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Redefining the Structure–Activity Relationships of 2,6-Methano-3-benzazocines. Part 2: 8-Formamidocyclazocine Analogues

Mark P. Wentland,[a,*] Xufeng Sun,[a] Yingchun Ye,[a] Rongliang Lou[a] and Jean M. Bidlack[b]

[a]*Department of Chemistry, 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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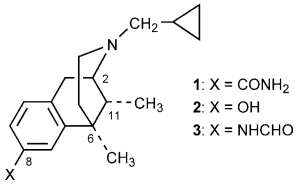
Abstract—High affinity binding for μ and κ opioid receptors has been observed in analogues of cyclazocine, ethylketocyclazocine and naltrexone where the prototypic (of opiates) phenolic OH group was replaced with a formamide (–NHCHO) group. For the 8-formamide analogue of cyclazocine, binding is highly enantiospecific (eudismic ratios ~2000 for μ and κ) with K_i values ≤1 nM observed for the (2*R*,6*R*,11*R*)-isomer, (−)-**4**. A preliminary SAR revealed that affinity is very sensitive to substitution on the formamide appendage.

As part of our broad goal to identify long-acting opioid-receptor interactive agents useful in the treatment of cocaine and heroin addiction in humans, we recently reported the synthesis, receptor binding and in vivo properties of 8-carboxamidocyclazocine (8-CAC; **1**).[1,2] Our observations that replacement of the 8-OH of cyclazocine **2**[3] and the corresponding phenolic OH of certain 4,5α-epoxymorphinans (e.g., naltrexone,[4] buprenorphine,[5] and naltrindole[5]) with $CONH_2$ resulted in sustained high affinity binding was unexpected; this is based on previous teachings that the prototypic phenolic OH of opiates was an important determinant for recognition to opioid receptors.[6] In addition to high binding affinity (K_i values <1 nM for μ and κ),[1] 8-CAC showed potent antinociception in mice (icv) and a much longer duration of antinociception action (15 h vs 2 h) compared to cyclazocine when both were dosed ip in mice.[2]

In hopes of gaining additional insight into this new SAR surrounding the 8-position of 2,6-methano-3-benzazocines (a.k.a. benzomorphans), we now report analogues of **1** where the 8-$CONH_2$ group has been 'reversed' via incorporation of a formamido substituent (8-NHCHO) to provide **3**. This concept of reversed amide bioisosterism has been successfully applied to other systems.[7] We also prepared the (−)- and (+)-enantiomers of **3** to assess enantiospecificity of binding and we evaluated a small series of variants of 8-formamidocyclazocine with differing alkyl and/or aryl groups on the N and C of the formamide to probe steric and/or conformational effects on binding affinity. In addition, we made and evaluated the corresponding formamido variants **9** and **12** of ethylketocyclazocine (**11**; EKC) and naltrexone **14**, respectively.



With the exception of target **3**, whose utility as a synthetic intermediate (no biological data reported) we reported some time ago,[8] no reports of 8-formamido-2,6-methano-3-benzazocines or the corresponding 3-formamido-morphinans have appeared in the literature. Several 8-acetamido-2,6-methano-3-benzaocines have been reported[8–11] as well as 8- and 3-alkoxycarbonylamino derivatives of 2,6-methano-3-benzazocines[11] and morphinans,[12] respectively. Other amide derivatives that have been reported are an 8,9-fused isatin derivative of a 2,6-methano-3-benzaocine[13] and a (+)-3-[4-oxo-2-phenyl-3(4*H*)-quinazolinyl]-17-methylmorphinan.[14]

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

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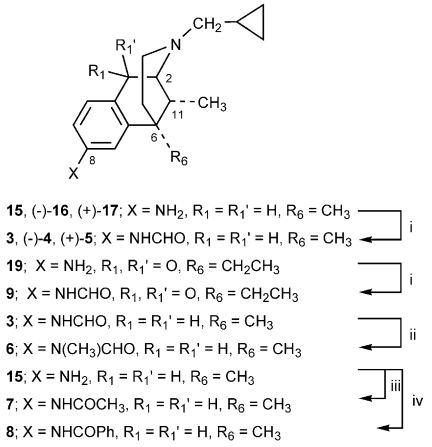
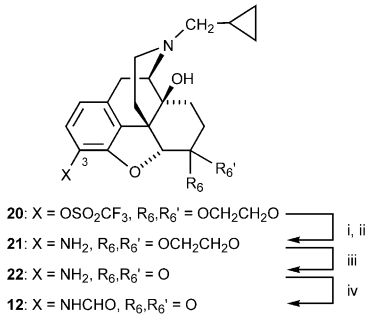
Formamide targets **3**, (−)-**4**, (+)-**5** and **9** were prepared in straightforward manner (Scheme 1) by treating amines **15**,[8,15] (−)-**16**,[15] (+)-**17**,[15] and **19**,[15] respectively, with neat formic acid at 100 °C;[17] yields ranged from 76 to 98%. Amide derivatives **7** and **8** were made by treating **15**[8,15] with acetyl chloride and benzoyl chloride, respectively, in pyridine at 25 °C in yields of 94 and 64%, respectively. The *N*-methyl derivative **6** was made via base-induced alkylation of **3** in 79% yield. For the naltrexone core, the 3-formamido analogue **12** was also made by treating the corresponding primary amine (i.e., **22**) with formic acid (Scheme 2) in 42% yield. Novel primary amine **22** was made using a Pd-catalyzed amination procedure similar to one recently reported[18] where triflate **20**[12] was first treated with Pd_2dba_3, 2-di(cyclophosphino)biphenyl, $LiN(SiMe_3)_2$ and Ph_3SiNH_2 (as ammonia equivalent) to give a silylated amine, which, without isolation, was treated in the same reaction vessel with 1 M TBAF/THF to give aminated derivative **21**[12] in 23% overall yield. The low yield in this sequence was a result of formation of a significant amount of naltrexone, which we assume arises from a competing reaction (with amination) of triflate cleavage by an, as yet, unidentified nucleophile. Cleavage of substrate was not observed in the Buchwald study since these substrates were aryl chlorides and bromides.[18] Compound **21**, when exposed to 6 N HCl/acetone, gave primary amine **22** in 84% yield.



Scheme 1. Reagents: (i) HCO_2H, 100 °C; (ii) NaH, DMSO, CH_3I, 25 °C; (iii) Ch_3COCl, pyr, 25 °C; (iv) PhCOCl, pyr, 25 °C.



Scheme 2. Reagents: (i) Pd_2dba_3, 2-di(cyclophosphino)biphenyl, $LiN(SiMe_3)_2$, Ph_3SiNH_2, tol, 100 °C; (ii) 1 M TBAF/THF, 25 °C; (iii) 6 N HCl/acetone, 56 °C; (iv) HCO_2H, 100 °C.

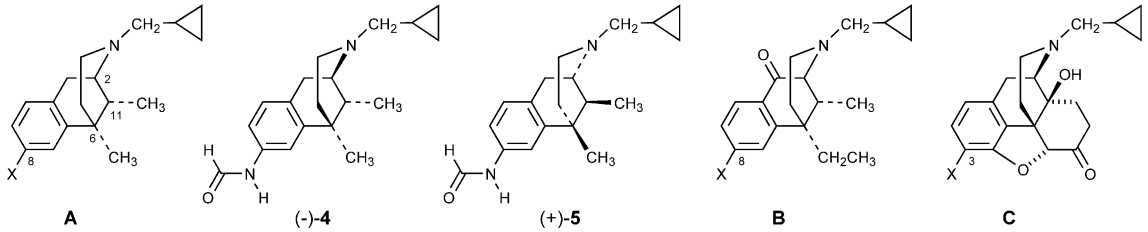
Affinities of target compounds for μ, δ and κ opioid receptors were assessed by generating K_i values using well-documented receptor binding assays. These data are presented in Table 1 along with comparative K_i data for the corresponding known carboxamido and OH analogues. The 8-formamido analogue **3** of cyclazocine displayed very high affinity for μ and κ receptors with K_i values of 1.9 and 0.85 nM, respectively. Affinity for δ was considerably lower ($K_i = 37$ nM) consistent with known receptor type selectivity for the 2,6-methano-3-benzazocine (a.k.a. benzomorphans) class.[3] Compared to the 8-carboxamido derivative **1** and cyclazocine itself (**2**), affinity of **3** was somewhat lower (4- to 5-fold) for μ and only slightly lower (2- to 4-fold) for κ. The 8-formamido analogue **9** of EKC had somewhat lower affinity ($K_i = 6.2$ nM) for μ than its corresponding carboxamide (**10**: $K_i = 1.2$ nM) or EKC itself (**11**: $K_i = 0.78$ nM). However, for κ, these three EKC derivatives had similar affinities near 1 nM. Like the two benzomorphans, the 3-formamido reversed amide isostere **12** had 4-fold lower affinity for μ than naltrexone-3-carboxamide **13**, however, a divergence in affinity for κ was observed. Here, formamide **12** had 5-fold higher affinity for κ than **13**, whereas the formamide and carboxamide benzomorphan pairs **1**/**3** and **9**/**10** had comparable affinity. We also evaluated novel compound **22**, the 3-NH_2 analogue of naltrexone, in receptor binding assays where good affinity for μ and κ was observed.

Several analogues of 8-formamidocyclazocine **3** were also made as probes to identify a preliminary SAR. Analysis of the opioid binding data for the enantiomers (−)-**4** (2*R*,6*R*,11*R*) and (+)-**5** (2*S*,6*S*,11*S*) of **3** revealed a very large enantiopreference for binding to all three receptors with eudismic ratios ~2000 for μ and κ. The active enantiomer (−)-**4** had, as expected, K_i values for μ and κ roughly half of those for the corresponding racemate **3**.

When the formamide nitrogen of **3** was methylated to give **6**, affinity for μ and κ decreased nearly 250-fold. This result suggests the NH of **3** contributes to receptor recognition as an H-bond donor to a complimentary acceptor site on the protein; this is in concert with known SAR studies concerning the role of similar H-bonding of the prototypic phenolic OH of opiates[6] and 8-alklyamino (RNH) analogues of 2,6-methano-3-benzazocines.[15] Alternatively, it is possible that the reduced affinity of **6** is a result of negative steric interaction of newly introduced methyl group with the protein; however, we found this part of receptor space can reasonably accommodate alkyl and aryl groups having steric bulk much larger than the methyl of **6**.[15]

We also replaced the hydrogen on the formamide carbon of **3** with methyl and phenyl appendages to provide acetamido derivative **7** and benzamide **8**, respectively. Data in Table 1 reveal that acetamido derivative **7** has very low affinity for μ and κ with K_i values of 240 and 130 nM, respectively. Comparing these values to formamide **3**, addition of the methyl group translates to between 126- and 153-fold lower affinity. The magnitude of this decrease is much greater than that observed by *N*-methylating carboxamide **1** to give **A** (X = CONHMe), the

Table 1. Opioid binding data for 8-formamido-2,6-methano-3-benzazocines and related compounds



Compd		K_i (nM ± SEM)[a]		
		[^{3}H]DAMGO (μ)	[^{3}H]Naltrindole (δ)	[^{3}H]U69,593 (κ)
1 (8-CAC)[b]	**A**: X = $CONH_2$	0.41 ± 0.07	8.3 ± 0.49	0.53 ± 0.06
2 (cyclazocine)[c]	**A**: X = OH	0.32 ± 0.02	1.1 ± 0.04	0.18 ± 0.020
3[d,e]	**A**: X = NHCHO	1.9 ± 0.14	37 ± 3.9	0.85 ± 0.080
(−)-**4**[e,f]		1.1 ± 0.04	9.8 ± 0.28	0.49 ± 0.012
(+)-**5**[e,g]		2300 ± 180	> 10,000	900 ± 8.7
6[e]	**A**: X = $N(CH_3)CHO$	550 ± 23	6200 ± 810	200 ± 8.7
7[d,e]	**A**: X = $NHCOCH_3$	240 ± 17	1700 ± 410	130 ± 4.6
8[e]	**A**: X = $NHCOC_6H_5$	570 ± 53	2400 ± 174	820 ± 20
9[e]	**B**: X = NHCHO	6.1 ± 0.83	52 ± 3.4	1.2 ± 0.11
10[b]	**B**: X = $CONH_2$	1.2 ± 0.12	9.8 ± 0.50	0.70 ± 0.08
11(EKC)[h]	**B**: X = OH	0.78 ± 0.10	3.4 ± 0.41	0.62 ± 0.11
12[e]	**C**: X = NHCHO	8.3 ± 0.89	420 ± 46	4.2 ± 0.18
13[i]	**C**: X = $CONH_2$	1.9 ± 0.21	110 ± 8.1	22 ± 0.85
14 (naltrexone)[j]	**C**: X = OH	0.17 ± 0.03	11 ± 1.1	0.31 ± 0.03
22[e]	**C**: X = NH_2	5.6 ± 0.32	880 ± 67	5.8 ± 0.69

[a]Binding assays (guinea pig brain membranes incubated with the appropriate radiolabeled ligand) used to screen compounds are similar to those previously reported (see refs [1 and 19]). Data are the mean ± SEM from at least three experiments performed in triplicate.
[b]Known compound—see ref 1.
[c]Known compound—see ref 3 and references therein for preparation.
[d]Known compound (see ref 8) used as synthetic intermediate; no characterization reported.
[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.
[f]$[\alpha]_D^{25} = -115.5°$ (*c* 1.0, $CHCl_3$).
[g]$[\alpha]_D^{25} = +117.3°$ (*c* 1.0, $CHCl_3$).
[h]Known compound—see ref 16 for preparation.
[i]Known compound—see ref 4.
[j]Source: Sigma Aldrich.

corresponding reversed amide of **7**, in this case, adding a methyl in an equivalent place resulted in a 59-fold reduction in μ binding affinity and only a 5-fold decrease for κ.[1]

Rationale for making the 8-NHCOPh analogue **8** centered around an earlier observation in our 8-amino-2,6-methano-3-benazocine series that a significant increase in affinity (7-fold for μ) resulted from substituting one H of the 8-NH_2 group with a phenyl or benzyl appendage [K_i ~1.3 nM (μ) for **A** where X = NHPh or $NHCH_2Ph$].[15] However, the benefits of aryl substitution did not transfer to the present series in that **8** had very poor affinity (300- and 965-fold for μ and κ, respectively) relative to formamide **3**. The reversed amide **A** (X = CONHPh) of **8** also showed very low affinity for μ and κ (K_i values of 740 and 460 nM, respectively).[1]

Analysis of opioid receptor binding data for this series of 8-formamido-2,6-methano-3-benzazocines and 3-formamidonaltrexone lead us to conclude that a formamido group (–NHCHO) is an effective bioisosteric replacement for the a carboxamido group (–$CONH_2$). Binding to opioid receptors is highly enantioselective with the (2*R*,6*R*,11*R*) isomer of the cyclazocine analogue (−)-**4** being the active enantiomer. For the cyclazocine core, substitution on N– or C– of formamide **3** substantially decreases binding affinity and that bioisosterism observed for the 8-NHCHO versus 8-$CONH_2$ pair did not translate to the corresponding methylated reversed amide pair, 8-$NHCOCH_3$ versus 8-$CONHCH_3$.

Our data suggest the importance of the formamide NH is due to H-bond donation; however, the role of the formyl group of **3** is unclear. For example, its electron withdrawing properties would certainly impart greater H-bond donation ability of the formamide NH and thereby enhance binding affinity. An alternative role would be direct interaction with the receptor via, for example, the oxygen of the formyl group acting as H-bond acceptor to a putative complimentary donor site on protein. The different conformational effects (e.g., restricted rotation about an amide C–N bond) and somewhat divergent SARs noted between the 8-formamido, carboxamido, and amino subseries, leads us to conclude that affinity is not only dependent on the size of an alkyl or aryl appendage, but its orientation in receptor space is crucial for efficient recognition. For example, the steric bulk, as quantified by the physico-

chemical descriptor MR,[20] of aromatic–NHCHO ($MR = 10.3$), $-CONH_2$ (9.8), and $-NHCH_3$ (10.3) groups are very similar, yet divergent K_i (μ) values are observed (1.9, 0.41, and 13 nM,[15] respectively). Work in our laboratories is continuing to gain a better understanding of the role of the formamido and carboxamido groups in recognition to the receptors. Judicious selection of analogues for future evaluation will allow us to address these hypotheses and will include those with different 8-substituents (e.g., urea) and conformational restrictions (to probe the bioactive conformation).

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References and Notes

1. Part I: Wentland, M. P.; Lou, R.; Ye, Y.; Cohen, D. J.; Richardson, G. P.; Bidlack, J. M. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 623.
2. Bidlack, J. M.; Cohen, D. J.; McLaughlin, J. P.; Lou, R.; Ye, Y.; Wentland, M. P. *J. Pharmacol. Exp. Ther.* **2002**, *302*, 374.
3. Archer, S.; Glick, S. D.; Bidlack, J. M. *Neurochem. Res.* **1996**, *21*, 1369.
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.; Lou, R.; Dehnhardt, C.; Cohen, D. J.; Bidlack, J. M. *Abstracts of Papers,* Proceedings of the 64th Annual Meeting of the College on Problems of Drug Dependence, Québec City, Québec, Canada, June 8–13, 2002; p 724 [Abstract also published in: *Alcohol Drug Depend*, **2002**, *66* (Suppl. 1) S192].
6. Aldrich, J. V. In *Burger's Medicinal Chemistry and Drug Discovery*; Wolff, M. E., Ed.; John Wiley & Sons: New York, 1996; Vol. 3, p 321.
7. Patani, G. A.; LaVoie, E. J. *Chem. Rev.* **1996**, *96*, 3147.
8. Wentland, M. P.; Albertson, N. F.; Pierson, A. K. *J. Med. Chem.* **1980**, *23*, 71.
9. Danso-Danquah, R.; Bai, X.; Zhang, X.; Mascarella, S. W.; Williams, W.; Sine, B.; Bowen, W. D.; Carroll, F. I. *J. Med. Chem.* **1995**, *38*, 2978.
10. Carter, A.; Ensinger, H.; Grauert, M.; Kuhn, F. J.; Merz, H.; Mueller, E.; Stransky, W.; Streller, I. Eur. Pat. Appl. 521422, 1993; *Chem. Abstr.* **1993**, *119*, 117602.
11. Vyas, D.; Joshi, Y. C.; Joshi, B. C. *J. Inst. Chem. (India)* **1982**, *54*, 213.
12. McCurdy, C. R.; Jones, R. M.; Portoghese, P. S. *Org. Lett.* **2000**, *2*, 819.
13. Allen, R. C.; Wettlaufer, D. G. US Patent 4,929,622, 1990; *Chem. Abstr.* **1990**, *113*, 212033.
14. Newman, A. H.; Bevan, K.; Bowery, N.; Tortella, F. C. *J. Med. Chem.* **1992**, *35*, 4135.
15. (a) Wentland, M. P.; Ye, Y.; Cioffi, C. L.; Lou, R.; Zhou, Q.; Xu, G.; Duan, W.; Dehnhardt, C. M.; Sun, X.; Cohen, D. J.; Bidlack, J. M. *J. Med. Chem.* **2003**, *46*, 838. (b) Wentland, M. P.; Xu, G.; Cioffi, C. L.; Ye, Y.; Duan, W.; Cohen, D. J.; Colasurdo, A. M.; Bidlack, J. M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 183.
16. (a) Michne, W. F.; Albertson, N. F. *J. Med. Chem.* **1972**, *15*, 1278. (b) Albertson, N. F. US Patent 4,161,598, 1979; *Chem. Abstr.* **1979**, *91*, 175227.
17. Chakrabarty, M.; Khasnobis, S.; Harigaya, Y.; Konda, Y. *Syn. Commun.* **2000**, *30*, 187.
18. Huang, X. H.; Buchwald, S. L. *Org. Lett.* **2001**, *3*, 3417.
19. Archer, S.; Seyed-Mozaffari, A.; Jiang, Q.; Bidlack, J. M. *J. Med. Chem.* **1994**, *37*, 1578.
20. Hansch, C.; Leo, A.; Hoekman, D. In *Exploring QSAR: Hydrophobic, Electronic, and Steric Constants*; American Chemical Society: Washington, 1995; p 217.