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Redefining the structure–activity relationships of 2,6-methano-3-benzazocines. 5. Opioid receptor binding properties of *N*-((4′-phenyl)-phenethyl) analogues of 8-CAC

Melissa A. VanAlstine,[a] Mark P. Wentland,[a,*] Dana J. Cohen[b] and Jean M. Bidlack[b]

[a]*Department of Chemistry and Chemical Biology, Rensselaer Polytechnic Institute, Troy, NY 12180, USA*
[b]*Department of Pharmacology and Physiology, School of Medicine and Dentistry, University of Rochester, Rochester, NY 14642, USA*

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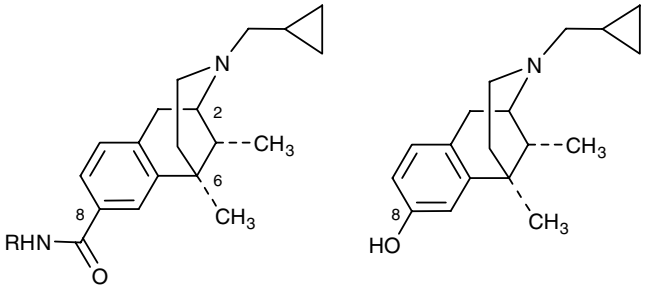
Abstract—A series of aryl-containing *N*-monosubstituted analogues of the lead compound 8-[*N*-((4′-phenyl)-phenethyl)]-carboxamidocyclazocine were synthesized and evaluated to probe a putative hydrophobic binding pocket of opioid receptors. Very high binding affinity to the μ opioid receptor was achieved though the *N*-(2-(4′-methoxybiphenyl-4-yl)ethyl) analogue of 8-CAC. High binding affinity to μ and very high binding affinity to κ opioid receptors was observed for the *N*-(3-bromophenethyl) analogue of 8-CAC. High binding affinity to all three opioid receptors were observed for the *N*-(2-naphthylethyl) analogue of 8-CAC.

We recently reported the synthesis and opioid receptor binding properties of **1**, an analogue of 8-carboxamidocyclazocine (8-CAC, **2**), having an *N*-((4′-phenyl)-phenethyl) substitution.[1] 8-CAC is a long-acting[2] analogue of cyclazocine (**3**)[3] with high affinity for μ and κ opioid receptors[4] having the potential to treat cocaine addiction in humans. Based on the long-standing knowledge that a phenolic hydroxyl group was required for the high affinity binding of many opioid receptor interactive ligands,[5] 8-CAC's high affinity for μ and κ opioid receptors was unexpected. Until recently all substitution of the carboxamide nitrogen of **2** were detrimental toward opioid receptor binding except *N*-((4′-phenyl)-phenethyl) (**1**), which produced high binding affinity to μ and δ, and moderate affinity to κ[1] (Fig. 1).



1: R = CH_2CH_2-4-$C_6H_4C_6H_5$ **3** (Cyclazocine)
2: R = H (8-CAC)

Figure 1. Structures of lead compounds for this study.

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**, we now report the synthesis and opioid receptor binding properties of a series of *N*-monosubstituted carboxamide analogues of 8-CAC (Table 1). Design of targets was based on the substitution on either of the aryl rings, both in its nature and its placement with respect to the ethylene linker. Specifically, we chose derivatives with different attachment points, substituents, and analogues where the aryl group was switched from biphenyl to the naphthyl or bromophenyl.

Using a one-step procedure (Scheme 1), novel racemic targets **7**–**9**, **11**, and **14**–**19** were conveniently made by treatment of triflate **4**[6] with the appropriate amine, dichloro[1,1′-bis(diphenylphosphino)-ferrocene] palladium (II) dichloromethane adduct, triethylamine, and carbon monoxide in dimethylsulfoxide. Amines were commercially available or made using known procedures.

Keywords: Opiate; SAR.
* Corresponding author. Tel.: +1 518 276 2234; fax: +1 518 276 4887; e-mail: wentmp@rpi.edu

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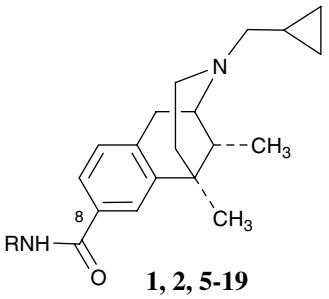
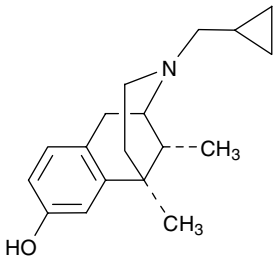
Table 1. Comparative opioid receptor binding data for 2,6-methano-3-benzazocine derivatives



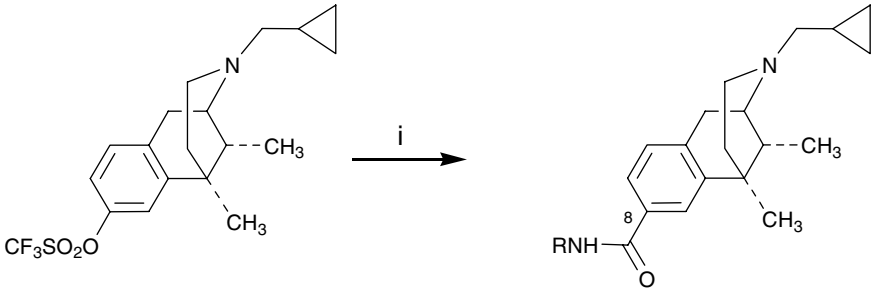
1, 2, 5-19



3 (Cyclazocine)

Compound	R	K_i[a] (nM)		
		[^{3}H]DAMGO (μ)	[^{3}H]Naltrindole (δ)	[^{3}H]U69,593 (κ)
1[b]	$(CH_2)_2(4\text{-}C_6H_4C_6H_5)$	0.30 ± 0.036	0.74 ± 0.019	1.8 ± 0.19
2[c]	H	0.31 ± 0.03	5.2 ± 0.36	0.06 ± 0.001
3[d]	Cyclazocine	0.16 ± 0.01	2.0 ± 0.22	0.07 ± 0.002
5[e]	$(CH_2)_2(3\text{-}C_6H_4C_6H_5)$	0.95 ± 0.15	5.9 ± 1.2	2.2 ± 0.14
6[e]	$(CH_2)_2(2\text{-}C_6H_4C_6H_5)$	6.7 ± 0.49	21 ± 3.1	2.4 ± 0.28
7[e]	$(CH_2)_2(4\text{-}BrC_6H_4)$	2.4 ± 0.33	2.5 ± 0.28	0.38 ± 0.060
8[e]	$(CH_2)_2(3\text{-}BrC_6H_4)$	0.35 ± 0.021	3.5 ± 0.19	0.063 ± 0.006
9[e]	$(CH_2)_2(2\text{-}BrC_6H_4)$	4.0 ± 0.36	150 ± 6.2	19 ± 1.3
10[e]	$(CH_2)_2(4\text{-}C_6H_4\text{-}4\text{-}CH_3OC_6H_4)$	0.084 ± 0.012	0.18 ± 0.022	1.5 ± 0.10
11[e]	$(CH_2)_2(4\text{-}C_6H_4\text{-}4\text{-}ClC_6H_4)$	0.20 ± 0.038	0.71 ± 0.046	3.2 ± 0.67
12[e]	$(CH_2)_2(4\text{-}C_6H_4\text{-}3,4\text{-}Cl_2C_6H_4)$	0.98 ± 0.13	2.5 ± 0.38	1.1 ± 0.087
13[e]	$(CH_2)_2(4\text{-}C_6H_4\text{-}4\text{-}CH_3C_6H_4)$	0.29 ± 0.075	0.72 ± 0.027	3.3 ± 0.20
14[e]	CH_2(2-naphthyl)	1.3 ± 0.29	31 ± 2.0	19 ± 1.7
15[e]	$(CH_2)_2$(2-naphthyl)	0.18 ± 0.009	0.90 ± 0.020	0.20 ± 0.056
16[e]	$(CH_2)_3$(2-naphthyl)	1.9 ± 0.19	18 ± 1.2	0.18 ± 0.016
17[e]	CH_2(1-naphthyl)	2.5 ± 0.31	52 ± 3.4	18 ± 0.63
18[e]	$(CH_2)_2$(1-naphthyl)	4.2 ± 0.13	24 ± 1.2	2.4 ± 0.46
19[e]	$(CH_2)_3$(1-naphthyl)	2.4 ± 0.45	18 ± 1.0	1.9 ± 0.077

[a] The K_d values for [^{3}H]DAMGO, [^{3}H]U69,593, and [^{3}H]naltrindole were 0.56, 0.34, and 0.10 nM, respectively. These values were used to calculate the K_i values.
[b] See Ref. 1.
[c] See Ref. 4.
[d] See Ref. 3.
[e] 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.



4

7: R = $(CH_2)_2(4\text{-}BrC_6H_4)$
8: R = $(CH_2)_2(3\text{-}BrC_6H_4)$
9: R = $(CH_2)_2(2\text{-}BrC_6H_4)$
11: R = $(CH_2)_2(4\text{-}C_6H_4\text{-}4\text{-}ClC_6H_4)$
14: R = CH_2(2-naphthyl)
15: R = $(CH_2)_2$(2-naphthyl)
16: R = $(CH_2)_3$(2-naphthyl)
17: R = CH_2(1-naphthyl)
18: R = $(CH_2)_2$(1-naphthyl)
19: R = $(CH_2)_3$(1-naphthyl)

Scheme 1. Syntheses of target compounds via Pd-catalyzed carboxamidation procedures. Reagents and condition: (i) RNH_2, $PdCl_2$(dppf), Et_3N, DMSO, CO, 70 °C.

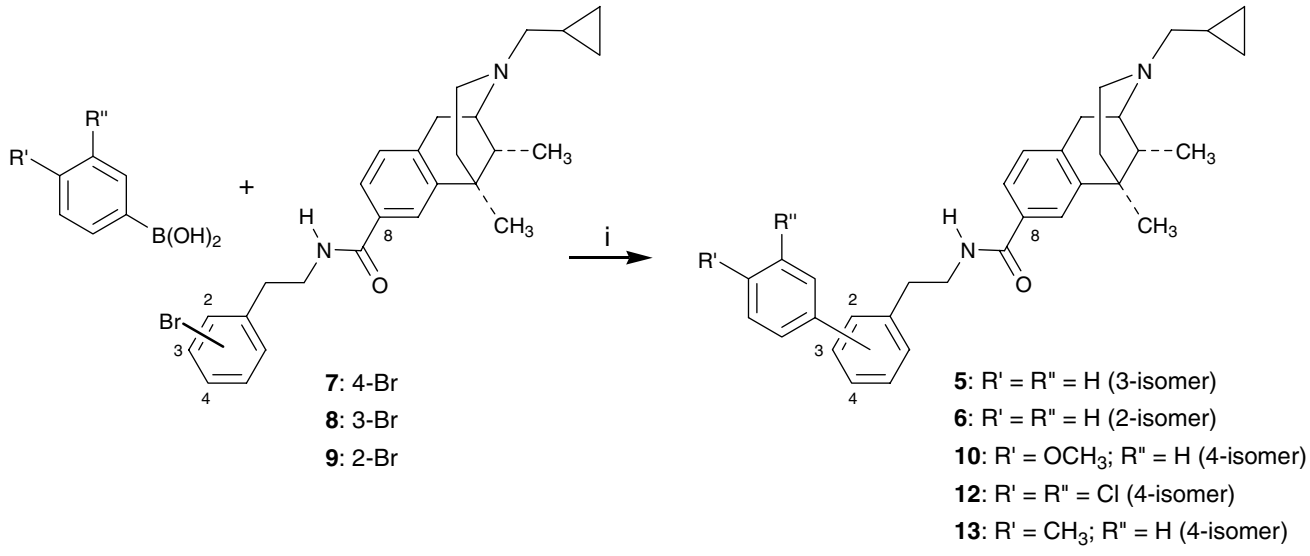
As shown in Scheme 2, targets **5** and **6** were prepared by treating **8** and **9**, respectively, with phenylboronic acid, palladium acetate, triphenylphosphine, and sodium carbonate in toluene (microwaves) at 120 °C for 20 min. Targets **10**, **12**, and **13** were similarly prepared from **7** using 4-methoxyphenylboronic acid, 3,4-dichlorophenylboronic acid, and 4-methylphenylboronic acid, respectively. Yields in these Suzuki couplings were in the 64–80% range.

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.[7] Data are summarized in Table 1. All compounds in Table 1 are racemic including cyclazocine. For comparison purposes, opioid binding affinity data for 8-[*N*-((4′-phenyl)-phenethyl)]-CAC (**1**),[1] 8-CAC (**2**),[4] and cyclazocine (**3**)[3] are included. As stated previously, *N*-((4′-phenyl)-phenethyl) substitution on the carboxamide group of 8-CAC had similar binding affinity for μ, slightly weaker for δ and 30-fold less affinity for κ opioid receptors, compared to 8-CAC (**2**). The optimal linker length between the carboxamide nitrogen and the biphenyl group has been shown to be a two-methylene spacer.[1] To test the optimal orientation of the distal phenyl group, the *N*-((3′-phenyl)-phenethyl) and *N*-((2′-phenyl)-phenethyl) analogues **5** and **6**, respectively, were synthesized. When the distal phenyl group was in the 3-position (**5**), binding affinity decreased only slightly for μ, δ, and κ (3-, 8-, and 1.2-fold, respectively) compared to **1**. However, when the distal phenyl group is in the 2-position (**6**) binding affinity is significantly decreased by 22-, 28-, and 1.3-fold for μ, δ, and κ receptors, respectively. These data suggest the receptors can accommodate the distal phenyl in the 2- and 3-positions; however, the 4-position is optimal.

To ascertain whether the role of the distal phenyl group in binding was mainly hydrophobics, π–π stacking or both, the corresponding bromophenyl analogues **7**–**9** were synthesized. When the distal phenyl group of **1** was replaced by bromide (**7**), binding affinity was reduced for μ and δ, 8- and 3-fold, respectively, but increased 5-fold for κ. This was the first instance of subnanomolar potency for the κ opioid receptor in this series. The 2- and 3-bromophenethyl analogues of 8-CAC were synthesized as well. Compared to **1**, the 3-bromophenethyl analogue **8** showed comparable affinity for μ, 5-fold decreased affinity for δ, and 29-fold increased affinity for κ. With a binding affinity of 0.063 nM, compound **8** had comparable affinity for the κ receptor as cyclazocine (**3**). The 2-bromophenethyl analogue **9** had decreased affinity for μ, δ, and κ receptors, 13-, 203-, and 10-fold, respectively, compared to **1**.

The Topliss approach[8] was applied to this series and the 4′-methoxy, 4′-chloro, 3′,4′-dichloro, and 4′-methyl analogues of **1** were synthesized. The 4′-methoxy analogue **10** had 4-fold increased binding affinity for μ and δ opioid receptors compared to **1** while against κ receptors, similar affinity was observed. The 4′-chloro and 4′-methyl analogues, **11** and **13**, respectively, had very similar binding to all three opioid receptors compared to **1**. The 3′,4′-dichloro analogue **12** showed 3-fold lower affinity to both μ and δ opioid receptors and similar affinity to the κ opioid receptor compared to **1**. From this activity pattern at the μ receptor, the probable operative parameter is deduced to be $-\pi$, implying that a more hydrophilic group would increase potency.

A series of naphthyl derivatives of **1** were made to further probe this putative hydrophobic interaction with the receptors. The 2-naphthylmethyl analogue **14** showed decreased binding affinity for μ, δ, and κ (4-, 42-, and 11-fold, respectively). The 2-naphthylethyl analogue **15** showed similar binding affinity to μ and δ opioid receptors compared to **1** and affinity increased by 9-fold for the κ opioid receptor. The 2-naphthylpropyl analogue **16** had decreased affinity for μ and δ, 6- and 24-fold, respectively, but increased affinity (10-fold) for κ.



Scheme 2. Syntheses of target compounds via Suzuki coupling. Reagents and condition: (i) $Pd(OAc)_2$, PPh_3, Na_2CO_3, tol, microwaves (20 W), 20 min, 120 °C.

All three 1-naphthyl analogues of 8-CAC, 1-naphthylmethyl (**17**), 1-naphthylethyl (**18**), and 1-naphthylpropyl (**19**) had decreased affinity for μ (8- to 14-fold), δ (24- to 70-fold), and κ (1- to 10-fold) opioid receptors compared to **1**.

In [^{35}S]GTPγS functional assays (Table 2),[1] compound **1** showed antagonist properties at μ while the 4-bromo analogue **7** showed only agonist properties at μ receptor. Compounds **5**, **6**, **8**, **10**, **11**, and **16** showed partial agonist properties as well as antagonist properties at μ receptor. All of the compounds were pure agonists at the κ opioid receptor. They had similar E_{max} values, ranging from 87% to 110% stimulation over control, which were slightly greater than the E_{max} value of 77% stimulation produced by the κ-selective agonist U50,488. There was not a strong correlation between the EC_{50} values for stimulating [^{35}S]GTPγS binding mediated by the κ receptor and the K_i values for the inhibition of [^{3}H]U69,593 binding to the κ receptor. For example, compounds **8** and **16** had the highest affinity for the κ receptor in the receptor binding assay. In the [^{35}S]GTPγS binding assay, compounds **1**, **8**, **10**, **11**, and **16** had similar EC_{50} values. Due to their lower affinity for the δ receptor, compounds **6** and **16** were not tested in the [^{35}S]GTPγS binding assay in CHO membranes expressing the δ opioid receptor. As observed with the κ receptor, compounds **1**, **5**, **7**, **8**, **10**, and **11** were shown to be pure agonists at the δ opioid receptor. Compound **10**, which had the highest binding affinity for the δ receptor within the series, also was the most potent at δ in the [^{35}S]GTPγS assay. Other than this observation, there was little correlation at the δ opioid receptor between binding affinity and functional activity.

Valuable insights into the SAR of the 8-position substituent of 8-CAC have been made by examination of the opioid receptor binding properties of a series of *N*-monosubstituted carboxamide analogues of 8-CAC. Our observation that the *N*-((3′-phenyl)-phenethyl) and *N*-((2′-phenyl)-phenethyl) groups were less potent than the *N*-((4′-phenyl)-phenethyl) analogue leads us to believe that the alkyl biaryl groups need to be in a near-linear arrangement. The loss in activity in the 4-bromophenylethyl analogue **7** compared to **1** suggests

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, κ, and δ 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
1	NA[c]	5.6 ± 3.4	150 ± 25	99 ± 1.3
5	6.8 ± 2.7	41 ± 2.3	NA	72 ± 11 at 10 μM
6	12 ± 2.8	36 ± 8.3	NA	73 ± 4.0 at 10 μM
7	16 ± 1.7	73 ± 5.3	NI	NI
8	5.6 ± 0.93	24 ± 2.0	230 ± 36	85 ± 8.1
10	11 ± 2.4	27 ± 1.3	24 ± 7.0	82 ± 9.3
11	2.5 ± 0.73	31 ± 2.2	NA	71 ± 5.6 at 10 μM
16	24 ± 5.0	31 ± 0.62	320 ± 62	78 ± 3.4
δ *Opioid receptor*				
SNC 80	4.8 ± 0.60	120 ± 4.7	NI	NI
1	3.0 ± 0.24	69 ± 8.6	NI	NI
5	18 ± 3.7	43 ± 3.4	NI	NI
6	NT[d]	NT	NT	NT
7	20 ± 5.0	120 ± 21	NI	NI
8	14 ± 3.5	85 ± 1.4	NI	NI
10	0.68 ± 0.15	70 ± 3.6	NI	NI
11	2.0 ± 0.027	45 ± 5.5	NI	NI
16	NT	NT	NT	NT
κ *Opioid receptor*				
U50,488	36 ± 5.0	77 ± 11	NI	NI
1	4.4 ± 0.73	87 ± 6.5	NI	NI
5	12 ± 1.7	95 ± 1.8	NI	NI
6	42 ± 3.0	97 ± 3.4	NI	NI
7	20 ± 1.5	110 ± 14	NI	NI
8	4.6 ± 0.40	110 ± 4.3	NI	NI
10	1.1 ± 0.24	98 ± 4.4	NI	NI
11	6.4 ± 2.2	96 ± 7.1	NI	NI
16	6.3 ± 0.30	98 ± 10	NI	NI

[a] See Ref. 1. Data are 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. SNC 80 and U50,488 at final concentrations of 10 and 100 nM were used for the δ and κ receptors, respectively.

[b] NI, no inhibition.

[c] NA, not applicable.

[d] NT, not tested.

that molecular recognition may not be purely hydrophobic in nature but could also involve π–π stacking. Within this series of *N*-monosubstituted carboxamide analogues of 8-CAC, the 3-bromophenylethyl analogue **8** was the first in this series to show subnanomolar affinity to the κ receptor. Application of the Topliss approach showed the physicochemical parameter $-\pi$ appears to be the most important physicochemical parameter for binding affinity. This information will guide future efforts. Evaluation of napthyl derivatives **14**–**19** showed that binding affinity was much higher when the naphthyl ring was attached to the linker via the 2-position and that the two carbon linker (ethylene) was optimal.

Results from this study will facilitate the design of new high affinity opioid receptor ligands. The synthesis and evaluation of new targets related to **1** is ongoing in our laboratories to further explore this novel SAR. New targets will include those analogues with a diverse array of (hetero) aryl groups on the 8-carboxamido group of 2,6-methano-3-benzazocines as well as the corresponding 3-carboxamido morphinans and 4,5α-epoxymorphinans.

Acknowledgments

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