen bond acceptor to achieve high MOR binding affinity and selectivity.[36,39] The docking study of NAP to the homology model of MOR also showed that the nitrogen atom on the pyridyl ring may serve as a hydrogen bond acceptor.[36] Further pharmacological characterization of NAP indicated that it can act as a competitive, high affinity MOR antagonist while carrying low efficacy at the DOR and KOR.[40] This information encouraged us to apply NAP as the lead for our next generation of molecular design for the development of MOR selective neutral antagonists. The aim of the current study is to further explore the structure selectivity relationship (SSR) of NAP for its MOR selectivity. Ten derivatives with different modifications toward 4-pyridyl side chain in NAP were designed and synthesized based on the following considerations: (1) the electronic/steric character of the aromatic ring system; (2) the amide spacer between the C(6) side chain and the morphinan skeleton; (3) the necessity of an aromatic system. Biological evaluations of this series of derivatives were then carried out. Two ligands with comparable binding affinity, selectivity and efficacy to NAP were identified and selected for future in vivo study to further characterize their pharmacological profiles.

Using naltrexamine as the starting material, the syntheses of these designed ligands were straightforward. First, the stereo-

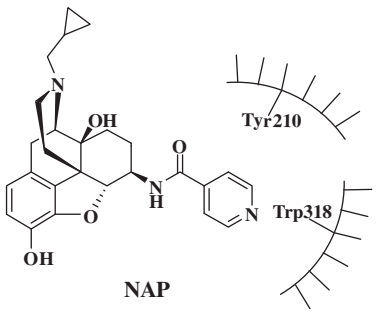
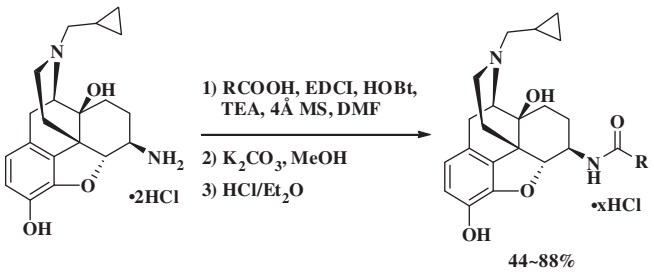


Figure 2. NAP and its interaction with the proposed alternative MOR 'address' domain.

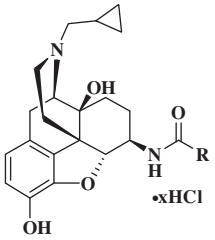


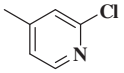
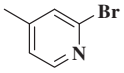
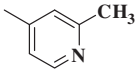
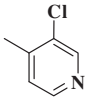
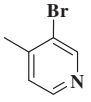
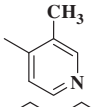
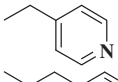
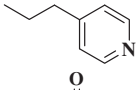
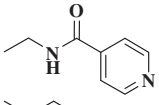
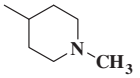
Scheme 1. Synthetic route for NAP derivatives.

selective synthesis of 6β-naltrexamine dihydrocholoride salt was successfully achieved according to the literature.[41] Then a variety of acids, either commercially available or prepared following reported protocols (see Supplementary data), were coupled with 6β-naltrexamine dihydrochloride salt via EDCI/HOBt method. The 6-monosubstituted target compounds were eventually obtained with reasonable yields after treating the coupling mixture under basic condition (Scheme 1).

The competitive radioligand binding assay was first applied to determine the binding affinity and selectivity of these NAP derivatives using monocloned opioid receptor expressing CHO cells. [^{3}H]naloxone, [^{3}H]NTI, and [^{3}H]norBNI were used to label MOR, DOR and KOR, respectively. Table 1 shows that all NAP derivatives retained subnanomolar affinity for the MOR, while there were some dramatic selectivity differences (MOR over KOR and DOR, respectively) for some of them. Because the binding pocket in both MOR and KOR can form aromatic stacking interaction with the ligand,[36] most of the derivatives displayed very low selectivity for MOR over KOR, except for compounds **6** (NMP) and **9** (NGP). Introduction of substitutions with different electronic and steric characteristics

Table 1
The binding affinity and selectivity of NAP derivatives (n = 3)[a]



Compd	R	K_i ± SEM (nM)			Selectivity ratio	
		MOR	KOR	DOR	κ/μ	δ/μ
NTX	N/A	0.26 ± 0.02	5.15 ± 0.26	117.06 ± 8.94	20	450
β-FNA	N/A	0.41 ± 0.04	0.94 ± 0.05	27.78 ± 4.60	2	68
CTAP	N/A	2.02 ± 0.71	1012.7 ± 174.8	1441.0 ± 106.1	501	713
NAP[b]		0.37 ± 0.07	60.72 ± 5.58	277.51 ± 7.97	163	747
1		0.10 ± 0.04	0.15	602.2 ± 22.33	1.5	6110.7
2		0.63 ± 0.18	0.18 ± 0.03	173.7 ± 134.9	0.3	276.0
3		0.39 ± 0.20	0.58 ± 0.12	90.1 ± 17.1	1.5	232.1
4		0.67 ± 0.28	19.93 ± 7.66	502.4 ± 70.1	29.6	745.8
5		0.45 ± 0.13	2.83 ± 0.60	128.0 ± 61.2	6.3	284.7
6 (NMP)		0.58 ± 0.25	96.7 ± 12.2	273.6 ± 1.81	166.1	469.9
7		0.85 ± 0.16	9.01 ± 0.61	865.6 ± 135.2	10.6	1016.9
8		0.57 ± 0.41	1.65 ± 0.19	294.0 ± 75.4	2.9	516.1
9 (NGP)		0.73 ± 0.59	203.2 ± 67.0	526.1 ± 78.3	277.6	718.8
10		0.87 ± 0.38	6.49 ± 2.36	2586 ± 1704	7.5	2968.1

[a] The average K_i values (n = 3) are reported ± standard error of the means (SEM) for each individual compound. Naltrexone, β-FNA, and CTAP were tested along as controls under the same conditions.
[b] Published data[36] using the same protocol.

onto the pyridyl ring did not influence MOR binding affinity very much, indicating non-affected hydrogen-bonding and aromatic stacking interactions. Yet the selectivity of MOR over KOR was achieved for compound **6** through a decreased KOR affinity. The observation that m*eta*-substituted compounds **4**, **5**, and **6** were less selective than their *ortho*-substituted counterparts **1**, **2**, and **3** for KOR (K_i value ratios, **4**/**1** ≈ 133, **5**/**2** ≈ 16, **6**/**3** ≈ 167) suggests an impaired aromatic stacking interaction between ligands and KOR caused by the steric hindrance of the *meta*-substitution. Adding one or two methylene group(s) between the C(6) side chain and the morphinan skeleton, as seen in compounds **7** and **8**, did not significantly affect on MOR affinity, but the selectivities for MOR over KOR were decreased 15- and 56-fold, respectively, compared to NAP. Whereas a considerable selectivity improvement for MOR over KOR was observed as the amide spacer went one atom longer (compound **9** vs **8**). Taken together, the effect of the amide spacer on MOR selectivity over KOR implies the aromatic stacking interaction between MOR and ligands is more flexible, while it prefers a certain distance for KOR binding. As the hydrogen bonding is a crucial interaction in MOR binding mode, the replacement of an aromatic system with a saturated cyclic ring (compound **10**) still leads to high affinity for MOR with relatively lower affinity to KOR. Actually, the nitrogen atom in the piperidinyl ring is a much stronger hydrogen acceptor than the nitrogen atom in the pyridyl ring.

Due to a lack of possible hydrogen bonding between the C(6) side chain moiety and DOR, as well as the non-aromatic binding locus to which the C(6) side chain pointed in DOR,[36] MOR over DOR selectivity of NAP derivatives is much easier to achieve compared to MOR over KOR selectivity. Among these NAP derivatives, only compounds **2**, **3**, and **5** showed a slight increase in DOR binding affinity, yet the selectivity ratios of MOR over DOR for all the derivatives are still above 200-fold. Furthermore, compound **10** appeared to be least potent for DOR in this series. Its binding affinity is only about 10% of that of NAP, which means the introduction of a hydrophilic moiety is unfavorable within the hydrophobic environment of DOR binding pocket.

Because hydrogen bonding plays an essential role among the interactions between ligands and MOR, all the NAP derivatives, which contain a hydrogen bond acceptor in each of the structures, still retained the high affinity for MOR (at the subnanomolar level). The introduction of halogen atoms or methyl group onto the aromatic ring, different distances between C(6) and the morphinan skeleton, and even the replacement of the aromatic system with a saturated cyclic system are all well tolerated for MOR binding. However, these modifications affected KOR binding affinity more profoundly either due to the weakened or the enhanced aromatic stacking interaction, which yields the varied MOR selectivity over KOR. As for DOR, the lacking of hydrogen bonding and aromatic stacking interaction in its binding pocket guaranteed all the NAP derivatives had high selectivity for MOR over DOR.

To determine whether each new ligand acts as an agonist, partial agonist, or antagonist of MOR, the ^{35}S-GTP[γS] binding assay was performed on the MOR-expressing CHO cells by determining its efficacy for G-protein activation relative to a full agonist at the MOR (Table 2). The results were interpreted as the relative efficacy of each new ligand to the full agonist DAMGO in the ability to produce the stimulation. Naltrexone (NTX; 0.1–100 nM) and CTAP (1–300 nM) were tested as controls, and produced minimal stimulation such that the concentration–effect curves could not be fit using non-linear regression (data not shown). Their maximal stimulation, observed at 100 nM of each ligand (Table 2), was less than 10% relative to DAMGO (100 ± 5.4%). As shown in Table 2, all NAP derivatives showed partial agonism for MOR in this in vitro assay, with compounds **8** and **9** displaying the lowest relative efficacy. It appeared that introducing some substitution at the *meta* position to the nitrogen atom of the pyridine ring of the side chain would lead to higher efficacy, as shown by compound **1**, **2**, and **3**. The results from our modeling study[36] indicated that the nitrogen atom might act as a hydrogen bonding acceptor to interact with amino acid residues Trp319 and/or Tyr210. Considering conformation change of trans-membrane helix 7 of the G-protein Coupled Receptors (where Trp319 locates in MOR) might induce the activation of the receptor[42,43] such adjacent substitution may further disturb the hydrogen bonding interaction of the pyridine ring system with Trp318 to induce potential agonism. The fact that compound **4**, **5**, and **6** carried similar substitution at ortho position while showing no significant change in their efficacy compared with NAP is in agreement with this hypothesis. On the other hand, it seemed that such hydrogen bonding might not be sensitive to the spacer of NAP analogues since compound **7**, **8**, and **9** also showed comparable agonism to that of NAP. Naturally the current effort is focusing on the integration of structural features from compound **6** and **9** to further test the above hypothesis and to hopefully further reduce the agonism of the next generation ligands.

Table 2
The efficacy and potency of NAP derivatives in ^{35}S-GTP[γS]-binding assay in MOR expressing CHO cells (n = 3)

Compd	EC_{50} ± SEM (nM)	% Max of DAMGO ± SEM
DAMGO	45.06 ± 6.63	100.0 ± 6.2
NTX	N.D.	7.75 ± 0.20 at 100 nM
CTAP	N.D.	1.99 ± 0.92 at 100 nM
NAP[a]	1.14 ± 0.38	22.72 ± 0.84
1	0.83 ± 0.03	58.20 ± 1.47
2	1.19 ± 0.57	50.80 ± 2.85
3	1.13 ± 0.14	43.32 ± 2.80
4	2.32 ± 1.76	24.86 ± 1.83
5	1.31 ± 0.59	23.23 ± 0.35
6 (NMP)	1.52 ± 0.26	30.63 ± 0.55
7	2.32 ± 0.64	32.78 ± 1.80
8	1.46 ± 0.11	22.62 ± 1.24
9 (NGP)	2.84 ± 0.53	22.62 ± 0.66
10	5.08 ± 0.43	37.24 ± 0.97

[a] Published data[36] using the same protocol. N.D. could not be determined.

Based on the binding and functional assay results, two NAP derivatives, namely compounds **6** (NMP) and **9** (NGP), were selected for further pharmacological evaluation. Both NMP and NGP showed sub-nanomolar binding affinity to the MOR. Compared with NAP, NMP's selectivity for the MOR was similar while the selectivity of NGP for MOR over KOR was considerably enhanced. Their efficacy at the MOR was also similar to that of NAP. Based on the recent report of the potential peripheral nervous system (PNS) activity of NAP,[39] it would be interesting to exploit both NMP and NGP for their application in PNS diseases treatment.

In summary, the important role of MOR antagonists in both the opioid receptor study and their clinical values makes the development of a non-peptidic, potent, selective, neutral, and reversible MOR antagonists highly desirable. A series of NAP derivatives were synthesized to explore its structure selectivity relationships (SSR) in an effort to develop such a MOR antagonist. The preliminary SSR observed for NAP derivatives provides solid evidence to support our hypothesis that hydrogen bonding and aromatic stacking interactions occurring between an alternative 'address' domain in MOR and ligands may lead to MOR selectivity over DOR and KOR. The currently ongoing studies of the next generation of NAP derivatives might shed light onto this aspect more comprehensively. Moreover, some of these derivatives exhibited considerable efficacies as MOR partial agonists in the in vitro study. These ligands may potentially be applied to treat opioid dependence and addiction.

Acknowledgments

We are grateful for the generous gift of the opioid receptor expressed CHO cell lines from Dr. Lee-Yuan Liu-Chen at Temple

University and Dr. Ping-Yee Law at University of Minnesota. The research was supported by PHS grant DA024022.

Supplementary data

Supplementary data (chemical synthesis and biological screening) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.06.135.

References and notes

1. Lasagna, L.; Beecher, H. K. *J. Pharmacol. Exp. Ther.* **1954**, *112*, 356.
2. Foldes, F. F.; Lunn, J. N.; Moore, J.; Brown, I. M. *Am. J. Med. Sci.* **1963**, *245*, 57.
3. Peng, X.-M.; Knapp, B. I.; Bidlack, J. M.; Neumeyer, J. L. *J. Med. Chem.* **2007**, *50*, 2254.
4. Blumberg, H.; Pachter, I. J.; Matossiank, Z. U.S. Pat. 3332950, **1967**.
5. Martin, W. R.; Jasinski, D. R.; Mansky, P. A. *Arch. Gen. Psychiat.* **1973**, *28*, 784.
6. Comer, S. D.; Sullivan, M. A.; Hulse, G. K. *Expert Opin. Investig. Drugs* **2007**, *16*, 1285.
7. Adi, Y.; Juarez-Garcia, A.; Wang, D.; Jowett, S.; Frew, E.; Day, E.; Bauliss, S.; Roberts, T.; Burls, A. *Health Technol. Assess* **2007**, *11*, 1.
8. Johansson, B. A.; Berglund, M.; Lindgren, A. *Addiction* **2006**, *101*, 491.
9. Kerkhof, A. J. *Eur. Neuropsychopharmaol.* **2006**, *16*, 311.
10. Garbutt, J. C. *Curr. Pharm. Des.* **2010**, *16*, 2091.
11. Ray, L. A.; Chin, P. F.; Miotto, K. C. N. S. *Neurol. Disord. Drug Targets* **2010**, *9*, 13.
12. Pettinati, H. M.; O'Brien, C. P.; Rabinowitz, A. R.; Wortman, S. P.; Oslin, D. W.; Kampman, K. M.; Dackis, C. A. *J. Clin. Psychopharmacol.* **2006**, *26*, 610.
13. Littleton, J.; Zieglgänsberger, W. *Am. J. Addict.* **2003**, *12*, s3.
14. Davis, R. L.; Buck, D. J.; Saffarian, N.; Mohan, S.; DeSilva, U.; Fernando, S. C.; Stevens, C. W. *J. Neuroimmune Pharmacol.* **2008**, *3*, 150.
15. Benamar, K.; Geller, E. B.; Adler, M. W. *Life Sci.* **2002**, *70*, 2139.
16. Mague, S. D.; Pliakas, A. M.; Todtenkopf, M. S.; Tomasiewicz, H. C.; Zhang, Y.; Stevens, W. C., Jr.; Jones, R. M.; Portoghese, P. S.; Carlezon, W. A., Jr. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 323.
17. Marin, S.; Marco, E.; Biscaia, M.; Fernandez, B.; Rubio, M.; Guaza, C.; Schmidhammer, H.; Viveros, M. P. *Pharmacol. Biochem. Behavior* **2003**, *74*, 649.
18. Zhang, H.-N.; Shi, Y.-G.; James, J. H.; Watson, S. J.; Ko, M.-C. *Eur. J. Pharmacol.* **2007**, *570*, 89.
19. Knoll, A. T.; Meloni, E. G.; Thomas, J. B.; Carroll, F. I.; Carlezon, W. A., Jr. *J. Pharmacol. Exp. Ther.* **2007**, *323*, 838.
20. Carr, G. V.; Bangasser, D. A.; Bethea, T.; Young, M.; Valentino, R. J.; Lucki, I. *Neuropsychopharmacology* **2010**, *35*, 752.
21. Beardsley, P. M.; Pollard, G. T.; Howard, J. L.; Carroll, F. I. *Psychopharmacology* **2010**, *210*, 189.
22. Sandi, C.; Borrell, J.; Guaza, C. *Life Sci.* **1990**, *46*, 1119.
23. Walker, B. M.; Koob, G. F. *Neuropsychopharmacology* **2008**, *33*, 643.
24. Walker, B. M.; Zorrilla, E. P.; Koob, G. F. *Addiction Biol.* **2011**, *16*, 116.
25. Kuzmin, A. V.; Gerrits, M. A. F. M.; Van Ree, J. M. *Eur. J. Pharmacol.* **1998**, *358*, 197.
26. Carey, A. N.; Borozny, K.; Aldrich, J. V.; McLaughlin, J. P. *Eur. J. Pharmacol.* **2007**, *569*, 84.
27. Raffa, R. B.; Stagliano, G. W.; Ross, G.; Powell, J. A.; Phillips, A. G.; Ding, Z.; Rawls, S. M. *Brain Res.* **2008**, *1193*, 51.
28. Aldrich, J. V.; Patkar, K. A.; McLaughlin, J. P. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 18396.
29. Jackson, K. J.; Carroll, F. I.; Negus, S. S.; Damaj, M. I. *Psychopharmacology* **2010**, *210*, 285.
30. Suzuki, T.; Mori, T.; Funada, M.; Misawa, M.; Nagase, H. *Eur. J. Pharmacol.* **1994**, *263*, 207.
31. Krishnan-Sarin, S.; Jing, S.-L.; Kurtz, D. L.; Zweifel, M.; Portoghese, P. S.; Li, T.-K. *Psypharmcology* **1995**, *120*, 177.
32. Schulteis, G.; Martinez, J. L., Jr. *Psypharmcology* **1990**, *100*, 102.
33. Verebey, K. *NIDA Research Monograph* **1980**, *28*, 147.
34. Jones, R. M.; Hjorth, S. A.; Schwartz, T. W.; Portoghese, P. S. *J. Med. Chem.* **1998**, *41*, 4911.
35. Portoghese, P. S.; Sultana, M.; Nagase, H.; Takemori, A. E. *J. Med. Chem.* **1988**, *31*, 281.
36. Li, G.; Aschenbach, L. C.; Chen, J.-Y.; Cassidy, M. P.; Stevens, D. L.; Gabra, B. H.; Selley, D. E.; Dewey, W. L.; Westkaemper, R. B.; Zhang, Y. *J. Med. Chem.* **2009**, *52*, 1416.
37. Schmidhammer, H.; Burkard, W. P.; Eggstin-Aeppli, L.; Smith, C. F. C. *J. Med. Chem.* **1989**, *32*, 418.
38. Marki, A.; Monory, K.; Otvos, F.; Toth, G.; Krassnig, R.; Schmidhammer, H.; Traynor, J. R.; Roques, B. P.; Maldonado, R.; Borsodi, A. *Eur. J. Pharmacol.* **1999**, *383*, 209.
39. Li, G.; Aschenbach, L. C. K.; He, H.-J.; Selley, D. E.; Zhang, Y. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1825.
40. Li, G.; Yuan, Y.; He, H.; Stevens, D. L.; Kozak, P.; Scoggins, K. L.; Mitra, P.; Gerk, P. M.; Selley, D. E.; Dewey, D. L.; Zhang, Y. *ACS Chem. Neurosci.* 2011, Epub May 6, as Articles ASAP.
41. Sayre, L. M.; Portoghese, P. S. *J. Org. Chem.* **1980**, *45*, 3366.
42. Park, J. H.; Scheerer, P.; Hofmann, K. P.; Choe, H. W.; Ernst, O. P. *Nature* **2008**, *454*, 183.
43. Simpson, L. M.; Wall, I. D.; Blaney, F. E.; Reynolds, C. A. *Proteins* **2011**, *79*, 1441.