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Redefining the structure–activity relationships of 2,6-methano-3-benzazocines. Part 6: Opioid receptor binding properties of cyclic variants of 8-carboxamidocyclazocine

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Abstract—A series of 7,8- and 8,9-fused pyrimidinone, aminopyrimidine and pyridone derivatives of 8-carboxamidocyclazocine (8-CAC) have been prepared and evaluated in opioid receptor binding assays. Targets were designed to corroborate a pharmacophore hypothesis regarding the bioactive conformation of the carboxamide of 8-CAC. In addition to the results from this study strongly supporting this pharmacophore hypothesis, a number of novel compounds with high affinity to opioid receptors have been identified.
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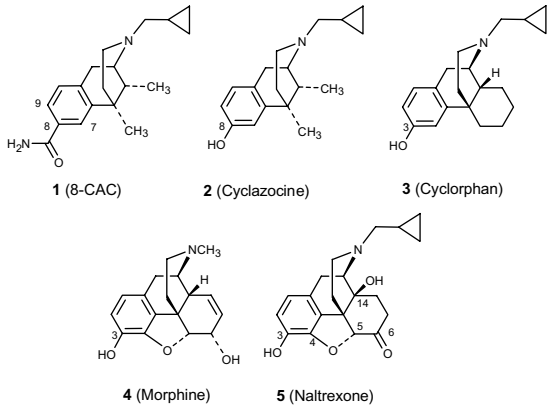
1. Introduction

We reported our observation in 2001[1] that the prototypic phenolic OH group of certain opioids can be replaced by a carboxamide group ($CONH_2$) and retain high affinity binding to opioid receptors. For example, binding affinities for 8-carboxamidocyclazocine (8-CAC, **1**) were K_i (nM) = 0.31, 5.2 and 0.06 for μ, δ and κ, respectively, while for cyclazocine (**2**),[2] the K_i values were within twofold. For other 2,6-methano-3-benzazocines (a.k.a. benzomorphans)[1] as well as quadracyclic morphinans [e.g., cyclorphan (**3**)],[3] the ratio of binding affinities [K_i ($CONH_2$)/K_i (OH)] for μ and κ receptors was also near unity in most cases. However, for pentacyclic 4,5α-epoxymorphinans [e.g., morphine (**4**) and naltrexone (**5**)], that ratio was much higher indicating the $CONH_2$ derivative displayed much lower affinity than its corresponding phenolic OH counterpart. For example, the K_i ($CONH_2$)/K_i (OH) ratio for μ was 35 and 7 for the morphine and naltrexone pairs, respectively.[4] For the $CONH_2$ partner **6** of naltrexone (**5**), we observed that the most stable conformation of the unbound ligand was that represented by **6** which is stabilized via intramolecular H-bonding of the furan O to the carboxamido NH.[5] Since this compound had much lower binding affinity than would be predicted from SAR studies, we reasoned that the putative bioactive conformation was **7** rather than **6** and that **6** must pay an energy penalty to adopt the putative bioactive conformation **7** resulting in lower affinity. For 2,6-methano-3-benzazocines and morphinans [e.g., 8-CAC (**1**)] the putative carboxamide bioactive conformation (as shown in **1**) is among many a number of stable conformations and is one that can be easily attained since there is no barrier created by H-bonding to a neighboring ether bridge.



Keywords: Opioid; SAR; Pharmacophore hypothesis.
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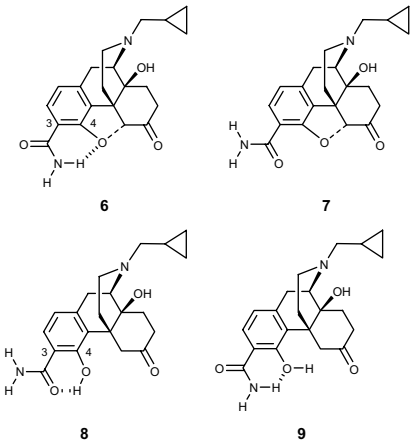
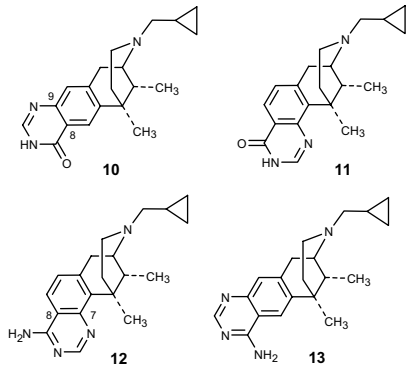


To test this conformational hypothesis, we designed, prepared and evaluated the 4-hydroxy-3-caboxamidonaltrexone analogue **8** in which the newly introduced 4-OH was found to stabilize the carboxamide in the putative bioactive conformation shown in **8** and not the alternative conformation **9**.[5] Compound **8** displayed extraordinarily high affinity for μ receptors (K_i = 0.052 nM) and high affinity for δ and κ receptors. When compared to **6**, compound **8** had binding affinities 14-, 212-, and 50-fold higher against μ, δ, and κ, respectively. We also showed that the benefit of the 4-OH was to stabilize the putative bioactive conformation and not through direct contact with the receptor.

We now report additional studies where the overall goal was to confirm or refute this conformational hypothesis. Objectives to meet this goal were (a) the design and preparation of analogues where we constrained the carboxamido (or surrogate) group of 8-CAC in the putative bioactive conformation **1** through covalent bonds rather than through non-covalent H-bonds as discussed above and (b) evaluation of new targets in opioid receptor binding assays. Previously reported carboxamide SAR studies revealed that the H-bond donating and accepting properties of the $CONH_2$ group were important for recognition by opioid receptors and that highly basic groups at the 8-position were not tolerated.[1,6] Keeping these SAR trends in mind, we designed the 8,9- and 7,8-fused 8-CAC derivatives **10** and **12**, respectively, as mimics of the putative carboxamide bioactive conformation as shown is **1**. We also made derivatives **11** and **13** as controls (i.e., forcing the carboxamide into a conformation believed not to be the bioactive one). Further validation of our pharmacophore hypothesis would be evident if **10** and **12** had higher affinity for opioid receptors than **11** and **13**, respectively.



2. Results

2.1. Chemistry

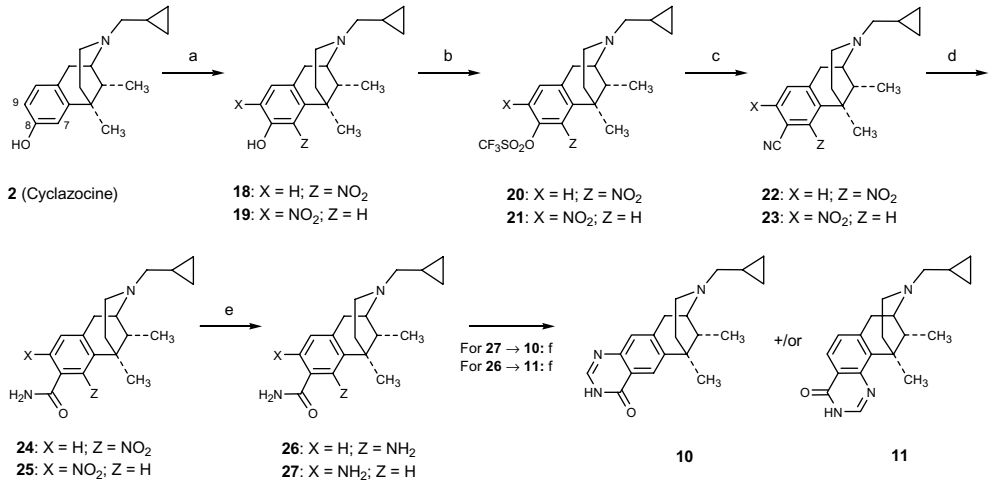
The first step in the syntheses of racemic target compounds **10**–**13** involved the nitration of cyclazocine (**2**) under standard conditions to provide a mixture of nitro derivatives **18** and **19** easily separated using silica gel flash chromatography (Scheme 1). Compounds **18** and **19** were treated with $PhN(SO_2CF_3)_2$ and triethylamine in CH_2Cl_2 to provide triflate esters **20** and **21**, respectively, in high yields. Compounds **20** and **21** were then converted to nitriles **22** and **23**, respectively, by the use of $Zn(CN)_2$ and $Pd(PPh_3)_4$ in DMF under microwave radiation. Partial hydrolysis of nitriles **22** and **23**, using KOH and *t*-BuOH gave carboxamides **24** and **25**, respectively. Subsequent reduction of the nitro groups of **24** and **25** using standard conditions provided the corresponding amines **26** and **27** which upon treatment with formic acid under microwave radiation provided the target derivatives **11** and **10**, respectively.

Target compound **12** was prepared as shown in Scheme 2. Carboxamide derivative **26** (from Scheme 1) was dehydrated using $POCl_3$ and pyridine under microwave radiation to provide nitrile intermediate **28** which was then treated with $HC(OMe)_3$ to provide **29**. Compound **29** was treated with ammonia to give the target pyrimidine derivative **12**. To assess the effect of N-substitution of target **12**, we made the (4′-phenyl)-phenethyl and benzyl derivatives **14** and **15** by treating intermediate **29** with (4′-phenyl)-phenethylamine and benzylamine, respectively.

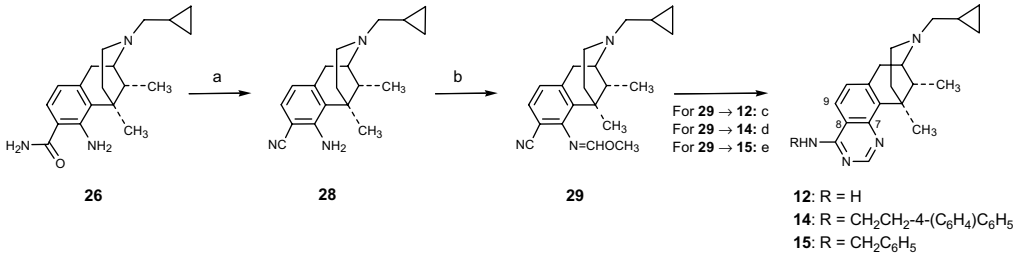
Target **13** having the same aminopyrimidine ring fusion as **12** but at the 8,9-positions was made using a slight modification of the methodology just described. As shown in Scheme 3 intermediate **23** (from Scheme 1) was reduced using hydrogen and 10% Pd/C in methanol to provide intermediate **30** which was then treated with trimethyl orthoformate to provide imidate **31**. Exposure of **31** to ammonia and methanol under microwave radiation provided target **13**.

As shown in Scheme 4, we also made the (4′-phenyl)-phenethyl and benzyl derivatives **16** and **17**, respectively, by treating intermediate **32** with (4′-phenyl)-phenethylamine and benzylamine, respectively. Using conditions similar to those previously reported,[7] compound **32** was made by exposing **27** (from Scheme 1) to $POCl_3$ and DMF under microwave radiation.

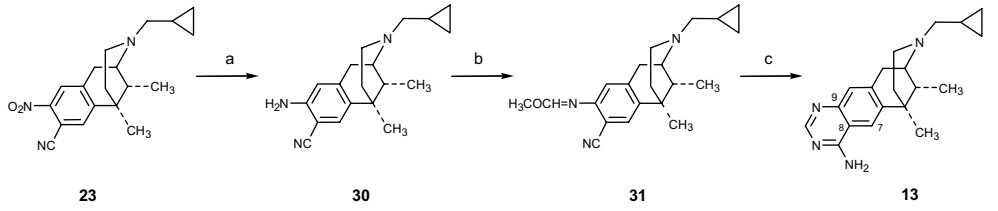
Lastly, novel fused 8,9-pyridinone analogue **37** was prepared as shown in Scheme 5. Activated ester intermediate **33**[8] was treated with methoxylamine hydrochloride in pyridine to afford *N*-methoxycarboxamide **34**. Using a general method previously described for making pyridinones,[9] intermediate **34** was lithiated at the sterically



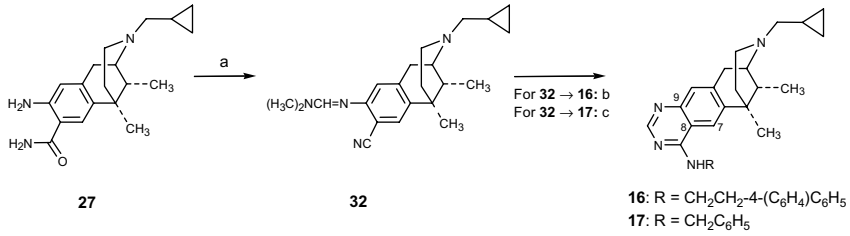
Scheme 1. Reagents and conditions: (a) 69% HNO_3, CH_3CO_2H, 25 °C; (b) $PhN(Tf)_2$, Et_3N, CH_2Cl_2, 25 °C; (c) $Zn(CN)_2$, $Pd(PPh_3)_4$, microwaves, 150 °C; (d) *t*-BuOH, KOH, 82 °C; (e) MeOH, 10% Pd/C, H_2, 25 °C; (f) 88% HCO_2H; microwaves, 120 °C.



Scheme 2. Reagents and conditions: (a) $POCl_3$, pyridine, microwaves, 100 °C; (b) $CH(OCH_3)_3$, 4 Å molecular sieves, 140 °C; (c) CH_3OH, NH_3, HOAc, microwaves, 100 °C; (d) CH_3OH, $H_2NCH_2CH_2$-4-(C_6H4)C_6H_5, HOAc, microwaves, 160 °C; (e) CH_3OH, $PhCH_2NH_2$, HOAc, microwaves, 160 °C.

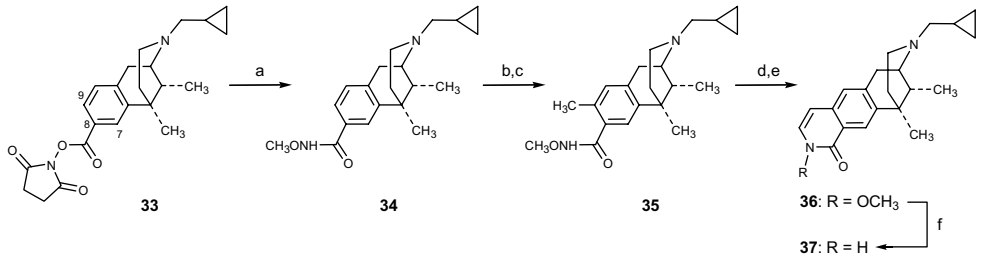


Scheme 3. Reagents and conditions: (a) 10% Pd/C, CH_3OH, H_2, 25 °C; (b) $CH(OCH_3)_3$, 4 Å molecular sieves, 140 °C; (c) CH_3OH, NH_3, microwaves, 120 °C.



Scheme 4. Reagents and conditions: (a) $POCl_3$, DMF, microwaves radiation, 100 °C; (b) CH_3CO_2H, $H_2NCH_2CH_2$-4-(C_6H_4)C_6H_5, CH_3CN, microwaves, 160 °C; (c) CH_3CO_2H, $PhCH_2NH_2$, CH_3CN, microwaves, 160 °C.

less encumbered 9-position and at nitrogen using *sec*-butyllithium in the presence of TMEDA at −20 °C. The resulting dianion was quenched with methyl iodide to provide the 9-methylated derivative **35**. Lithiation of **35** with excess *sec*-butyllithium at −78 °C followed by a DMF quench gave the *N*-methoxypyridinone derivative **36**. Titanium trichloride reduction of **36** provided target **37**.



Scheme 5. Reagents and conditions: (a) CH_3ONH_2HCl, pyr; (b) *sec*-BuLi, TMEDA, THF, −78 °C; (c) MeI; (d) *sec*-BuLi, THF, −78 °C; (e) DMF; (f) $TiCl_3$, HCl, EtOH, 100 °C, microwaves.

2.2. Biology

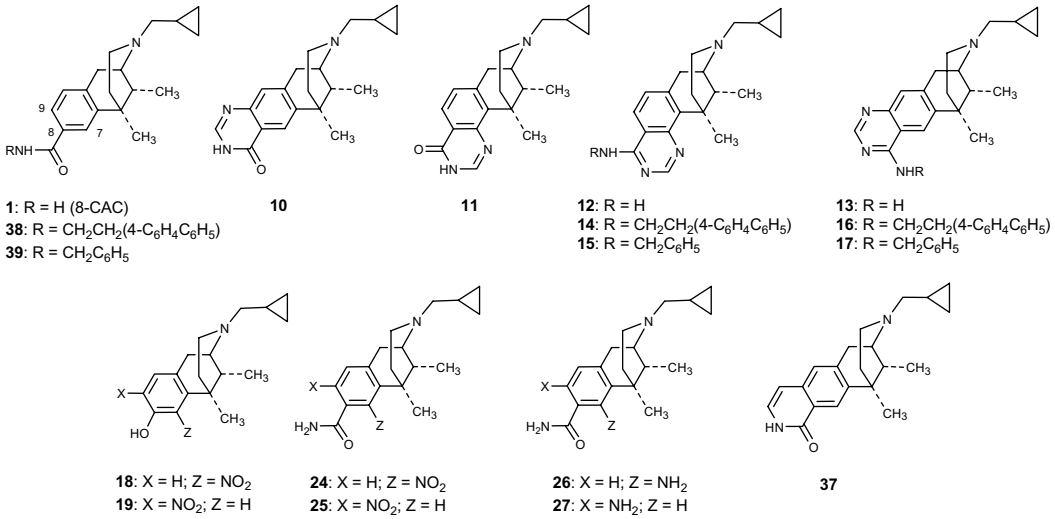
Target compounds were evaluated for their affinity and selectivity for μ, δ and κ opioid receptors stably expressed in Chinese hamster ovary (CHO) cell membranes. The details of these assays are found in the experimental section and the data are summarized in Table 1. Opioid binding affinity data for 8-CAC (**1**) and the two N-alkylated 8-CAC analogues **38** and **39** are also included. All the compounds in Table 1 are racemic. Against the δ receptor, binding affinity for all the new targets in Table 1 is low (K_i = 35 to >10,000 nM) relative to their affinities for μ and κ opioid receptors. Therefore, we focused our analysis of the data on the μ and κ receptors. For target compounds **10**–**13**, binding affinities for the μ opioid receptor ranged from very high (e.g., K_i = 0.55 nM for **12**) to very low (e.g., K_i = 890 nM for **13**). Affinity for the κ receptor was good for targets **10** and **12** with K_i values of 12 nM and 1.0 nM, respectively, while for compounds **11** and **13**, affinity was very low (K_i values of 160 nM and 560 nM, respectively). With the exception of target **14**, binding affinities of the N-substituted aminopyrimidine analogues **14**–**17** were relatively weak for μ and κ receptors (K_i = 28–88 nM and 48–240 nM, respectively). For **14**, however, affinities for μ and κ were good with K_i values of 6.9 nM and 8.6 nM, respectively. The pyridinone target **37** had high affinity μ and κ receptors (K_i values of 5.5 nM and 0.74 nM, respectively). Lastly, the binding affinities of a number of synthetic intermediates were assessed. For μ and κ receptors, 7-nitro-containing compounds **18** (K_i = 32 nM and 3.2 nM, respectively) and **24** (K_i = 20 nM and 34 nM, respectively) had reasonably good affinity, while the corresponding 9-nitro-containing compounds **19** and **25** had very poor affinities (K_i values in the range of 110–3800 nM). The 7-amino variant **26** of 8-CAC had very high affinity for μ and κ with K_i = 0.55 nM and 0.70 nM, respectively, while the affinities of its regioisomer **27** were much lower (K_i = 88 nM and 32 nM, respectively).

Three compounds, **12**, **26**, and **37**, that displayed high affinity for μ and κ receptors were characterized in a [^{35}S]GTPγS assay to assess functional activity. These data are summarized in Table 2. Due to the relatively poor binding affinity to δ receptors, these compounds were not evaluated for functional activity at δ. Target compound **12** was found to be an antagonist at both μ and κ receptors and **26** to be an antagonist at μ and moderate agonist at κ. Compound **37** displayed weak mixed agonist/antagonist properties at μ and for κ, it was an agonist. Functional activity for **38**, another highly potent in the binding assays, has been previously reported.[12,13] It is an antagonist at μ and an agonist at δ and κ receptors.

3. Discussion

For the two 8,9- and 7,8-fused-pyrimindinone targets **10** and **11**, respectively, our pharmacophore hypothesis predicts the former to have relatively high affinity for μ and κ receptors and the latter predicted to have low affinity. Against the κ receptor we do, in fact, observe a significant difference in the binding affinities of **10** and **11**. As predicted, target **10** has reasonably high affinity (K_i = 12 nM) and compound **11** has low affinity (K_i = 160 nM). Against the μ receptor, however, we do not observe the same divergence. While target **11** did, as predicted, exhibit low affinity for μ (K_i = 270 nM) target **10** did so as well (K_i = 170 nM). It may well be that the carboxamide group embedded in **10** is in the proper bioactive conformation and its poor binding affinity is due to the substantial structural change at position-9 relative to 8-CAC (9-H). In other words, unlike the κ receptor, μ poorly accommodates substitution at position-9 of 2,6-methano-3-benzaocines. There are several data points in this study that support such an argument. As shown in Table 1, the 9-nitro variants **19** and **25** of cyclazocine and 8-CAC, respectively, have much lower affinity for μ and κ receptors than their 7-nitro counterparts **18** and **24**. Also, the 9-amino variant **27** of 8-CAC displays considerable lower affinity for the receptors than its 7-amino counterpart **26**. There are data, however, that contradict this argument. Opioids with a fused 8,9-fused aminothiazole ring (2,6-methano-3-benzaocine numbering) or aminooxazole ring are reported to have high affinity for μ receptors.[10] In another study, cyclazocine derivatives with a 8,9-fused imidazole or triazole ring are also characterized by having high affinity for the μ receptor.[11] Besides one based on an invalid pharmacophore hypothesis, the only other explanation that comes to mind regarding the poor activity of **10** at the μ receptor is that the electron withdrawing imine part of the pyrimidinone ring reduces the H-bond accepting ability of the carboxamide oxygen. To test this premise, we prepared the pyridinone derivative **37** where the imine N of **10** is replaced by a non-electron withdrawing CH. Physical data that support such a premise is seen in

Table 1. Opioid receptor binding data for 7,8- and 8,9-ring fused 2,6-methano-3-benzazocines and related compounds



Compound	K_i[a] (nM ± SE)		
	[^{3}H]DAMGO (μ)	[^{3}H]Naltrindole (δ)	[^{3}H]U69,593 (κ)
1 (8-CAC)[b]	0.31 ± 0.03	5.2 ± 0.36	0.06 ± 0.001
10[c]	170 ± 6.7	780 ± 32	12 ± 0.65
11[c]	270 ± 33	2000 ± 49	160 ± 7.1
12[c]	0.55 ± 0.018	120 ± 8.5	1.0 ±0.071
13[c]	890 ± 39	>10 μM	560 ± 23
14[c]	6.9 ± 0.33	52 ± 2.6	8.6 ± 1.5
15[c]	44 ± 0.76	1500 ± 68	240 ± 1.8
16[c]	28 ± 1.9	410 ± 61	140 ± 4.4
17[c]	88 ± 7.2	1000 ± 37	48 ± 2.3
18[c]	32 ± 2.6	1900 ± 204	3.2 ± 0.14
19[c]	3800 ± 166	>10 μM	580 ± 5.4
24[c]	20 ± 1.2	220 ± 20	34 ± 1.2
25[c]	630 ± 41	730 ± 21	110 ± 11
26[c]	0.55 ± 0.029	35 ± 0.036	0.70 ± 0.036
27[c]	88 ± 5.2	2000 ± 12	32 ± 1.7
37[c]	5.5 ± 0.67	74 ± 6.8	0.74 ± 0.10
38[d]	0.30 ± 0.036	0.74 ± 0.019	1.8 ± 0.19
39[d]	27 ± 5.5	210 ± 55	36 ± 1.1

[a] See Section 5.
[b] See Ref. 13.
[c] Proton NMR, IR and MS were consistent with the assigned structures of all new compounds. C, H, and N elemental analyses were obtained for all new targets and most intermediates and were within ±0.4% of theoretical values.
[d] See Ref. 12.

the downfield chemical shift [δ 8.05 (s, 1H)] of the CH on the pyrimidinone ring of **10** relative to that [δ 7.15 (d, 1H, J = 7.0 Hz)] of the corresponding CH of **37**. Compound **37** has high affinity for the μ receptor (K_i = 5.5 nM) and is 31-fold more potent than the corresponding pyrimidinone **10**. Pyridinone **37** also has very high affinity for the κ receptor (K_i = 0.74 nM) and has 16-fold higher affinity than **10**. These results fit nicely with the premise that an electron withdrawing group at position-9 is detrimental for binding. These also support of the overall hypothesis that the carboxamide structure embedded in **37** is in the bioactive conformation. We attempted to make the analogue of **37** having a 7,8-fusion, however, we were unsuccessful using a similar method to that used to make **37**.

For the 7,8- and 8,9-fused-aminopyrimidine targets **12** and **13**, respectively, our pharmacophore hypothesis predicts **12** to have relatively high affinity for μ and κ receptors and **13** predicted to have low affinity. This is precisely what we observe. Compound **12** has K_i values of 0.55 nM and 1.0 nM against μ and κ, respectively and **13** has K_i values of 890 nM and 560 nM against μ and κ, respectively. For an aminopyrimidine surrogate of a carboxamide, these data indicate our pharmacophore hypothesis is reinforced.

In earlier SAR studies, we reported that N-substitution of the carboxamide of 8-CAC (**1**), with groups such as methyl, OH, NH_2 or phenyl greatly reduced binding affinity, however, when the substituent was a (4′-phenyl)-phenethyl group (**38**) binding affinity for μ and κ receptors was very high (K_i values of 0.30 nM and 1.8 nM, respectively).[1,12,13] We hypothesized that the *N*-(4′-phenyl)-phenethyl appendage occupies a previously unexplored hydrophobic pocket in opioid

Table 2. EC_{50} and E_{max} values for the stimulation of [^{35}S]GTPγS binding and IC_{50} and I_{max} values for the inhibition of agonist-stimulated [^{35}S]GTPγS binding to the human μ and κ opioid receptors[a]

Compound	EC_{50} (nM)	E_{max} (% maximal stimulation)	IC_{50} (nM)	I_{max} (% maximal inhibition)
μ *Opioid receptor*				
DAMGO	55 ± 7	116 ± 4	NI[b]	NI
12	NA[c]	0.64 ± 1.0	28 ± 1.1	88 ± 3.1
26	NA	4.6 ± 1.7	31 ± 6.5	93 ± 1.5
37	NA	27 ± 3.5	850 ± 270	69 ± 6.7
κ *Opioid receptor*				
U50,488	36 ± 5.0	77 ± 11	NI	NI
12	NA	6.3 ± 0.6	1300 ± 290	75 ± 4.7
26	15 ± 6.9	38 ± 3.0	NI	NI
37	60 ± 9.3	91 ± 9.8	NI	NI

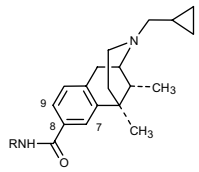
[a] See Section 5. Data are the mean values ± SEM from at least three separate experiments, performed in triplicate. For calculation of the E_{max} values, the basal [^{35}S]GTPγS binding was set at 0%. For inhibition studies, 200 nM DAMGO was used as the agonist for the μ receptor and U50,488 at final concentration of 100 nM was used for the κ receptor.

[b] NI → no inhibition.

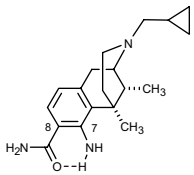
[c] NA → not applicable.

receptors.[12,13] We also made the corresponding *N*-benzyl 8-CAC analogue **39** which had much lower affinity for μ and κ receptors (K_i values of 27 nM and 36 nM, respectively).[12] To study the additivity of SAR between the 8-CAC and 7,8-fused-aminopyrimidine platforms, we prepared and evaluated analogues **14** and **15** of aminopyrimidine target **12** by appending (4′-phenyl)-phenethyl and benzyl groups to the exocyclic nitrogens which correspond to the carboxamide N of 8-CAC. Data from Table 1 reveal that the introduction of a (4′-phenyl)-phenethyl group in the 7,8-fused aminopyrimidine core (compare **12** and **14**) results in a 13- and 48-fold decrease in binding affinity against μ and κ, respectively. These data contrast the 8-CAC core μ results (compare **1** and **38**) where N-substitution with a (4′-phenyl)-phenethyl group results in comparable binding affinity; against κ there is a similar decrease (30-fold) in binding affinity. With a *N*-benzyl substituent (compare **12** and **15**), there is a 80- and 240-fold decrease in binding affinity against μ and κ, respectively. This decrease in binding affinity parallels the decrease observed upon the introduction of an *N*-benzyl group into 8-CAC (compare **1** and **39**) where an 87- and 600-fold decrease in binding affinity against μ and κ, respectively, is observed. We also made and tested the *N*-(4′-phenyl)-phenethyl and *N*-benzyl analogues in the much less active 8,9-fused-aminopyrimidine platform **13**. In contrast to the 7,8-fused system **12**, the addition of the hydrophobic appendages to **13** enhances binding affinity for μ and κ 32- and 4-fold, respectively, for the (4′-phenyl)-phenethyl derivative **16** and 10- and 12-fold, respectively, for the benzyl analogue **17**. Absolute binding affinities, however, are relatively weak.

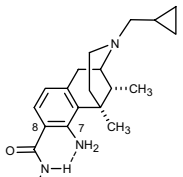
Examination of binding data for synthetic intermediates **18**, **19**, and **24**–**27**, revealed that nitro-substitution on the 7- or 9-positions of the aromatic ring was detrimental to binding for both cyclazocine (compare **2**–**18** and **2**–**19**) and 8-CAC (compare **1**–**24** and **1**–**25**). It is noteworthy that when nitro is at position-7 in cyclazocine and 8-CAC (i.e., **18** and **24**, respectively), binding affinities are much higher than the corresponding 9-nitro derivatives **19** and **25**. The observation that the 7-amino variant **26** of 8-CAC had very high affinity for μ and κ (K_i = 0.55 nM and 0.70 nM, respectively) suggests the amino group stabilizes the carboxamide in the putative bioactive conformation as depicted in **26a**. While the proton NMR of **26** in $CDCl_3$ suggests the presence of an intramolecular H-bond between the $CONH_2$ and adjacent NH_2, we cannot tell whether the most stable form is **26a** or **26b**. Abraham aromatic H-bond structural constants for $ArCONH_2$ are 0.49 (H-bond acidity) and 0.53 (H-bond basicity) and for $ArNH_2$, they are 0.26 (H-bond acidity) and 0.27 (H-bond basicity).[14] These data would lead one to conclude that **26a** and **26b** would have similar stabilities, however, the $ArCONH_2$ and $ArNH_2$ groups are considered in isolation in this analysis. In **26**, the groups are, of course, conjugated which may well tip the scale in favor of **26a**; this would be highly consistent with our view on stabilization of the carboxamide group in the bioactive conformation by H-bonding with an adjacent OH group (e.g., **8**). The relatively poor activity of the 9-amino regioisomer **27** (K_i = 88 nM and 32 nM, respectively) may be due to (a) poor tolerance of the receptor to 9-substitution and/or (b) the 9-amino group stabilizing the carboxamide in a conformation other than the bioactive one.



38: R = $CH_2CH_2(4\text{-}C_6H_4C_6H_5)$
39: R = $CH_2C_6H_5$



26a



26b

All three compounds, **12**, **26**, and **37**, that were characterized in the [^{35}S]GTPγS assay, were found to be antagonists at the μ receptor, although **37** was a mixed agonist/antagonist. For the three compounds at the κ receptor, however, a divergence in functional activity was observed. Whereas **26** and **37** were agonists at κ, compound **12** was an antagonist.

4. Conclusions

Opioid receptor binding affinity data for novel target compounds **10**–**14** and **37** were used in this SAR study to substantiate and strengthen our pharmacophore hypothesis that the carboxamide bioactive conformation of 8-CAC and related opioids is that depicted by **1** versus rotomers where the carboxamide is rotated about the C–C bond to the aryl ring. This conclusion is based on the observed (and predicted) high affinity binding of (a) compound **12** for μ and κ relative to **13** and (b) compound **10** for κ relative to **11**. The poor affinity of **10** for μ was not as predicted and seemingly contradicts our underlying hypothesis. However, by the design and evaluation of **37**, a highly active close analogue of **10**, we now believe the poor μ affinity of **10** is due to a weakening of the H-bond accepting ability of its carboxamide due to the presence of the electron withdrawing imine moiety embedded in the heterocyclic ring. Not only does the observed high μ affinity of **37** help in explaining the poor activity of **10**, but it also strengthens our underlying pharmacophore hypothesis since this compound rigidifies the carboxamide group in the putative bioactive conformation.

From our data, it is apparent that target **12** and 8-CAC (**1**) share a common pharmacophore. Therefore, we expected that the effect of substituting the carboxamide N of 8-CAC with, for example, a (4′-phenyl)-phenethyl group (i.e., **38**) would be very similar to that same substitution on the exocyclic N of **12** (e.g., **14**). While the impact on κ affinity is similar across both platforms (i.e., 30-fold decrease for the **1** → **38** conversion and ninefold decrease for **12** → **14**), there is a significant divergence on μ affinity (i.e., **1** and **38** have same affinity and a 13-fold decrease is seen for the **12** → **14** conversion). This divergence may be a consequence of a conformational change of the carboxamide group of **38** (relative to 8-CAC) to facilitate interaction of the *N*-(4′-phenyl)-phenethyl group with its putative complimentary hydrophobic binding site. For **14**, the aminopyrimidine surrogate of the carboxamide cannot undergo conformational change due to its rigidified nature. This may weaken the stability of the putative hydrophobic interaction between the (4′-phenyl)-phenethyl group and the receptor. In summary, there appears to be no benefit in binding affinity when a (4′-phenyl)-phenethyl or benzyl group is appended to the exocyclic nitrogen of **12**. While binding affinity is increased when the groups are attached to **14**, absolute potency is relatively weak.

Assuming the intramolecular H-bond between the neighboring amino and carboxamide groups of **26** and **27** is due to H-bond donation by the amine and accepting by the carboxamide oxygen, the observation that **26** has much higher affinity for μ and κ receptors than **27** adds further credence to our pharmacophore hypothesis.

In summary, the value of the SAR data generated in this study is not only the strengthening of our underlying pharmacophore hypothesis, but also in the identification of a number of novel opioids having high affinity to μ and κ receptors. These novel compounds (e.g., **12** and **26**) have drug-like structures suitable for additional studies to aid in the selection of clinical candidates. Additional SAR studies in this area are ongoing in our laboratories and will be the subject of future communications.

5. Experimental

5.1. Chemistry

Proton NMR spectra and in certain cases ^{13}C NMR were obtained on a Varian Unity-300 or 500 NMR spectrometer with tetramethylsilane as an internal reference for samples dissolved in $CDCl_3$. The samples dissolved in CD_3OD and DMSO-d_6 were referenced to the solvent. Proton NMR multiplicity data are denoted by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublets), and br (broad). Coupling constants are in Hertz. Direct insertion probe chemical ionization mass spectral data were obtained on a Shimadzu GC-17A GC–MS mass spectrometer. Direct infusion electrospray ionization (in positively charged ion mode) mass spectral data were obtained on an Agilent 1100 series LC/MSD system (Germany). Melting points were determined on a Meltemp capillary melting point apparatus and were uncorrected. Infrared spectral data were obtained on a Perkin-Elmer Paragon 1000 FT-IR spectrophotometer. Reactions performed under microwave radiation were done on a Personal Chemistry Creator system (20 W). The assigned structure of all the test compounds and intermediates were consistent with the spectral data. Carbon, hydrogen, and nitrogen elemental analyses for all novel targets were performed by Quantitative Technologies Inc., Whitehouse, NJ, and were within ±0.4% of theoretical values except as noted; the presence of water or other solvents was confirmed by proton NMR. Reactions were generally performed in an argon or nitrogen atmosphere. Commercially purchased chemicals were used without purification unless otherwise noted. Silica gel (Bodman Industries, ICN SiliTech 2-63 D 60A, 230–400 Mesh) was used for all flash chromatography. Where indicated, an Isco CombiFlash Companion was used for purification of reaction products. Toluene and Et_2O were distilled from sodium metal. THF was distilled from sodium/benzophenone ketyl. Pyridine was distilled from KOH. Methylene chloride was distilled from CaH_2. DMF and DMSO were distilled from CaH_2 under reduced pressure. Methanol was dried over 3 Å molecular sieves prior to use.

5.1.1. *cis*-(±)-3-(Cyclopropylmethyl)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-7-nitro-2,6-methano-3-benzazocine-8-ol (18) and *cis*-(±)-3-(cyclopropylmethyl)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-9-nitro-2,6-methano-3-benzazocin-8-ol (19). A solution of 69% nitric acid (0.20 g) in

2.0 mL glacial acetic acid was added to a solution of cyclazocine[2] (**1**; 0.542 g, 2.0 mmol) in 3.0 mL glacial acetic acid at 25 °C. After stirring at 25 °C for 3 h, TLC indicated the presence of starting material and an additional 0.10 gm of 69% nitric acid was added. After stirring for 2 h at 25 °C, TLC indicated all starting material was consumed and the reaction mixture was poured into a mixture of ice and excess concentrated ammonium hydroxide. The mixture was treated with ethyl acetate and the organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated to give a crude solid product which was purified by gradient silica gel flash chromatography (CH_2Cl_2/CH_3OH; 20:1 → 10:1) to give **18** (0.26 g, 40%) as a brownish solid and **19** (0.35 g, 54%) as a brownish foam: recrystallization from MeOH/CH_2Cl_2 gave yellow crystals having mp 145 °C and mp 175 °C, respectively.

For **18**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.98 (d, 1H, J = 8.3 Hz), 6.83 (d, 1H, J = 8.5 Hz), 3.10 (m, 1H), 2.84 (d, 1H, J = 18.8 Hz), 2.81–2.57 (m, 2H), 2.46 (m, 1H), 2.32 (m, 1H), 2.03 (m, 3H), 1.86–1.66 (m, 1H), 1.31 (s, 3H), 1.25 (m, 1H), 0.87 (m, 4H), 0.51 (m, 2H), 0.11 (m, 2H); MS (ESI) m/z 317 $(M+H)^+$; Anal. Calcd. for $C_{18}H_{24}N_2O_3 \cdot 0.75H_2O$: C, 65.53; H, 7.79; N, 8.49. Found: C, 65.27; H, 7.41; N, 8.23.

For **19**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.36 (s, 1H), 7.80 (s, 1H), 7.03 (s, 1H), 3.16 (m, 1H), 2.95 (d, 1H, J = 18.8 Hz), 2.79–2.56 (m, 2H), 2.48 (m, 1H), 2.32 (m, 1H), 1.96 (m, 3H), 1.39 (s, 3H), 1.36 (m, 1H), 0.85 (m, 4H), 0.52 (m, 2H), 0.11 (m, 2H); MS (ESI) m/z 317 $(M+H)^+$; Anal. Calcd. for $C_{18}H_{24}N_2O_3 \cdot 0.5H_2O$: C, 66.44; H, 7.74; N, 8.61. Found: C, 66.03; H, 7.33; N, 8.48.

5.1.2. Trifluoromethanesulfonic acid, *cis*-(±)-3-(cyclopropylmethyl)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-7-nitro-2,6-methano-3-benzazocine-8-yl ester (20). Triethylamine (0.22 g, 2.22 mmol) was added to a solution of **18** (0.47 g, 1.48 mmol) dissolved in 20 mL of CH_2Cl_2. $PhN(SO_2CF_3)_2$ (0.58 g, 1.63 mmol) was then added and the resulting mixture stirred at 25 °C for 4 h. The solvent was removed on a rotary evaporator and the resulting mixture was purified by gradient silica gel flash chromatography (CH_2Cl_2/CH_3OH; 80:1 → 40:1) to give **20** (0.59 g, 88%) as a yellow foam: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 (d, 1H, J = 8.5 Hz), 7.24 (d, 1H, J = 8.6 Hz), 3.56 (m, 1H), 3.17 (m, 1H), 3.05 (m, 2H), 2.81 (m, 1H), 2.66 (m, 1H), 2.29–2.04 (m, 2H), 1.90 (m, 1H), 1.34 (m, 4H), 0.87 (m, 4H), 0.69 (m, 2H), 0.28 (m, 2H).

5.1.3. Trifluoromethanesulfonic acid, *cis*-(±)-3-(cyclopropylmethyl)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-9-nitro-2,6-methano-3-benzazocine-8-yl ester (21). Using a procedure similar to that used to prepare **20**, compound **19** was converted to **21** (93%) as a yellow foam: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.27 (s, 1H), 3.60 (m, 1H), 3.22–2.94 (m, 3H), 2.84 (m, 1H), 2.68 (m, 1H), 2.30 (m, 1H), 2.11 (m, 2H), 1.41 (s, 3H), 1.38 (m, 1H), 0.84 (m, 4H), 0.69 (m, 2H), 0.29 (m, 2H).

5.1.4. *cis*-(±)-3-(Cyclopropylmethyl)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-7-nitro-2,6-methano-3-benzazocine-8-carbonitrile (22). To a tube containing **20** (0.27 g, 0.61 mmol) was added under a N_2 blanket, $Zn(CN)_2$ (0.14 g, 1.22 mmol) and $Pd(PPh_3)_4$ (0.07 g, 0.061 mmol). DMF (degassed with N_2, 3.0 mL) was then added via a cannula under N_2. The resulting mixture was irradiated with microwaves at 150 °C for 15 min. The resulting mixture was partitioned between water and EtOAc. The organic phase was washed with water (X2) and brine, and then dried over Na_2SO_4, filtered, and concentrated to give a crude product which was purified by silica gel flash chromatography ($CH_2Cl_2/CH_3OH/NH_4OH$; 80:1:0.1) to give **22** (0.14 g, 70%) as an off-white foam: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52 (d, 1H, J = 8.1 Hz), 7.35 (d, 1H, J = 8.1 Hz), 3.20 (m, 1H), 3.04 (d, 1H, J = 19.1 Hz), 2.86 (m, 1H), 2.68 (m, 2H), 2.47 (m, 1H), 2.34 (m, 1H), 2.10–1.74 (m, 3H), 1.34 (m, 4H), 0.89 (m, 1H), 0.84 (d, 3H, J = 7.1 Hz), 0.54 (m, 2H), 0.12 (m, 2H). MS (ESI) m/z 326 $(M+H)^+$.

5.1.5. *cis*-(±)-3-(Cyclopropylmethyl)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-9-nitro-2,6-methano-3-benzazocine-8-carbonitrile (23). Using a procedure similar to that used to prepare **22**, compound **21** was converted to **23** (quantitative yield) as an off-white foam: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.75 (s, 1H), 3.22 (m, 1H), 3.08 (d, 1H, J = 19.8 Hz), 2.79 (m, 2H), 2.47 (m, 1H), 2.32 (m, 1H), 2.10–1.78 (m, 3H), 1.46 (s, 3H), 1.33 (m, 1H), 0.87 (m, 1H), 0.83 (d, 3H, J = 7.1 Hz), 0.54 (m, 2H), 0.12 (m, 2H).

5.1.6. *cis*-(±)-3-(Cyclopropylmethyl)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-7-nitro-2,6-methano-3-benzazocine-8-carboxamide (24). A solution of **22** (0.11 g, 0.33 mmol) dissolved in *t*-BuOH (2.0 mL) was heated at 58 °C and KOH (0.056 g, 1.0 mmol) was added. After stirring at 58 °C for 1 h, brine and EtOAc were added. The organic phase was dried over Na_2SO_4, filtered, and concentrated to give a crude product which was purified by silica gel flash chromatography ($CH_2Cl_2/CH_3OH/NH_4OH$; 20:1:0.1) to give **24** as an off-white solid (0.098 g, 85%). Crystallization of this solid from acetone followed by a recrystallization from *i*-PrOH/*t*-BuOH gave crystals having mp 190 °C: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (s, 1H), 7.54 (s, 1H), 7.38 (s, 2H), 3.04 (m, 1H), 2.96 (d, 1H, J = 19.5 Hz), 2.76 (m, 2H), 2.38 (m, 1H), 2.24 (m, 1H), 2.06–1.56 (m, 3H), 1.19 (m, 4H), 0.79 (m, 1H), 0.73 (d, 3H, J = 6.8 Hz), 0.44 (m, 2H), 0.07 (m, 2H); MS (ESI) m/z 344 $(M+H)^+$; Anal. Calcd. for $C_{19}H_{25}N_3O_3 \cdot 0.5H_2O$: C, 64.75; H, 7.44; N, 11.92. Found: C, 64.47; H, 7.21; N, 11.56.

5.1.7. *cis*-(±)-3-(Cyclopropylmethyl)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-9-nitro-2,6-methano-3-benzazocine-8-carboxamide (25). Using a procedure similar to that used to prepare **24**, compound **23** was converted to **25** (45%) as an off-white foam: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.42 (s, 1H), 5.89 (m, 2H), 3.20 (m, 1H), 3.03 (d, 1H, J = 19.0 Hz), 2.75 (m, 2H), 2.47 (m, 1H), 2.33 (m, 1H), 2.06–1.82 (m, 3H), 1.43 (s, 3H), 1.34 (m, 1H), 0.87 (m, 1H), 0.83 (d, 3H, J = 7.1 Hz), 0.53 (m, 2H), 0.12 (m, 2H); MS (ESI) m/z 344 $(M+H)^+$; Anal.

Calcd. for $C_{19}H_{25}N_3O_3 \cdot 0.25H_2O$: C, 65.59; H, 7.39; N, 12.08. Found: C, 65.39; H, 7.38; N, 11.93.

5.1.8. *cis*-(±)-3-(Cyclopropylmethyl)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-7-amino-2,6-methano-3-benzazocine-8-carboxamide (26). To a solution of **24** (0.15 g, 0.44 mmol) dissolved in MeOH (20 mL) was added 10% Pd/C (0.093 g). The resulting mixture was subjected to 55 psi H_2 in a Parr shaker for 3d at 25 °C. The mixture was filtered and concentrated to give a crude product that was purified by silica gel flash chromatography ($CH_2Cl_2/CH_3OH/NH_4OH$; 30:1:0.1) giving **26** (0.060 g, 44%) as a white foam: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.14 (d, 1H, J = 8.1 Hz), 6.42 (d, 1H, J = 8.1 Hz), 6.12 (s, 1H), 5.58 (br s, 2H), 3.09 (m, 1H), 2.76 (m, 3H), 2.24 (m, 1H), 2.28 (m, 1H), 2.06–1.70 (m, 3H), 1.59 (s, 3H), 1.58 (m, 1H), 0.91 (d, 3H, J = 7.1 Hz), 0.86 (m, 1H), 0.51 (m, 2H), 0.10 (m, 2H); MS (ESI) m/z 314 $(M+H)^+$; Anal. Calcd. for $C_{19}H_{27}N_3O \cdot 0.25H_2O$: C, 71.78; H, 8.72; N, 13.22. Found: C, 72.00; H, 8.84; N, 12.98.

5.1.9. *cis*-(±)-3-(Cyclopropylmethyl)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-9-amino-2,6-methano-3-benzazocine-8-carboxamide (27). Using a procedure similar to that used to prepare **26**, compound **25** was converted to **27** (63%) as an off-white foam: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.20 (s, 1H), 6.42 (s, 1H), 5.61 (br s, 2H), 5.42 (s, 2H), 3.10 (m, 1H), 2.84 (d, 1H, J = 18.8 Hz), 2.75–2.53 (m, 2H), 2.46 (m, 1H), 2.30 (m, 1H), 2.06–1.80 (m, 3H), 1.35 (s, 3H), 1.27 (m, 1H), 0.87 (m, 1H), 0.84 (d, 3H, J = 7.1 Hz), 0.51 (m, 2H), 0.10 (m, 2H); MS (ESI) m/z 314 $(M+H)^+$; Anal. Calcd. for $C_{19}H_{27}N_3O \cdot 0.25H_2O$: C, 71.78; H, 8.72; N, 13.22. Found: C, 72.00; H, 8.73; N, 13.27.

5.1.10. 7,8-Fused pyrimidinone derivative 11. A mixture of **26** (0.035 g, 0.11 mmol) and 2.0 mL of 88% formic acid was heated at 120 °C under microwave radiation for 30 min. The reaction mixture was basified using excess NH_4OH and the organic material was extracted into ethyl acetate. The organic phase was washed with brine, dried over Na_2SO_4, and concentrated giving a crude product that was purified by silica gel chromatography (Combiflash—$CH_2Cl_2/CH_3OH/NH_4OH$; 20:1:0.1) giving **11** (0.020 gm, 54%) as an off-white foam. Further crystallization from acetone gave white crystals (mp 220 °C): ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.90 (br s, 1H), 8.08 (d, 1H, J = 8.1 Hz), 7.99 (s, 1H), 7.25 (d, 1H, J = 8.1 Hz), 3.19 (m, 1H), 2.93 (m, 2H), 2.77 (m, 1H), 2.48 (m, 1H), 2.29 (m, 1H), 2.06 (m, 1H), 1.90 (m, 2H), 1.81 (s, 3H), 1.64 (m, 1H), 0.90 (d, 3H, J = 7.1 Hz), 0.88 (m, 1H), 0.51 (m, 2H), 0.10 (m, 2H); MS (ESI) m/z 324 $(M+H)^+$; Anal. Calcd. for $C_{20}H_{25}N_3O$: C, 74.27; H, 7.79; N, 12.99. Found: C, 73.95; H, 7.86; N, 12.78.

5.1.11. 8,9-Fused pyrimidinone derivative 10. Using a procedure similar to that used to prepare **11**, compound **27** (0.021 g, 0.067 mmol) was converted to **10** (0.010 g, 46%) as an off-white foam: ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.10 (br s, 1H), 8.19 (s, 1H), 8.05 (s, 1H), 7.48 (s, 1H), 3.23 (m, 1H), 3.14 (d, 1H, J = 19.3 Hz), 2.91–2.72 (m, 2H), 2.51 (m, 1H), 2.35 (m, 1H), 2.08–1.86 (m, 3H), 1.52 (s, 3H), 1.38 (m, 1H), 0.90 (m, 1H), 0.87 (d, 3H, J = 7.1 Hz), 0.88 (m, 1H), 0.53 (m, 2H), 0.13 (m, 2H); MS (ESI) m/z 324 $(M+H)^+$; Anal. Calcd. for $C_{20}H_{25}N_3O \cdot 0.25H_2O$: C, 73.25; H, 7.84; N, 12.81. Found: C, 73.14; H, 7.90; N, 12.38.

5.1.12. *cis*-(±)-7-Amino-3-(cyclopropylmethyl)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-2,6-methano-3-benzazocine-8-carbonitrile (28). A mixture of **26** (0.22 g, 0.70 mmol), $POCl_3$ (0.11 g, 0.70 mmol), and pyridine (2.0 mL) was heated at 100 °C for 20 min under microwave radiation and concentrated. The residue was dissolved in 1.0 N HCl and stirred for 1 h at 25 °C. The reaction mixture was made basic with saturated $NaHCO_3$/crushed ice and the organic material was extracted into CH_2Cl_2. The organic layer were washed with brine, dried over Na_2SO_4, filtered and concentrated to give a crude product that was purified by silica gel chromatography (Combiflash—$CH_2Cl_2/CH_3OH/NH_4OH$) to give **28** as a brownish oil (0.11 g) in 54% yield: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.07 (d, 1H, J = 8.5 Hz), 6.41 (d, 1H, J = 8.8 Hz), 3.16 (m, 1H), 2.70 (m, 4H), 2.46 (m, 1H), 2.30 (m, 1H), 2.07–1.70 (m, 3H), 1.64 (m, 4H), 0.94 (d, 3H, J = 6.8 Hz), 0.88 (m, 1H), 0.53 (m, 2H), 0.12 (m, 2H).

5.1.13. *cis*-(±)-9-Amino-3-(cyclopropylmethyl)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-2,6-methano-3-benzazocine-8-carbonitrile (30). A mixture of **23** (0.180 g, 0.55 mmol), 10% Pd/C and CH_3OH (20 mL) was subjected to 40 psi H_2 in a Parr shaker at 25 °C for 15 h. The mixture was filtered and concentrated to give **30** as a crude product that was purified by silica gel chromatography (Combiflash—$CH_2Cl_2/CH_3OH/NH_4OH$) to give a brownish solid (0.070 g, 47%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 (s, 1H), 6.47 (s, 1H), 4.18 (s, 2H), 3.15 (m, 1H), 2.86 (d, 1H, J = 19.0 Hz), 2.80–2.58 (m, 2H), 2.48 (m, 1H), 2.33 (m, 1H), 1.94 (m, 3H), 1.32 (s, 3H), 1.25 (m, 1H), 0.90 (m, 1H), 0.81 (d, 3H, J = 7.1 Hz), 0.53 (m, 2H), 0.12 (m, 2H); MS (ESI) m/z 296 $(M+H)^+$.

5.1.14. 7,8-Fused aminopyrimidine derivative 12. A mixture of **28** (0.11 g, 0.38 mmol), $CH(OCH_3)_3$ (2 mL) and 4 Å molecular sieves was heated at 140 °C for 48 h. The reaction mixture was filtered and concentrated to give imidate intermediate **29** (0.120 g) which, without further purification, was combined with methanol saturated with ammonia gas. The resulting mixture was heated for 1 h at 100 °C under microwave radiation and then made basic with concentrated ammonia. After dilution with H_2O, the organic material was extracted into CH_2Cl_2 and the organic layer was washed with brine, dried over Na_2SO_4, and concentrated to give mixture that was purified by silica gel chromatography (Combiflash—$CH_2Cl_2/CH_3OH/NH_4OH$) and crystallization. The desired product **12** (0.074 gm) was obtained in 56% yield (two steps) as an off-white solid: mp 190 °C: NMR (500 MHz, $CDCl_3$) δ 8.56 (s, 1H), 7.49 (d, 1H, J = 8.3 Hz), 7.20 (d, 1H, J = 8.3 Hz), 5.54 (s, 2H), 3.20 (m, 1H), 2.92 (m, 2H), 2.76 (m, 1H), 2.48 (m, 1H), 2.29 (m, 1H), 2.19 (m, 1H), 1.94 (m, 4H), 1.89 (s, 3H),

0.91 (d, 3H, J = 7.1 Hz), 0.89 (m, 1H), 0.51 (m, 2H), 0.10 (m, 2H); MS (ESI) m/z 323 $(M+H)^+$; Anal. Calcd. for $C_{20}H_{26}N_4 \cdot 0.25H_2O$: C, 73.47; H, 8.17; N, 17.14. Found: C, 73.59; H, 8.04; N, 16.92.

5.1.15. 8,9-Fused aminopyrimidine derivative 13. Using a procedure similar to that used to prepare **12**, compound **30** was converted to imidate intermediate **31** which was then converted to **13** (86%) as an off-white foam: ^1H NMR (500 MHz, $CDCl_3$) δ 8.56 (s, 1H), 7.62 (s, 1H), 7.58 (s, 1H), 6.00 (s, 2H), 3.23 (m, 1H), 3.18 (d, 1H, J = 19.0 Hz), 2.89 (m, 1H), 2.73 (m, 1H), 2.51 (m, 1H), 2.35 (m, 1H), 2.01 (m, 3H), 1.48 (s, 3H), 1.35 (m, 1H), 0.89 (m, 1H), 0.87 (d, 3H, J = 7.3 Hz), 0.53 (m, 2H), 0.13 (m, 2H); MS (ESI) m/z 323 $(M+H)^+$; $C_{20}H_{26}N_4 \cdot 0.25H_2O$: C, 73.47; H, 8.17; N, 17.14. Found: C, 73.33; H, 8.03; N, 16.85.

5.1.16. 7,8-Fused biphenylethylaminopyrimidine derivative 14. Using a procedure similar to that used to prepare **12** (except acetic acid was added), compound **29** was treated with 4-biphenylethylamine to provide to **14** (71%) as an off-white foam: NMR (500 MHz, $CDCl_3$) δ 8.64 (s, 1H), 7.58 (m, 4H), 7.45 (m, 2H), 7.34 (m, 3H), 7.29 (d, 1H, J = 8.5 Hz), 7.13 (d, 1H, J = 8.5 Hz), 5.56 (m, 1H), 3.93 (m, 2H), 3.19 (m, 1H), 3.06 (t, 2H, J = 6.6 Hz), 2.89 (m, 2H), 2.77 (m, 1H), 2.47 (m, 1H), 2.28 (m, 1H), 2.21 (m, 1H), 1.90 (s, 3H), 1.87 (m, 1H); 1.63 (m, 2H), 0.90 (d, 3H, J = 7.1 Hz), 0.88 (m, 1H), 0.51 (m, 2H), 0.10 (m, 2H); MS (ESI) m/z 503 $(M+H)^+$; Anal. Calcd. for $C_{34}H_{38}N_4 \cdot 0.5H_2O$: C, 79.81; H, 7.68; N, 10.95. Found: C, 79.88; H, 7.66; N, 10.83.

5.1.17. 7,8-Fused benzylaminopyrimidine derivative 15. Using a procedure similar to that used to prepare **12**, compound **29** was treated with benzylamine to provide **15** (69%) as an off-white foam: NMR (500 MHz, $CDCl_3$) δ 8.64 (s, 1H), 7.44 (d, 1H, J = 8.5 Hz), 7.40–7.30 (m, 5H); 7.15 (d, 1H, J = 8.3 Hz), 5.81 (m, 1H), 4.83 (d, 2H, J = 5.4 Hz), 3.20 (m, 1H), 2.91 (m, 2H), 2.77 (m, 1H), 2.48 (m, 1H), 2.29 (m, 1H), 2.22 (m, 1H), 1.93 (m, 2H), 1.90 (s, 3H), 1.88 (m, 1H); 0.90 (d, 3H, J = 7.1 Hz), 0.88 (m, 1H), 0.51 (m, 2H), 0.10 (m, 2H); MS (ESI) m/z 413 $(M+H)^+$; Anal. Calcd. for $C_{27}H_{32}N_4 \cdot 0.5H_2O$: C, 76.92; H, 7.89; N, 13.29. Found: C, 76.77; H, 7.99; N, 12.90.

5.1.18. 8,9-Fused biphenylethylaminopyrimidine derivative 16. A mixture of **27** (0.084 g, 0.27 mmol), $POCl_3$ (0.41 g, 2.7 mmol), and DMF (3.0 mL) was heated at 100 °C under microwave radiation for 10 min and concentrated. The resulting dark oil was dissolved in H_2O, made basic with Na_2CO_3 and extracted (X3) with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4 and concentrated to give mixture that was purified by silica gel chromatography (CH_2Cl_2/CH_3OH/NH_4OH) giving the desired amidine intermediate **32** in 89% yield. Compound **32** (0.15 g, 0.43 mmol) was treated with 4-biphenylethylamine (0.10 g, 0.51 mmol) and 30% HOAc in CH_3CN (3.0 mL) at 160 °C under microwave radiation for 5 min. The reaction mixture was cooled down and partitioned between saturated Na_2CO_3 and CH_2Cl_2. The organic phase was washed with brine, dried over Na_2SO_4 and concentrated to give a crude product that was purified by silica gel chromatography (Combiflash—hexane/EtOAc/Et_3N 80:20:0.5 to 50:50:0.5) to provide to **16** (0.18 g, 86%) as an off-white foam: NMR (500 MHz, $CDCl_3$) δ 8.63 (s, 1H), 7.58 (m, 4H), 7.54 (s, 1H), 7.45 (m, 2H), 7.36 (m, 4H), 5.72 (m, 1H), 3.96 (m, 2H), 3.20 (m, 1H), 3.16 (d, 1H, J = 19.1 Hz), 3.09 (t, 2H, J = 7.1 Hz), 2.80 (m, 1H), 2.71 (m, 1H), 2.50 (m, 1H), 2.34 (m, 1H), 2.04–1.94 (m, 3H), 1.43 (s, 3H), 1.30 (m, 1H), 0.88 (m, 1H), 0.85 (d, 3H, J = 7.1 Hz), 0.52 (m, 2H), 0.12 (m, 2H); MS (ESI) m/z 503 $(M+H)^+$; Anal. Calcd. for $C_{34}H_{38}N_4 \cdot 0.5H_2O$: C, 79.81; H, 7.68; N, 10.95. Found: C, 79.52; H, 7.64; N, 10.83.

5.1.19. 8,9-Fused benzylaminopyrimidine derivative 17. Using a procedure similar to that used to prepare **16**, compound **32** was treated with benzylamine to provide to **17** (92 %) as an off-white solid: NMR (500 MHz, $CDCl_3$) δ 8.64 (s, 1H), 7.56 (m, 1H), 7.50 (s, 1H), 7.44 (d, 2H, J = 7.3 Hz), 7.39 (t, 2H, J = 7.3 Hz), 7.34 (d, 1H, J = 7.3 Hz), 5.94 (br s, 1H), 4.90 (m, 2H), 3.22 (m, 1H), 3.17 (d, 1H, J = 19.0 Hz), 2.88 (m, 1H), 2.72 (m, 1H), 2.51 (m, 1H), 2.34 (m, 1H), 1.99 (m, 3H), 1.47 (s, 3H), 1.33 (m, 1H), 0.88 (m, 1H), 0.86 (d, 3H, J = 7.1 Hz), 0.52 (m, 2H), 0.12 (m, 2H); MS (ESI) m/z 413 $(M+H)^+$; $C_{27}H_{32}N_4 \cdot H_2O$: C, 75.31; H, 7.96; N, 13.01. Found: C, 75.64; H, 7.73; N, 13.02.

5.1.20. *cis*-(±)-3-(Cyclopropylmethyl)-1,2,3,4,5,6-hexahydro-*N*-methoxy-6,11-dimethyl-2,6-methano-3-benzazocine-8-carboxamide (34). A solution of **33**[8] (240 mg, 0.606 mmol) and methoxylamine hydrochloride (61 mg, 0.727 mmol) in 3 mL of dry pyridine was stirred at room temperature for 12 h. The solvent was removed in vacuo and the residue was taken up in methylene chloride (40 mL), and washed with saturated sodium bicarbonate solution, water, and brine. The organic phase was dried over sodium sulfate, filtered, and concentrated to give a brown residue, which was purified by flash chromatography (CH_2Cl_2/CH_3OH/NH_4OH 20:1:0.1) to give **34** as an off-white foam (159 mg, 0.485 mmol, 80%): ^1H NMR (500 MHz, $CDCl_3$) δ 8.64 (br s, 1H), 7.64 (d, 1H, J = 1.5 Hz), 7.42 (dd, 1H, 7.8, 2.5 Hz), 7.12 (d, 1H, J = 7.8 Hz), 3.89 (s, 3H), 3.15 (m, 1H), 2.96 (d, 1H, J = 18.6 Hz), 2.70 (m, 2H), 2.46 (m, 1H), 2.32 (m, 1H), 1.91 (m, 3H), 1.42 (s, 3H), 1.33 (m, 1H), 0.86 (m, 1H), 0.82 (d, 3H, J = 7.2 Hz), 0.51 (m, 2H), 0.11 (m, 2H); MS (ESI) m/z 329 $(M+H)^+$; IR (CH_2Cl_2) ν_{max} 3464, 3196, 1643 cm^{-1}; Anal. Calcd. for $C_{20}H_{28}N_2O_2 \cdot 0.25H_2O$: C, 72.15; H, 8.63; N, 8.41. Found: C, 72.15; H, 8.67; N, 8.13.

5.1.21. *cis*-(±)-3-(Cyclopropylmethyl)-1,2,3,4,5,6-hexahydro-*N*-methoxy-6,9,11-trimethyl-2,6-methano-3-benzazocine-8-carboxamide (35). Conditions of Fisher et al. were used.[9] *sec*-Butyllithium (1.4 M in cyclohexane, 4.6 mL, 4.57 mmol) was added to a mixture of **34** (100 mg, 0.305 mmol) and TMEDA (530 mg, 4.57 mmol) in dry THF at −78 °C under nitrogen atmosphere. The resulting mixture was warmed to −20 °C, stirred for 10 min and cooled to −78 °C again. Iodomethane (649 mg, 4.57 mmol) was added dropwise and the resulting mix-

ture was stirred for 5 min. The reaction was quenched with 20 mL of saturated ammonia chloride and extracted with EtOAc (3× 20 mL). The combined organic phases were washed twice with water and once with brine, dried over sodium sulfate, filtered, and concentrated to give **35** as a brown oil (146 mg, estimated yield 74%). There was small amount of starting material in this oil and it is difficult to separate starting material from product using flash chromatography because these two compounds have the same R_f value on silica gel TLC plate. Therefore, **35** was used in next step without further purification: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.19 (s, 1H), 6.90 (s, 1H), 3.86 (s, 3H), 3.12 (m, 1H), 2.87 (d, 1H, J = 19 Hz), 2.64 (m, 2H), 2.42 (m, 1H), 2.36 (s, 3H), 2.28 (m, 1H), 1.86 (m, 3H), 1.34 (s, 3H), 1.26 (m, 1H), 0.85 (m, 1H), 0.85 (d, 3H, J = 6.8 Hz), 0.50 (m, 2H), 0.10 (m, 2H); MS (ESI) m/z 343 $(M+H)^+$.

5.1.22. 8,9-Fused *N*-methoxypyridinone derivative 36. Conditions of Fisher et al. were used.[9] *sec*-Butyllithium (1.4 M in cyclohexane, 2.3 mL, 2.99 mmol) was added dropwise to a solution of **35** (146 mg, 0.427 mmol) in 2 mL of dry THF under argon at −78 °C. The resulting mixture was stirred for 5 min. DMF (0.23 mL) was added and the reaction mixture was stirred for additional 5 min. Then the reaction was quenched with 20 mL of saturated ammonia chloride and extracted with EtOAc (3× 20 mL). The combined organic phases were washed twice with water and once with brine, dried over sodium sulfate, filtered, and concentrated to give a brown oil, which was mixed with 1 mL of concentrated HCl and stirred for 1 h. The reaction mixture was then made basic using 5 N NaOH solution and extracted with EtOAc (3× 20 mL). The combined organic phases were washed twice with water and once with brine, dried over sodium sulfate, filtered, and concentrated to give a brown oil, which was purified by flash chromatography (CH_2Cl_2/CH_3OH/NH_4OH 25:1:0.1) to give **36** as a white foam (0.054 g, 0.153 mmol) in 50% overall from **34**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.25 (d, 1H, J = 8.0 Hz), 7.24 (s, 1H), 6.37 (d, 1H, J = 8.0 Hz), 4.08 (s, 3H), 3.19 (m, 1H), 3.08 (d, 1H, J = 19 Hz), 2.81 (m, 1H), 2.71 (m, 1H), 2.49 (m, 1H), 2.32 (m, 1H), 1.97 (m, 3H), 1.52 (s, 3H), 1.39 (m, 1H), 0.88 (m, 1H), 0.84 (d, 3H, J = 7.0 Hz), 0.52 (m, 2H), 0.11 (m, 2H); MS (ESI) m/z 353 $(M+H)^+$.

5.1.23. 8,9-Fused pyridinone derivative 37. Conditions of Fisher and coworkers were used.[9] A solution of titanium(III) chloride (0.197 g, 1.28 mmol) in 2.5 mL of 6 N HCl was added to a solution of **36** (45 mg, 0.128 mmol) in 1 mL of EtOH. The resulting mixture was irradiated with microwaves at 100 °C for 30 min. The cooled reaction mixture was poured onto a mixture of ice and water and basified with 5 N NaOH to approximately pH 13. Air was bubbled through the solution until the blue color disappeared. White precipitants were observed in solution. The mixture was extracted with EtOAc (3× 20 mL). The combined organic phases were washed twice with water and once with brine, dried over sodium sulfate, filtered, and concentrated to give a brown residue, which was purified by flash chromatography (CH_2Cl_2/CH_3OH/NH_4OH 25:1:0.1) to give **37** as a white foam (0.026 g, 0.0819 mmol, 64%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.89 (br s, 1H), 8.33 (s, 1H), 7.29 (s, 1H), 7.15 (d, 1H, J = 7.0 Hz), 6.49 (d, 1H, J = 7.0 Hz), 3.22 (m, 1H), 3.11 (d, 1H, J = 18.5 Hz), 2.84 (m, 1H), 2.74 (m, 1H), 2.51 (m, 1H), 2.35 (m, 1H), 2.00 (m, 3H), 1.54 (s, 3H), 1.41 (m, 1H), 0.89 (m, 1H), 0.87 (d, 3H, J = 7.0 Hz), 0.52 (m, 2H), 0.11 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.81, 143.04, 142.86, 135.93, 127.41, 124.74, 124.48, 124.22, 106.29, 60.11, 57.05, 45.91, 42.72, 41.86, 37.12, 26.01, 24.43, 14.44, 9.58, 4.22, 3.82; MS (ESI) m/z 323 $(M+H)^+$; Anal. Calcd. for $C_{21}H_{26}N_2O{\cdot}0.5H_2O$: C, 76.10; H, 8.21; N, 8.45. Found: C, 76.20; H, 8.36; N, 8.01.

5.2. Opioid receptor binding assays

Binding assays used to screen compounds are similar to those previously reported.[15] Membrane protein from CHO cells that stably expressed one type of the human opioid receptors was incubated with 12 different concentrations of the compound in the presence of either 1 nM [^{3}H]U69,593 (μ), 0.25 nM [^{3}H]DAMGO (δ) or 0.2 nM [^{3}H]naltrindole (κ) in a final volume of 1 mL of 50 mM Tris–HCl, pH 7.5 at 25 °C. Incubation times of 60 min were used for [^{3}H]U69,593 and [^{3}H]DAMGO. Because of a slower association of [^{3}H]naltrindole with the receptor, a 3 h incubation was used with this radioligand. Samples incubated with [^{3}H]naltrindole also contained 10 mM $MgCl_2$ and 0.5 mM phenylmethylsulfonyl fluoride. Non-specific binding was measured by inclusion of 10 μM naloxone. The binding was terminated by filtering the samples through Schleicher & Schuell No. 32 glass fiber filters using a Brandel 48-well cell harvester. The filters were subsequently washed three times with 3 mL of cold 50 mM Tris–HCl, pH 7.5, and were counted in 2 mL Ecoscint A scintillation fluid. For [^{3}H]naltrindole and [^{3}H]U69,593 binding, the filters were soaked in 0.1% polyethylenimine for at least 60 min before use. IC_{50} values will be calculated by least squares fit to a logarithm-probit analysis. K_i values of unlabeled compounds were calculated from the equation $K_i = (IC_{50})/1 + S$ where S = (concentration of radioligand)/(K_d of radioligand)[16] The K_d values for [^{3}H]DAMGO, [^{3}H]U69,593, and [^{3}H]naltrindole were 0.56 nM, 0.34 nM, and 0.10 nM, respectively. Data are means ± SEM from at least three experiments performed in triplicate.

5.3. [^{35}S]GTPγS Binding assays

Procedure similar to those previously reported was used.[12] In a final volume of 0.5 mL, 12 different concentrations of each test compound were incubated with 15 μg (κ) or 7.5 μg (μ) of CHO cell membranes that stably expressed either the human κ, or μ opioid receptor. The assay buffer consisted of 50 mM Tris–HCl, pH 7.4, 3 mM $MgCl_2$, 0.2 mM EGTA, 3 μM GDP, and 100 mM NaCl. The final concentration of [^{35}S]GTPγS was 0.080 nM. Non-specific binding was measured by inclusion of 10 μM GTPγS. Binding was initiated by the addition of the membranes. After an incubation of 60 min at 30 °C, the samples were filtered through Schleicher & Schuell No. 32 glass fiber filters. The filters

were washed three times with cold 50 mM Tris–HCl, pH 7.5, and were counted in 2 mL of Ecoscint scintillation fluid. Data are the mean E_{max} and EC_{50} values ± SEM from at least three separate experiments, performed in triplicate. For calculation of the E_{max} values, the basal [^{35}S]GTPγS binding was set at 0%. To determine antagonist activity of a compound at the μ opioid receptors, CHO membranes expressing the μ opioid receptor were incubated with 12 different concentrations of the compound in the presence of 200 nM of the μ agonist DAMGO. To determine antagonist activity of a compound at the κ opioid receptors, CHO membranes expressing the κ opioid receptor, were incubated with the compound in the presence of 100 nM of the κ agonist U50,488.

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References and notes

1. Wentland, M. P.; Lou, R.; Ye, Y.; Cohen, D. J.; Richardson, G. P.; Bidlack, J. M. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 623.
2. Archer, S.; Glick, S. D.; Bidlack, J. M. *Neurochem. Res.* **1996**, *21*, 1369.
3. Zhang, A.; Xiong, W.; Bidlack, J. M.; Hilbert, J. E.; Knapp, B. I.; Wentland, M. P.; Neumeyer, J. L. *J. Med. Chem.* **2004**, *47*, 165.
4. Wentland, M. P.; Lou, R.; Dehnhardt, C. M.; Duan, W.; Cohen, D. J.; Bidlack, J. M. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1717.
5. Wentland, M. P.; Lu, Q.; Lou, R.; Knapp, B. I.; Bidlack, J. M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2107.
6. Wentland, M. P.; Sun, X.; Bu, Y.; Lou, R.; Cohen, D. J.; Bidlack, J. M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2547.
7. (a) Bachmann, W. E.; Brockway, C. E. *J. Org. Chem.* **1948**, *13*, 384; (b) Zhang, Q.; Chen, Y.; Zheng, Y.; Xia, P.; Xia, Y.; Yang, Z.; Bastow, K. F.; Morris-Natschke, S. L.; Lee, K.-H. *Bioorg. Med. Chem.* **2003**, *11*, 1031.
8. Lou, R.; VanAlstine, M.; Sun, X.; Wentland, M. P. *Tetrahedron Lett.* **2003**, *44*, 2477.
9. Fisher, L. E.; Caroon, J. M.; Jahangir, S.; Stabler, R.; Lundberg, S.; Muchowski, J. M. *J. Org. Chem.* **1993**, *58*, 3643.
10. Peng, X.; Knapp, B. I.; Bidlack, J. M.; Neumeyer, J. L. *Bioorg. Med. Chem.* **2007**, *15*, 4106.
11. Ganorkar, R. R.; Lu, Q.; Wentland, M. P.; Bidlack, J. M. *Abstracts of Papers,* 234th ACS National Meeting, Boston, MA, United States, August 19–23, 2007; MEDI-140.
12. Wentland, M. P.; VanAlstine, M.; Kucejko, R.; Lou, R.; Cohen, D. J.; Parkhill, A. L.; Bidlack, J. M. *J. Med. Chem.* **2006**, *49*, 5635.
13. VanAlstine, M. A.; Wentland, M. P.; Cohen, D. J.; Bidlack, J. M. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6516.
14. Abraham, M. H.; Platts, J. A. *J. Org. Chem.* **2001**, *66*, 3484.
15. Neumeyer, J. L.; Zhang, A.; Xiong, W.; Gu, X.; Hilbert, J. E.; Knapp, B. I.; Negus, S. S.; Mello, N. K.; Bidlack, J. M. *J. Med. Chem.* **2003**, *46*, 5162.
16. Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.