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Redefining the structure-activity relationships of 2,6-methano-3-benzazocines. Part 9: Synthesis, characterization and molecular modeling of pyridinyl isosteres of *N*-BPE-8-CAC (**1**), a high affinity ligand for opioid receptors†

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Abstract

Derivatives of the lead compound *N*-BPE-8-CAC (**1**) where each CH of the biphenyl group was individually replaced by N were prepared in hopes of identifying high affinity ligands with improved aqueous solubility. Compared to **1**, binding affinities of the five possible pyridinyl derivatives for the μ opioid receptor were between 3-fold lower to 5-fold higher with the K_i of the most potent compound being 0.064 nM. Docking of 8-CAC (**2**) into the unliganded binding site of the mouse μ opioid receptor (pdb: 4DKL) revealed that 8-CAC and β -FNA (from 4DKL) make nearly identical interactions with the receptor. However, for **1** and the new pyridinyl derivatives **4–8**, binding is not tolerated in the 8-CAC binding mode due to the steric constraints of the large *N*-substituents. Either an alternative binding mode or rearrangement of the protein to accommodate these modifications may account for their high binding affinity.

N-(2-[1,1'-biphenyl]-4-ylethyl)-3-(cyclopropylmethyl)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-2,6-methano-3-benzazocine-8-carboxamide [*N*-BPE-8-CAC (**1**) in Figure 1] displays high binding affinity of for opioid receptors.¹ The observed high potency of **1** was quite unexpected based on our early understanding of the SAR associated with substitution of the carboxamide-N of 8-carboxamidocyclazocine (8-CAC, **2**).² 8-CAC is also a high affinity ligand for opioid receptors and is a derivative of the well-known racemic opioid cyclazocine (**3**)³ where its prototypic (of opioids) phenolic-OH is replaced by a carboxamido group.

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We hypothesized the biphenylethyl group of **1** occupies previously unexplored receptor space in opioid receptors that is largely hydrophobic in nature. There is evidence, however, that this pocket can tolerate polar groups on the distal phenyl ring. For example, we found that binding affinity was significantly increased by the introduction of a 2'-, 3'- or 4'-OR group (where R is OH or CH₃) into the distal phenyl group of **1**.^{4,5} To further probe opioid receptor space for what we believe contains a putative hydrophobic pocket complementary to the aryl groups on the 8-position of **1** as well as a potential H-bond/acceptor donor group(s) within this pocket, we now report the synthesis, opioid receptor binding properties and molecular modeling studies of a series of analogues of **1** (see Table 1 for structures) where each CH of the biphenyl group was replaced by N (total of five due to symmetry). If such pyridinyl substitution was, in fact, tolerated, an added benefit would be increased aqueous solubility of the ligands since the clog*P* of **1** (ACD LogD suite) is a very high 5.9.

Novel racemic targets **4–8** were conveniently made in one step from known intermediates as shown in Scheme 1. All reagents, including boronic acids, were commercially available; primary amines used to make targets **6–8** were made using known procedures.⁶ Target compounds **4** and **5** were made from N-bromophenethyl intermediate **9**⁴ using standard Suzuki coupling conditions in 35% and 70% yield, respectively. The triflic acid ester **10**² of cyclazocine **3**, was converted to target compounds **6** (45%) and **8** (33%) using the Pd-catalyzed conditions shown. Lastly, target **7** was made in 85% yield by treating activated ester **11**⁷ with NH₂(CH₂)₂(3-pyridinyl-4-C₆H₅).

Target compounds were evaluated for their affinity and selectivity for human μ , δ and κ opioid receptors stably expressed in Chinese hamster ovary (CHO) cell membranes.⁸ Data are summarized in Table 1. For comparison purposes, literature opioid receptor binding affinity data⁴ for lead compound (**1**) and 8-CAC (**2**) are included. High affinity binding to the μ receptor was observed for all target compounds **4–8**. For compounds **7** and **8** where the nitrogen substitution is in the proximal phenyl ring, binding affinity (K_i values of 0.61 nM and 0.82 nM, respectively) for μ was slightly lower than that observed for lead **1** (K_i = 0.31 nM). When the nitrogen substitution was placed in the distal phenyl ring, specifically at the “C” position to provide **6**, a nearly identical K_i value for μ of 0.33 nM was observed compared to **1**. However, for analogues **4** and **5** where the CH→N switch occurs at the “A” and “B” positions of the distal aromatic ring, binding affinity was significantly increased (K_i values of 0.065 nM and 0.064 nM, respectively) compared to **1**. At the κ receptor, target compounds **4–8** displayed comparable binding affinity which was very similar (K_i values within 2-fold) to that observed for **1**. Lastly, compounds **4–8** had similar K_i values at the δ receptor, however, affinity was much lower (9- to 19-fold) compared to **1**.

Target compounds were also evaluated in [³⁵S]GTP γ S assays for functional activity at the μ and κ opioid receptors (Table 2).¹ Due to the much weaker binding affinity for the δ receptor, new compounds were not evaluated for δ functional activity. In contrast to lead compound **1** which is an antagonist at μ , all new targets compounds **4–8** were partial agonists at μ having qualitatively similar agonist and antagonist potencies. Like lead compound **1**, targets compounds **4–8** were agonists at the κ receptor; potencies (EC₅₀ values = 2.6–5.0 nM) were very similar for compounds **1**, **4**, and **6**, while compounds **5**, **7** and **8** were somewhat less potent (EC₅₀ values = 7.8–19 nM).

We used the recently published crystal structure⁹ of the mouse μ opioid receptor- β -FNA complex to study the interactions of *N*-BPE-8-CAC (**1**), 8-CAC (**2**) and the new pyridinyl derivatives **4–8** with the mouse μ -opioid receptor. It is important to note that, as β -FNA is a functional antagonist of μ signaling, the conformation of the receptor is likely in an ‘inactive’ state. Docking¹⁰ of these compounds into the unliganded binding site of the mouse μ opioid receptor (pdb: 4DKL) revealed the following: The highest scoring pose for

8-CAC (**2**) is analogous to that of β -FNA (Figure 2). The protonated amine and cyclopropylmethyl groups of the two molecules occupy identical sites within the binding cavity and make identical interactions with the receptor. Slight differences in ring conformations arising from the differences in the ring systems result in a slight difference in the geometry at which the phenol in β -FNA and the carboxamide of **2** are projected. The key differences in the interactions of the two ligands with the receptor are due to the two additional heavy atoms of the carboxamide versus the phenolic hydroxyl. In the μ opioid receptor- β -FNA complex structure, the phenolic OH hydrogen bonds to His297 of the receptor via a hydrogen bonding network through two bridging waters. Due to the larger size of the carboxamide group, neither of the bridging waters can be accommodated in its crystallographic position. In contrast, the carboxamide of **2** can now directly hydrogen-bond to His297 (Figure 2A). Depending on the protonation state of His297, either the carbonyl oxygen or the NH₂ groups of the carboxamide can be hydrogen bonded.

Due to the steric constraints, substitutions on the carboxamide nitrogen are not tolerated in the 8-CAC binding mode, requiring either an alternative binding mode or rearrangement of the protein to accommodate the modifications. For a small modification, such as a N-methyl group, only a small perturbation in the compound orientation or proximal receptor side chains may be sufficient, at a small energetic cost, however, largely retaining the primary interactions observed for 8-CAC. Larger substitutions (e.g., phenyl) likely require an alternative binding mode, losing the hydrogen bond between the carboxamide and His297, whose interactions with the protein are suboptimal relative to those of 8-CAC, translating to a significantly reduced binding affinity, consistent with the observed SAR for these type of substitutions.² For example, the N-methyl and N-phenyl analogues of 8-CAC have binding affinities to μ that are 75-fold and 2300-fold, respectively, lower than 8-CAC.²

Docking of molecules **1** and **4–8**, with larger, more lipophilic modifications at the carboxamide N, suggests that these are now capable of reaching more distant binding subsites within the receptor, thus picking up additional interactions and regaining the affinity lost through substituting the carboxamide. Because of the large size of the opioid binding pocket and the presence of multiple subsites, various binding modes with small differences in docking scores are possible (Figure 3). Thus, 8-CAC derives its potency through fewer, higher affinity, and highly specific and directional interactions, whereas the *N*-BPE-8-CAC (**1**) and its pyridinyl analogues **4–8** may achieve a similar potency through many lower affinity interactions with reduced specificity.

As the crystallographically determined μ opioid structure is in complex with an antagonist (β -FNA), some structural changes would be required to achieve an active state. As 8-CAC (**2**) and the pyridinyl analogues **4–8** of *N*-BPE-8-CAC (**1**), are functional partial agonists at the μ receptor, they would be expected to either 1) induce conformational changes of the receptor upon binding to achieve a partially active state from a single binding mode, or 2) have distribution of binding modes, some of which are active and others which are inactive. It is difficult to determine the specific conformational changes that lead to the 'active' complexes of **4–8**, however the results from this study may suggest that the multiple binding modes which are possible with an inactive form of the receptor contribute to their inability to achieve full agonism, and may be a common mechanism for partial agonists.

Valuable insights into the SAR of the 8-position substituent of 8-CAC have been made by examination of the opioid receptor binding properties and molecular modeling studies of a series of pyridinyl analogues of lead compound **1**. At the κ receptor, where there is no significant difference (< 2-fold) in the binding affinities within the set of compounds **1**, **4–8**, the CH $\rightarrow$ N switch at each of the positions of the biphenyl appendage appears to be an effective bioisosteric replacement. Within this same set of compounds at the μ receptor,

however, the CH→N switch does not consistently provide analogues having qualitatively similar binding affinities suggesting that N is not a broadly applicable bioisosteric replacement for CH at opioid receptors. With regard to receptor selectivity, affinity of target compounds **4–8** for the μ receptor is, in general, much higher than for δ and somewhat higher than κ . This differs somewhat from the profile noted for lead **1** which is considerable less selective among the three receptors. As previously mentioned, we had noted that an H-bond donor or acceptor appendage (OH or OCH₃) at the para position of the distal phenyl group of **1**, was responsible for much higher affinity for the μ receptor compared to the unsubstituted appendage. It is interesting to speculate that the high binding affinity of analogues **7** and **8** for the μ receptor compared to **4**, **5** and **6** may well be a result of a similar molecular recognition element.

In addition to gaining a better understanding of the SAR in this novel series of high affinity ligands for opioid receptors, another objective was to identify analogues with a lower clog*P* (and by inference greater aqueous solubility) than **1** without a compromise in intrinsic activity. This objective has, in fact, been realized since the clog*P* of analogues **4–8** is 4.5 compared to 5.9 for **1**. We continue to explore the SAR of lead compound **1** with respect to substitution in the distal phenyl ring and will report our results in due course.

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10. Methods: Docking of molecules to mouse μ opioid receptor: All modeling and visualization was carried out in MOE 2011.10 (Chemical Computing Group Inc., Montreal, Quebec, Canada). Receptor structure preparation: The crystal structure of the mouse μ opioid receptor complexed with the covalently linked antagonist, β -FNA (pdb: 4DKL) was used for docking. Missing sidechain atoms, terminal caps and hydrogens were added using the standard structure preparation protocol and protonate 3D methods implemented in MOE v2011.10. The amino acid side chains proximal to the co-crystallized β -FNA and were minimized using the LigX protocol with AMBER99 force field and restraints on the binding pocket atoms with tethering weight set to

5000. Ligand and solvent atoms were initially restrained with a tethering weight of 10. The restraints on the ligand and solvent atoms were removed and the system was minimized with the MMFF94x forcefield. The covalent bond between ligand β -FNA and the receptor residue K233 was removed and hydrogens and bond order were corrected for both the ligand and receptor. In subsequent calculations, the ligand and the receptor were treated separately. Solvent molecules were removed prior to docking. Docking of molecules into the unliganded μ opioid protein structure: 3D structures of the molecules were built and minimized using MOE's molecule builder with the MMFF94x force field. Flexible docking was carried out using MOE's triangle matcher method for placement, force field method for refinement (MMFF94x), and GBVI/WSA binding energy for the final scores and ranking of the docked poses. Receptor atoms were treated as fixed.

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Figure 1.
Lead structures for this study

Figure 2.

Comparison between the binding position of β -FNA in the crystal structure (yellow carbons) and the docked binding mode of 8-CAC (**2**) (green carbons) in the mouse μ opioid receptor. (A) The receptor is represented as a ribbon with helices in red and loops in white. The molecular surface of the binding site (grey), selected amino acid side chains (grey carbons), and the two waters (red spheres) providing bridging hydrogen bonds between the β -FNA phenol and His297 are included for reference. The hydrogen bond between the carboxamide of **2** and His297 is highlighted by a dashed line. (B) The protein and crystallographic waters are removed for additional clarity.

Figure 3. Docking of N-BPE-8-CAC (**1**) and analogues **4–8** into the 4DKL μ -opioid structure. (A) The top scoring binding mode for **1** (yellow), **4** (green), **5** (magenta), **6** (cyan), **7** (orange), and **8** (pink). (B) Representative binding modes for **5**, highlighting the potential subsites which the biaryl moiety can occupy.

Scheme 1.
Synthetic procedures used to make compounds **4–8**.

Table 1

Comparative opioid receptor binding data for 2,6-methano-3-benzazocine derivatives.

compd	K_i (nM $\pm$ S.E.) ^a			
	[³ H]DAMGO (μ)	[³ H]Naltrindole (δ)	[³ H]U69,593 (κ)	μ : δ : κ
1 (N-BPE-8-CAC) ^b	0.30 $\pm$ 0.036	0.74 $\pm$ 0.019	1.8 $\pm$ 0.19	1:2:6
2 (8-CAC) ^b	0.31 $\pm$ 0.03	5.2 $\pm$ 0.36	0.06 $\pm$ 0.001	1:17:0.2
4 ^c	0.065 $\pm$ 0.0089	6.7 $\pm$ 0.58	1.8 $\pm$ 0.12	1:103:28
5 ^c	0.064 $\pm$ 0.0051	8.2 $\pm$ 0.50	2.2 $\pm$ 0.043	1:128:34
6 ^c	0.33 $\pm$ 0.032	9.2 $\pm$ 1.3	3.3 $\pm$ 0.089	1:28:10
7 ^c	0.61 $\pm$ 0.14	14 $\pm$ 1.2	2.6 $\pm$ 0.12	1:23:4
8 ^c	0.82 $\pm$ 0.095	6.5 $\pm$ 0.81	1.4 $\pm$ 0.16	1:8:2

^aBinding assays used to screen compounds are similar to those previously reported (see ref. 8). Membrane protein from CHO cells that stably expressed one type of the human opioid receptor were incubated with 12 different concentrations of the compound in the presence of either 1 nM [³H]U69,593 (μ), 0.25 nM [³H]DAMGO (δ) or 0.2 nM [³H]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 [³H]U69,593 and [³H]DAMGO. Because of a slower association of [³H]naltrindole with the receptor, a 3 h incubation was used with this radioligand. Samples incubated with [³H]naltrindole also contained 10 mM MgCl₂ and 0.5 mM phenylmethylsulfonyl fluoride. Nonspecific binding was measured by inclusion of 10 μ M 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 Ecocint A scintillation fluid. For [³H]naltrindole and [³H]U69,593 binding, the filters were soaked in 0.1% polyethylenimine for at least 60 min before use. IC₅₀ values were 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) - see ref. 11. The K_d values for [³H]DAMGO, [³H]U69,593, and [³H]naltrindole were 0.56 nM, 0.34 nM, and 0.10 nM, respectively. Data are the mean $\pm$ SEM from at least three experiments performed in triplicate.

^bSee reference 4.

^cProton 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 target compounds and most intermediates and were within $\pm 0.4\%$ of theoretical values.

Table 2

EC₅₀ and E_{max} values for the stimulation of [³⁵S]GTPγS binding and IC₅₀ and I_{max} values for the inhibition of agonist-stimulated [³⁵S]GTPγS binding to the human μ and κ opioid receptors.^a

compd	Functional description	EC ₅₀ (nM)	E _{max} (% maximal stimulation)	IC ₅₀ (nM)	I _{max} (% maximal inhibition)
Mu Opioid Receptor					
DAMGO	agonist	55 ± 7	116 ± 4	NI ^b	NI
1	antagonist	NA ^c	5.6 ± 3.4	150 ± 25	99 ± 1.3
2	partial agonist	2.7 ± 0.62	24 ± 1.9	5.6 ± 0.62	76 ± 1.1
4	partial agonist	2.2 ± 0.80	33 ± 0.68	120 ± 36	79 ± 3.3
5	partial agonist	0.85 ± 0.037	28 ± 2.2	110 ± 24	83 ± 2.9
6	partial agonist	1.7 ± 0.43	31 ± 1.8	150 ± 25	87 ± 3.1
7	partial agonist	4.5 ± 0.40	30 ± 2.3	250 ± 80	87 ± 1.2
8	partial agonist	19 ± 1.4	25 ± 1.1	75 ± 35	53 ± 5.4
Kappa Opioid Receptor					
U50,488	agonist	36 ± 5.0	77 ± 11	NI	NI
1	agonist	3.0 ± 0.50	76 ± 6.7	NI	NI
2	agonist	4.4 ± 0.73	87 ± 6.5	NI	NI
4	agonist	5.0 ± 3.0	64 ± 5.1	NI	NI
5	agonist	19 ± 4.1	52 ± 0.55	NI	NI
6	agonist	2.6 ± 0.27	50 ± 2.6	NI	NI
7	agonist	7.8 ± 1.2	52 ± 4.8	NI	NI
8	agonist	14 ± 1.4	64 ± 2.4	NI	NI

^aSee reference 1 for experimental details. Data are the mean values ± S.E.M. from at least three separate experiments, performed in triplicate. For calculation of the E_{max} values, the basal [³⁵S]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.

^bNI → No Inhibition.

^cNA → Not applicable.