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Redefining the structure-activity relationships of 2,6-methano-3-benzazocines. Part 8. High affinity ligands for opioid receptors in the picomolar K_i range: Oxygenated *N*-(2-[1,1'-biphenyl]-4-ylethyl) analogues of 8-CAC‡

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Abstract

N-(2-(4'-methoxy[1,1'-biphenyl]-4-yl)ethyl)-8-CAC (**1**) is a high-affinity ($K_i = 0.084$ nM) ligand for the μ opioid receptor and served as the lead compound for this study. Analogues of **1** were made in hopes of identifying an SAR within a series of oxygenated (distal) phenyl derivatives. A number of new analogues were made having single-digit pM affinity for the μ receptor. The most potent was the 3',4'-methylenedioxy analogue **18** ($K_i = 1.6$ pM).

The broad goal of our research is to identify orally-available, long-acting modulators of μ and κ opioid G-protein coupled receptors as potential anticocaine medications. In 2001, we reported the synthesis and biological properties of 8-carboxamidocyclazocine (8-CAC; **2** in Figure 1), an opioid modulator having a novel carboxamido (CONH₂) group at the 8-position of the 2,6-methano-3-benzazocine core in place of the prototypic (of opioids) phenolic-OH of cyclazocine (**3**).¹ At that time, cyclazocine was undergoing clinical evaluation to treat cocaine addiction, however, it was beset by having a short duration of action in humans.^{2,3} 8-CAC displays similar binding affinities to μ and κ receptors as cyclazocine and has a much longer duration of action in a mouse antinociception model,⁴ however; its utility as an anticocaine medication is questioned by having an undesirable side effect profile.⁵

While studying the effects of *N*-substitution of the carboxamido group of 8-CAC on binding affinity, we observed that affinity for opioid receptors significantly decreased upon mono-substitution (e.g., 8-CONHCH₃, 8-CONHPh) or di-substitution (e.g., 8-CON(CH₃)₂).¹ The discovery that an *N*-(2-[1,1'-biphenyl]-4-ylethyl) (a.k.a. *N*-BPE in this work) appendage (i.e., **4**) gave comparable binding affinity to μ as the unsubstituted carboxamide was clearly

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an unexpected finding based on our previous experience.⁶ Preliminary SAR studies regarding the substitution of the distal phenyl ring of **4** subsequently revealed that a 4'-methoxy group was very beneficial (3.7-fold decrease in K_i value) for binding affinity to μ and it is this derivative **1** that served as the lead compound for the current study. Herein we report the syntheses and pharmacological characterizations of oxygenated (distal) phenyl analogues of **1** to probe the SAR of this exciting series of high affinity ligands.

As shown in the Scheme 1, novel target compounds **5-13** and **15-20** were made from the racemic 4-bromophenethyl intermediate **23**⁷ using standard Suzuki coupling conditions (Methods A or B) in yields of 14–61%. All reagents, including boronic acids or esters, were commercially available. The optically active enantiomer **14** of racemate **13**, was made from the [(–)-[(2*R*,6*R*,11*R*)]-triflate ester of cyclazocine⁸ via a procedure very similar to that used to make **13**. The last coupling step was accomplished in 22% yield using Method B. Lastly, the methoxy-substituted naphthalene analogue **21** was prepared from the triflate ester (**24**) of cyclazocine⁸ and 6-methoxy-2-naphthaleneethanamine⁹ using the known conditions⁷ summarized in Scheme 2.

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. Data are summarized in Table 1. For comparison purposes, literature opioid receptor binding affinity data for lead compound **1**, 8-CAC (**2**), cyclazocine (**3**) and the unsubstituted *N*-BPE derivative **4** are included.⁷ High affinity binding to the μ receptor was observed for all new *N*-BPE target compounds **5-20**. With one exception, K_i values were < 1.0 nM and five compounds had values that were single digit picomolar. New compounds **5-20** displayed higher selectivity for the μ receptor over δ and κ receptors (see selectivity ratios in Table 1). Analogue **18** exhibited the highest selectivity for μ having a μ : δ : κ K_i ratio of 1:625:456. There was little consistency in this group of compounds as far as selectivity between δ and κ .

In the first set of structural variations, we studied the effect of methoxy substitution at different sites on the distal phenyl ring on binding affinity at μ . The 3'-derivative **5** was 5-fold more potent than the 4'-substituted lead **1** while the 2'-methoxy derivative **6** and **1** were equipotent. We also studied the effects of other alkoxy substituents at the 4'-position. Of the variations studied, the methoxy derivative **1** was 7.6-, 2.7-, 21- and 3.6-fold more potent than those analogues with 4' substituents being OCH₂CH₃ (**7**), OCH(CH₃)₂ (**8**), OCF₃ (**11**), and OCHF₂ (**12**), respectively. The only mono-4'-substituted compound to show better potency than lead **1** was the des-alkyl derivative **13**. The K_i value for this 4'-OH analogue **13** is a notable 0.0056 nM which represents a 15-fold increase in binding affinity at μ compared to **1**.

We previously reported the eudismic ratio of the enantiomers of the original lead *N*-BPE compound **4** was 26 at the μ receptor with the (–)-[(2*R*,6*R*,11*R*)]-enantiomer being the eutomer.⁶ Since SAR in this series was tracked using racemates, we wanted to confirm in at least one example, that the bulk of the observed activity of a racemate resided in (2*R*,6*R*,11*R*)-enantiomer. To that end, we made and tested the (–)-[(2*R*,6*R*,11*R*)]-enantiomer **14** of the highly potent 4'-hydroxy racemic derivative **13** and found K_i values of the optically active partner slightly less than those of the racemate against all three opioid receptors. Within the variability of the assay where a 2-fold difference in K_i values is not considered significantly different, these data confirm that, in at least one example, the (2*R*,6*R*,11*R*)-enantiomer exhibits very high potency.

For the 4'-isopropoxy and 4'-OH derivatives, **8** and **13**, respectively, we also studied the attachment of the group at the 3'- and 2'-positions on the distal phenyl. In contrast to the

trio of methoxy analogues **1**, **5** and **6** where the 3'-derivative was the most potent, in the hydroxy case, the 3'-derivative **15** was least potent having a K_i value 4-fold higher than both the 4'-OH (**13**) and 2'-OH (**16**) regioisomers. For the isopropoxy substituent, the K_i value for the 4'-isomer **8** was within 2-fold of the values for the 3'- and 2'-isomers, **9** and **10**, respectively.

We also studied the effects of 3',4'- and 2',4'-disubstitution on the distal phenyl ring. Addition of a methoxy group at the 3'-position of **1** to provide **17** resulted in a 12-fold increase in binding affinity at the μ receptor. An even greater enhancement of potency was realized when the 3',4'-methylenedioxy group was introduced on the distal phenyl ring. The resulting compound, **18**, exhibited a $K_i = 0.0016$ nM which represents a 53-fold increase in potency relative to **1**. We also studied methyl substitution at the 3'- and 2'-positions of lead **1** giving targets **19** and **20**, respectively. Compared to **1**, binding affinity at μ for **19** was comparable and was slightly decreased (2.7-fold) for **20**.

In our original report⁷ describing the discovery of lead compound **1**, we also provided data for the highly potent *N*-[2-(2-naphthalenyl)ethyl]-analogue **22** ($K_i = 0.18$ nM Table 1). In the current study, we investigated the effect of methoxy substitution at the 6'-position on the naphthalene ring of **22** resulting in **21**. Compared to **22**, binding affinity of **21** for μ increased 2.8-fold.

A number of target compounds were also evaluated in [³⁵S]GTP γ S assays for functional activity at the μ , κ and δ opioid receptors (Table 2). Like lead compound **1**, all new *N*-BPE derivatives tested (**5**, **8**, **14**, **16-19**) were partial agonists at the μ receptor, however, potencies for receptor stimulation (EC_{50} 's) were considerably more potent (4- to 200-fold) than **1**. In contrast to the partial agonist activity observed for the *N*-BPE derivatives, the 6'-methoxy naphthalene derivative **21** was an antagonist of moderate potency ($IC_{50} = 45$ nM). Like lead compound **1**, new compounds tested were agonists at the δ receptor with EC_{50} values in the 0.16 to 18 nM range. One exception is compound **17** which was a partial agonist at the δ receptor. Compound **17** had an I_{max} value of 32% in inhibiting agonist-stimulated [³⁵S]GTP γ S binding. When tested alone, compound **17** had an E_{max} value of 73%. These data demonstrate that compound **17** had both agonist and antagonist properties. A compound that produced both agonist and antagonist activity in our assay was regarded as a partial agonist. At the κ receptor, all compounds tested, included **1**, were agonists with EC_{50} values in the 0.086 to 5.4 nM range.

Certain trends in the SAR emerged during the course of this study. For 4'-monosubstituted *N*-BPE analogues, the rank order (from highest to lowest) of potency of these compounds (plus two from an earlier study⁷) is the following: OH (**13**) > OCH₃ (**1**) > OCH(CH₃)₂ (**8**) ~ Cl⁷ ~ CH₃⁷ ~ OCHF₂ (**12**) > OCH₂CH₃ (**7**) > OCF₃ (**11**). While certainly not a perfect correlation, a trend is evident that electron donating groups are beneficial for binding to the μ receptor. This trend is corroborated by the observation that addition of an alkoxy group at the 3'-position of **1** significantly enhances binding affinity (i.e., 3',4'-OCH₂O- (**18**) > 3',4'-OCH₃ (**17**) >> 4'-OCH₃ (**1**)). Despite the fact that substituents are tolerated at the 2'- or 3'-positions of the *N*-BPE group, however, substitution of **1** with a mildly electron donating methyl group whether it is at the 3'-position (**19**) or 2'-position (**20**) provides no benefit. From this limited data set, there is a tendency that a single electron donating alkoxy or hydroxy group is beneficial at any position on the distal phenyl ring as evidenced by the following rank orders: when Y = OCH₃ (3' > 4' ~ 2'); Y = OCH(CH₃)₂ (4' ~ 2' ~ 3'); and Y = OH (4' ~ 2' > 3'). Also, SAR appears to be additive regarding the benefit of a methoxy group on the distal part of the *N*-substituent as evidenced by 4'-OCH₃ (**1**) > 4'-H (**4**) by 3.7-fold when *N*-BPE and 4'-OCH₃ (**21**) > 4'-H (**22**) by 2.8-fold when *N*-2-naphthaleneethyl.

In conclusion, valuable insights into opioid SAR have been made by studying the opioid receptor binding properties of a series of 8-CAC analogues where the distal regions of the carboxamide *N*-substituent have been modified. In doing so, we have discovered a number of novel derivatives having single-digit pM K_i values for the μ receptor. We continue to explore the SAR of lead compound **1** to corroborate/refute our current hypothesis that the BPE group of **1** occupies previously unexplored receptor space in opioid receptors that is largely hydrophobic in nature and deep within this pocket lies the potential for polar non-covalent interactions (e.g., π -cation, H-bond acceptor/donor) responsible for enhancing binding affinity.

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Figure 1.

Scheme 1.

Scheme 2.

Table 1

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

compd ^b	Y	<i>K_i</i> (nM ± S.E.) ^d			μ:δ:κ ^c
		[³ H]DAMGO (μ)	[³ H]Naltrexole (δ)	[³ H]U69,593 (κ)	
1 ^d	4'-OCH ₃	0.084 ± 0.012	0.18 ± 0.022	1.5 ± 0.10	1:2:18
2 ^d	8-CAC	0.31 ± 0.03	5.2 ± 0.36	0.06 ± 0.001	1:17:0.2
3 ^d	Cyclazocine	0.16 ± 0.01	2.0 ± 0.22	0.07 ± 0.01	1:13:0.4
4 ^d	H	0.30 ± 0.036	0.74 ± 0.019	1.8 ± 0.19	1:2:6
5	3'-OCH ₃	0.018 ± 0.0009	1.3 ± 0.030	0.22 ± 0.016	1:72:12
6	2'-OCH ₃	0.12 ± 0.021	0.84 ± 0.082	0.38 ± 0.0078	1:7:3
7	4'-OCH ₂ CH ₃	0.64 ± 0.058	3.4 ± 0.039	3.3 ± 0.32	1:5:5
8	4'-OCH(CH ₃) ₂	0.23 ± 0.041	1.9 ± 0.21	1.6 ± 0.11	1:8:7
9	3'-OCH(CH ₃) ₂	0.43 ± 0.041	3.9 ± 1.4	2.4 ± 0.25	1:9:6
10	2'-OCH(CH ₃) ₂	0.12 ± 0.0018	0.55 ± 0.025	1.8 ± 0.17	1:8:15
11	4'-OCF ₃	1.8 ± 0.038	6.6 ± 0.21	5.2 ± 0.48	1:4:3
12	4'-OCF ₂	0.3 ± 0.017	0.80 ± 0.10	2.7 ± 0.075	1:3:9
13	4'-OH	0.0056 ± 0.00073	0.81 ± 0.12	0.49 ± 0.011	1:145:88
14		0.0049 ± 0.001	0.78 ± 0.05	0.36 ± 0.018	1:160:74
15	3'-OH	0.021 ± 0.00084	1.2 ± 0.024	1.3 ± 0.050	1:57:62
16	2'-OH	0.0056 ± 0.00064	0.24 ± 0.017	2.1 ± 0.12	1:43:375
17	3',4'-(OCH ₃) ₂	0.0071 ± 0.00043	1.5 ± 0.12	1.3 ± 0.075	1:211:183
18	3',4'-OCH ₂ O	0.0016 ± 0.0034	1.0 ± 0.12	0.73 ± 0.049	1:625:456

21: Z = OCH₃
22: Z = H

		K_i (nM $\pm$ S.E.) ^a			
compd ^b	Y	[³ H]DAMGO (μ)	[³ H]naltrindole (δ)	[³ H]U69,593 (κ)	$\mu:\delta:\kappa$ ^c
19	3'-CH ₃ , 4'-OCH ₃	0.059 $\pm$ 0.0050	1.5 $\pm$ 0.14	1.7 $\pm$ 0.14	1:25:29
20	2'-CH ₃ , 4'-OCH ₃	0.23 $\pm$ 0.0056	1.1 $\pm$ 0.18	1.3 $\pm$ 0.11	1:5:6
21		0.064 $\pm$ 0.003	2.2 $\pm$ 0.15	0.45 $\pm$ 0.028	1:34:7
22 ^d		0.18 $\pm$ 0.009	0.90 $\pm$ 0.020	0.20 $\pm$ 0.056	1:5:1

^a Binding assays used to screen compounds are similar to those previously reported (see ref. 10). 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 Ecoscint 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.

^b Proton NMR, IR and MS were consistent with the assigned structures of all new compounds. Purity for new compounds was assessed by LC/MS (98%) or C, H, and N elemental analyses (experimental values within $\pm 0.4\%$ of theoretical values).

^c $\mu:\delta:\kappa = [K_i(\mu)/K_i(\mu)]: [K_i(\delta)/K_i(\mu)]: [K_i(\kappa)/K_i(\mu)]$

^d See reference 7.

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, δ and κ opioid receptors.^a

Table 2

cmpd	Functional description	EC ₅₀ (nM)	E _{max} (% maximal stimulation)	IC ₅₀ (nM)	I _{max} (% maximal inhibition)
<u>Mu Opioid Receptor</u>					
DAMGO	agonist	55 ± 7	116 ± 4	NI ^b	NI
1	partial agonist	11 ± 2.4	27 ± 1.3	24 ± 7.0	82 ± 9.3
5	partial agonist	0.22 ± 0.04	25 ± 1.2	27 ± 7.5	75 ± 2.5
8	partial agonist	2.3 ± 0.60	35 ± 0.75	13 ± 2.6	72 ± 2.1
14	partial agonist	0.50 ± 0.0084	31 ± 0.93	4.5 ± 1.3	62 ± 2.6
16	partial agonist	1.5 ± 0.68	48 ± 0.68	64 ± 11	67 ± 0.85
17	partial agonist	1.6 ± 0.28	32 ± 1.3	29 ± 1.0	70 ± 0.60
18	partial agonist	0.15 ± 0.0098	29 ± 0.99	11 ± 2.0	75 ± 0.22
19	partial agonist	0.60 ± 0.14	21 ± 2.5	30 ± 9.2	81 ± 5.7
21	antagonist	NA ^c	6.6 ± 1.6	45 ± 14	76 ± 5.2
<u>Delta Opioid Receptor</u>					
SNC 80	agonist	4.8 ± 0.60	120 ± 4.7	NI	NI
1	agonist	0.68 ± 0.15	70 ± 3.6	NI	NI
5	agonist	1.2 ± 0.45	53 ± 1.2	NI	NI
8	agonist	18 ± 5.4	88 ± 4.9	NI	NI
14	agonist	0.31 ± 0.036	64 ± 2.7	NI	NI
16	agonist	0.16 ± 0.049	86 ± 0.67	NI	NI
17	partial agonist	6.2 ± 0.81	73 ± 1.4	70 ± 14	32 ± 1.8
18	agonist	0.80 ± 0.26	75 ± 2.1	NI	NI
19	agonist	2.8 ± 0.21	55 ± 2.1	NI	NI
21	agonist	7.5 ± 3.6	32 ± 2.7	NI	NI
<u>Kappa Opioid Receptor</u>					
U50,488	agonist	36 ± 5.0	77 ± 11	NI	NI
1	agonist	1.1 ± 0.24	98 ± 4.4	NI	NI
5	agonist	3.1 ± 0.38	59 ± 0.18	NI	NI
8	agonist	5.4 ± 0.72	79 ± 2.7	NI	NI

compd	Functional description	EC ₅₀ (nM)	E _{max} (% maximal stimulation)	IC ₅₀ (nM)	I _{max} (% maximal inhibition)
14	agonist	0.26 ± 0.066	100 ± 2.8	NI	NI
16	agonist	0.086 ± 0.0020	110 ± 0.33	NI	NI
17	agonist	3.0 ± 0.15	100 ± 2.6	NI	NI
18	agonist	0.34 ± 0.033	55 ± 0.34	NI	NI
19	agonist	0.62 ± 0.11	55 ± 2.8	NI	NI
21	agonist	1.3 ± 0.34	79 ± 1.8	NI	NI

^aSee reference 6 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, U50,488 at final concentration of 100 nM was used for the κ receptor and 10 nM SNC 80 was used to measure inhibition of [³⁵S]GTPγS binding mediated by the κ opitoid receptor.

^bNI → No Inhibition.

^cNA → Not applicable.