Bioorganic & Medicinal Chemistry Letters 19 (2009) 203–208

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Syntheses and opioid receptor binding properties of carboxamido-substituted opioids ☆

Mark P. Wentland [a,*], Rongliang Lou [a], Qun Lu [a], Yigong Bu [a], Melissa A. VanAlstine [a], Dana J. Cohen [b], 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

ARTICLE INFO

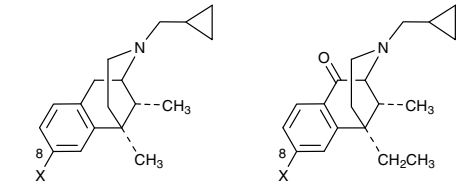
Article history:
Received 22 September 2008
Revised 22 October 2008
Accepted 27 October 2008
Available online 7 November 2008

Keywords:
Opioids
SAR

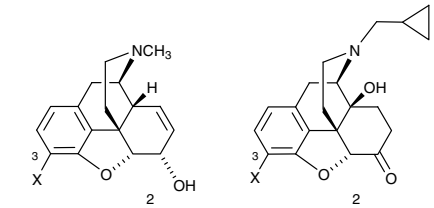
ABSTRACT

A series of 15 novel opioid derivatives were made where the prototypic phenolic-OH group of traditional opioids was replaced by a carboxamido ($CONH_2$) group. For 2,6-methano-3-benzazocines and morphinans similar or, in a few instances, enhanced affinity for μ, δ and κ opioid receptors was observed when the OH → $CONH_2$ switch was applied. For 4,5α-epoxymorphinans, binding affinities for the corresponding carboxamide derivatives were much lower than the OH partner consistent with our pharmacophore hypothesis concerning carboxamide bioactive conformation. The active metabolite of tramadol and its carboxamide counterpart had comparable affinities for the three receptors.

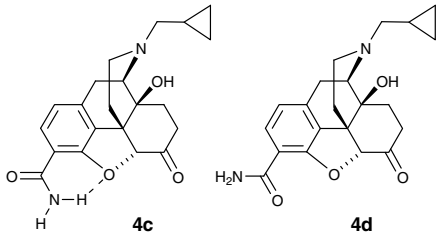
In 2001 we reported our observation that the prototypic phenolic hydroxyl group of certain opioids can be replaced by a carboxamide group and retain high affinity binding to opioid receptors.[1] When this OH → $CONH_2$ switch was applied to the cyclazocine core structure, for example, binding affinities for cyclazocine (**1a**)[2] and 8-carboxamidocyclazocine (8-CAC, **1b**) were comparable for μ, δ and κ receptors (i.e., K_i values within 2-fold – see first entry in Table 1). A similar result was seen for the OH → $CONH_2$ switch in other 2,6-methano-3-benzazocine (a.k.a. benzomorphan) core structures (e.g., ethylketocyclazocine **2a**).[1] However, when the core structure was the pentacyclic 4,5α-epoxymorphinan derived from natural products, a divergence in SAR was seen. For the morphine (**3a**/**3b**) and naltrexone (**4a**/**4b**) pairs, binding affinity for μ, for example, was reduced by 39- and 7-fold, respectively, when the OH → $CONH_2$ switch was applied.[3,4] We recently reported strong evidence that in the naltrexone case, this divergent SAR is likely a consequence of the furan O stabilizing, via a strong intramolecular H-bond, a carboxamide conformation (**4c**) that is not the bioactive one.[4] Therefore, 3-desoxy-3-carboxamido naltrexone **4b** and, by analogy, the morphine analogue **3b**, must pay an energy penalty to adopt the putative bioactive conformation depicted in **4d**. For the 2,6-methano-3-benzazocines 8-CAC (**1b**) and **2b**, the putative carboxamide bioactive conformation can easily be attained since there is no barrier created by H-bonding to a neighboring ether bridge.



1a: X = OH (cyclazocine) **2a**: X = OH (EKC)
1b: X = $CONH_2$ (8-CAC) **2b**: X = $CONH_2$



3a: X = OH (morphine) **4a**: X = OH (naltrexone)
3b: X = $CONH_2$ **4b**: X = $CONH_2$



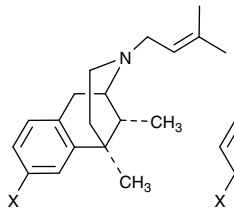
4c **4d**

☆ Presented, in part, at Wentland, M. P.; Lou, R.; Cohen, D. C.; Bidlack, J. M. Carboxamido Analogues of Nalbuphine, Butorphanol and Nor-BNI. Proceedings of the 66th Annual Meeting of the College on Problems of Drug Dependence, San Juan, PR, June 12–17, 2004.

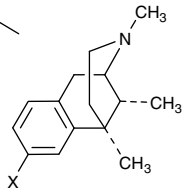
* Corresponding author. Tel.: +1 518 276 2234; fax: +1 518 276 4887.
E-mail address: wentmp@rpi.edu (M.P. Wentland).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.10.134

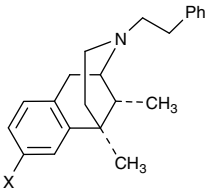
Table 1
Opioid binding data for carboxamido-substituted 2,6-methano-3-benzazocine, morphinan, 4,5α-epoxymorphinan and tramadol derivatives



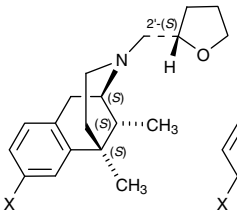
Pentazocine core:
5a: X = OH
5b: X = $CONH_2$



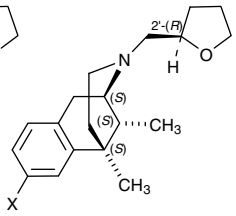
Metazocine core:
6a: X = OH
6b: X = $CONH_2$



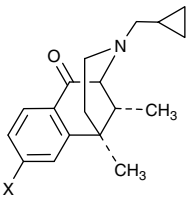
Phenazocine core:
7a: X = OH
7b: X = $CONH_2$



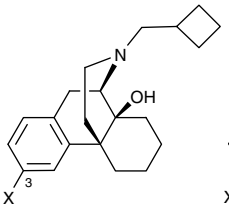
Mr2034 core:
8a: X = OH
8b: X = $CONH_2$



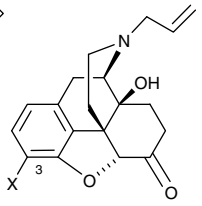
Mr2034 isomer core:
9a: X = OH
9b: X = $CONH_2$



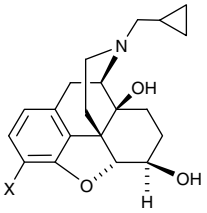
Ketocyclazocine core:
10a: X = OH
10b: X = $CONH_2$



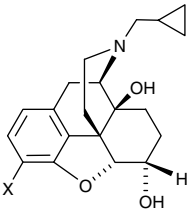
Butorphanol core:
11a: X = OH
11b: X = $CONH_2$



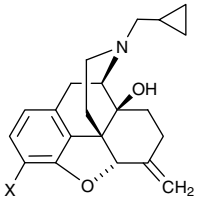
Naloxone core:
12a: X = OH
12b: X = $CONH_2$



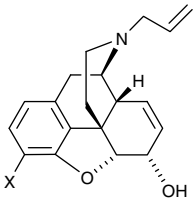
Naltrexone-6-β-ol core:
13a: X = OH
13b: X = $CONH_2$



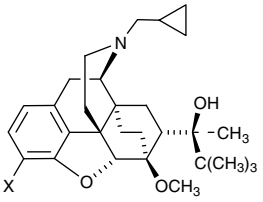
Naltrexone-6-α-ol core:
14a: X = OH
14b: X = $CONH_2$



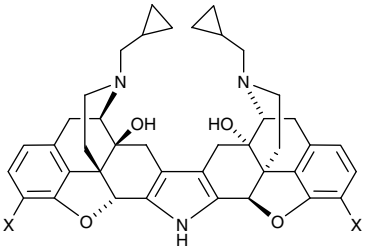
Nalmefene core:
15a: X = OH
15b: X = $CONH_2$



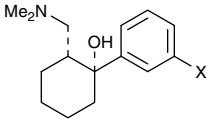
Nalorphine core:
16a: X = OH
16b: X = $CONH_2$



Buprenorphine core:
17a: X = OH
17b: X = $CONH_2$



n*or*-BNI (Norbinaltorphimine)core:
18a: X = OH
18b: X = $CONH_2$



Tramadol core:
19a: X = OH
19b: X = $CONH_2$
19c: X = OCH_3

Compound	K_i – nM ± SE[a] [*K*BR][b]						κ:μ[c]	κ:δ[d]
	[3H]DAMGO	[μ]	[^{3}H]Naltrindole	[δ]	[^{3}H]U69,593	[κ]		
Cyclazocine core								
1a:X = OH[e]	0.16 ± 0.01	[0.5]	2.0 ± 0.22	[0.4]	0.07 ± 0.01	[1.2]	2	30
1b: X = $CONH_2$[f]	0.31 ± 0.03		5.2 ± 0.36		0.06 ± 0.001		5	90
EKC core								
2a: X = OH[g,h]	0.78 ± 0.10	[0.7]	3.4 ± 0.41	[0.4]	0.62 ± 0.11	[0.9]	1	4
2b: X = $CONH_2$[g,h]	1.2 ± 0.12		9.8 ± 0.50		0.70 ± 0.08		2	14
Morphine core								
3a: X = OH[g,i]	0.88 ± 0.14	[0.03]	140 ± 18	[0.07]	24 ± 2.3	[0.01]	0.037	6
3b: X = $CONH_2$[g,i]	34 ± 1.8		1900 ± 81		2000 ± 97		0.017	1
Naltrexone core								
4a: X = OH[j]	0.11 ± 0.006	[0.15]	60 ± 3.2	[0.11]	0.19 ± 0.005	[0.02]	0.6	3320
4b: X = $CONH_2$[j]	0.71 ± 0.058		550 ± 40		0.36		0.065	50
Pentazocine core								
5a: X = OH	6.9 ± 0.64	[1.3]	180 ± 3.2	[0.8]	3.0 ± 0.18	[0.5]	2	60
5b: X = $CONH_2$[k]	5.2 ± 0.41		220 ± 10		5.6 ± 0.26		1	39
Metazocine core								
6a: X = OH	3.8 ± 0.66	[2.4]	140 ± 17	[0.4]	9.9 ± 0.22	[0.5]	0.4	14
6b: X = $CONH_2$[k]	1.6 ± 0.07		320 ± 43		19 ± 0.54		0.08	17
Phenazocine core								
7a: X = OH	0.20 ± 0.07	[13]	5.0 ± 0.88	[4.2]	2.0 ± 0.13	[3.6]	0.1	2.5
7b: X = $CONH_2$[k]	0.015 ± 0.0010		1.2 ± 0.27		0.55 ± 0.029		0.03	2
Mr2034 (2'S) core								
8a: X = OH[g]	0.19 ± 0.01	[3.7]	3.6 ± 0.40	[1.8]	0.09 ± 0.01	[1.0]	2	40
8b: X = $CONH_2$[g,k]	0.052 ± 0.013		2.0 ± 0.15		0.089 ± 0.004		0.6	22

Table 1 (*continued*)

Compound	K_i – nM ± SE[a] [*K*BR][b]						κ:μ[c]	κ:δ[d]
	[3H]DAMGO	[μ]	[^{3}H]Naltrindole	[δ]	[^{3}H]U69,593	[κ]		
Mr2034 diastereomer (2′R) core								
9a: X = OH[g]	4.0 ± 0.54	[1.4]	67 ± 4.3	[2.0]	1.5 ± 0.07	[0.5]	3	45
9b: X = $CONH_2$[g,k]	2.9 ± 0.17		34 ± 0.10		2.8 ± 0.24		1	12
Ketocyclazocine core								
10a: X = OH[g]	3.3 ± 0.66	[2.4]	20 ± 2.7	[1.0]	1.0 ± 0.24	[0.6]	3	20
10b: X = $CONH_2$[g,k]	1.4 ± 0.07		20 ± 2.3		1.8 ± 0.10		0.8	11
Butorphanol core								
11a: X = OH	0.12 ± 0.058	[0.8]	12 ± 3.8	[0.9]	0.22 ± 0.023	[0.6]	0.55	50
11b: X = $CONH_2$[k]	0.15 ± 0.019		14 ± 2.1		0.39 ± 0.057		0.38	40
Naloxone core								
12a: X = OH	0.66 ± 0.11	[0.1]	120 ± 13	[0.1]	1.2 ± 0.072	[0.03]	0.55	100
12b: X = $CONH_2$[k]	4.5 ± 0.18		1000 ± 59		46 ± 1.3		0.1	22
Naltrexone-6-β-ol core								
13a: X = OH	0.19 ± 0.016	[0.1]	53 ± 3.5	[<0.01]	0.48 ± 0.010	[0.02]	0.4	110
13b: X = $CONH_2$[k]	2.5 ± 0.95		>10 μM		30 ± 0.86		0.08	–
Naltrexone-6-α-ol core								
14a: X = OH	0.21 ± 0.09	[0.03]	56 ± 5.1	[<0.01]	0.56 ± 0.11	[0.01]	0.4	100
14b: X = $CONH_2$[k]	7.1 ± 0.88		>10 μM		100 ± 2.7		0.07	–
Nalmefene core								
15a: X = OH	0.44 ± 0.083	[0.2]	9.3 ± 1.5	[0.1]	0.12 ± 0.0042	[0.1]	4	78
15b: X = $CONH_2$[k]	2.6 ± 0.33		67 ± 2.6		2.1 ± 0.29		1	32
Nalorphine core								
16a: X = OH	0.19 ± 0.01	[0.01]	120 ± 17	[<.01]	0.38 ± 0.05	[0.004]	0.5	320
16b: X = $CONH_2$[k]	18 ± 2.2		>10 μM		100 ± 7.8		0.2	–
Buprenorphine core								
17a: X = OH	0.21 ± 0.024	[0.3]	2.9 ± 0.49	[0.1]	0.62 ± 0.073	[0.3]	0.3	5
17b: X = $CONH_2$[k]	0.77 ± 0.065		54 ± 8.4		2.1 ± 0.12		0.4	26
Nor-BNI core								
18a: X = OH	2.7 ± 0.33	[0.01]	24 ± 1.6	[0.02]	0.027 ± 0.008	[0.001]	100	900
18b: X = $CONH_2$[k]	400 ± 32		1000 ± 241		21 ± 4.7		19	50
Tramadol core								
19a: X = OH	8.6 ± 0.37	[0.9]	2900 ± 66	[1.2]	450 ± 43	[1.3]	0.02	6
19b: X = $CONH_2$[k]	9.5 ± 0.25		2400 ± 200		350 ± 10		0.03	7
19c: X = OCH_3 (tramadol)	1600 ± 241		9.4 ± 0.79		14 ± 0.44		114	0.7

[a] Binding assays used to screen compounds are similar to those previously reported (see Refs. 26 and 27). Membrane protein from CHO cells that stably expressed one type of the human opioid receptor or (as indicated) from guinea pig brain were 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. Nonspecific binding was measured by inclusion of 10 μM naloxone. The binding was terminated by filtering the samples through Schleicher and 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% polyethyleneimine 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) – see Ref. 28 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.

[b] *K*BR (K_i binding ratio) = K_i (OH)/K_i ($CONH_2$) for μ, δ or κ opioid receptors.

[c] κ:μ = K_i (μ)/K_i (κ).

[d] κ:δ = K_i (δ)/K_i (κ).

[e] See text for references to known phenolic-OH opioids.

[f] See Ref. 29.

[g] Guinea pig membranes.

[h] See Ref. 1.

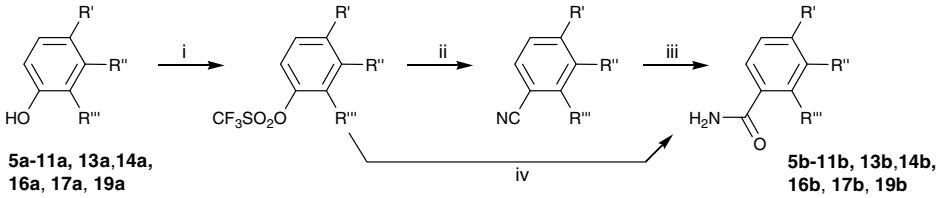
[i] See Ref. 3.

[j] See Ref. 4.

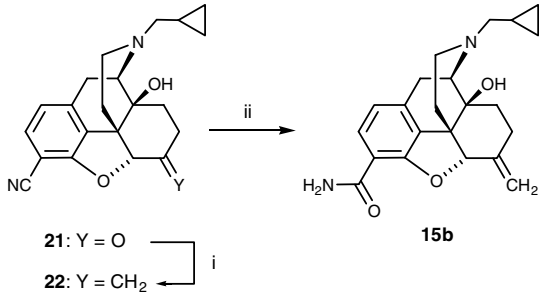
[k] Proton NMR, IR and MS were consistent with the assigned structures of all new compounds. C, H, and N elemental analyses were obtained for all new targets and most intermediates and were within ±0.4% of theoretical values.

Subsequent to these findings, other researchers have published studies where the OH → $CONH_2$ switch was applied to a number of other well-known opioid core structures; these include the morphinan class (e.g., levorphanol, cyclorphan, MCL-101),[5] phenylpiperidine class[6,7] and opioid peptides.[8,9] We now wish to report additional examples of carboxamido-substituted opioids. The objective of this study was to take 15 well-known phenolic-OH-containing opioids (structures shown in Table 1) having divergence in core structures to use as substrates for the OH → $CONH_2$ switch and compare affinity and selectivity for μ, δ and κ opioid receptors within each pair to determine if the SAR is consistent with that seen with the limited number of pairs previously reported. Intrinsic opioid-receptor mediated activity for a number of high affinity carboxamide-containing ligands compared to the corresponding phenolic-OH prototype was also determined using [^{35}S]GTPγS binding assays.

Carboxamide targets **5b–11b**, **13b**, **14b**, **16b**, **17b** and **19b** were made directly from their corresponding known phenols using methodology previously reported (Scheme 1).[1,3,4] Triflate ester formation was accomplished by treating the phenol with $(CF_3SO_2)_2O$



Scheme 1. Reagents and conditions: (i) $(CF_3SO_2)_2O$, pyr, CH_2Cl_2, 25 °C (Method A) or $PhN(Tf)_2$, Et_3N, CH_2Cl_2, 25 °C (Method B); (ii) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 120 °C, 20 h or under microwave radiation 150 °C for 15 min; (iii) *t*-BuOH, KOH, 82 °C (combined ii + iii = Method C); (iv) $CO/NH_3/Pd(OAc)_2$/DPPF in DMSO (Method D) or CO, $HN(SiMe_3)_2$, $PdCl_2$, PPh_3, DMF followed by 2 N H_2SO_4 (Method E).



Scheme 2. Reagents and conditions: (i) Ph_3PCH_3Br, $NaNH_2$, THF, 25 °C, 20 h; (ii) K_2CO_3, H_2O_2, DMSO, 25 °C, 3 h.

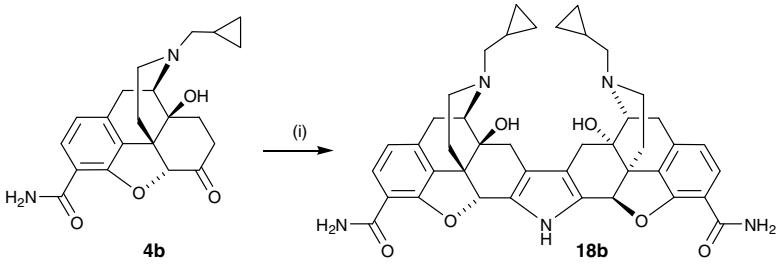
and pyridine in CH_2Cl_2 (Method A) or $PhN(SO_2CF_3)_2$ and triethylamine in CH_2Cl_2 (Method B). For targets **6b**–**11b**, **13b**, **14b** and **16b**, preparation of the corresponding carboxamide was enabled via a two-step procedure where the triflate was first converted to a nitrile using $Zn(CN)_2$, $Pd(PPh_3)_4$, in DMF followed by partial hydrolysis of the nitrile using KOH/*t*-BuOH (Method C). Alternatively, triflate esters were directly converted to the carboxamide targets **5b** and **17b** via palladium catalyzed procedures using $CO/NH_3/Pd(OAc)_2$/DPPF in DMSO (Method D) or $CO/HN(SiMe_3)_2/PdCl_2/PPh_3$ in DMF followed by workup with dilute sulfuric acid (Method E).[1,3]

Using the methodology described in Scheme 1, the following known phenolic-OH-containing opioids were converted to the corresponding novel carboxamide targets as follows: pentazocine (**5a**)[10] → **5b**, Methods A (93%) and D (18%); metazocine (**6a**)[11] → **6b**, Methods A (75%) and C (26% combined); phenazocine (**7a**)[12] → **7b**, Methods B (96%) and C (54%); Mr2034 (**8a**)[13] → **8b**, Methods A (95%) and C (98%); Mr2034 diastereomer (**9a**)[13] → **9b**, Methods A (96%) and C (88%); ketocyclazocine (**10a**)[14] → **10b**, Methods A (98%) and C (93%); butorphanol (**11a**)[15] → **11b**, Methods A (77%) and C (89%); naltrexone-6-β-ol (**13a**)[16] → **13b**, Methods B (99%) and C (65%); naltrexone-6-α-ol (**14a**)[17] → **14b**, Methods B (98%) and C (86%); nalorphine (**16a**)[10] → **16b**, Methods B (97%) and C (11%); buprenorphine (**17a**)[18] → **17b**, Methods B (95%) and E (48%); and tramadol active metabolite (**19a**)[19] → **19b**, Methods A (91%) and C (62%).

Carboxamide target **12b** corresponding to naloxone (**12a**)[20] was prepared from the known 6-ethylene ketal derivative[21] of naloxone. The OH → $CONH_2$ conversion was accomplished using Methods B (74%) and E (69%); deketalization using 6 N HCl in refluxing acetone for 4 h (87%) gave **12b**. As shown in Scheme 2, carboxamide target **15b** corresponding to nalmefene (**15a**)[22] was prepared from the known 3-desoxy-3-cyanonaltrexone **21**[23] by first using standard Wittig olefination conditions to provide intermediate **22** in 78% yield. Conversion of **22** to target **15b** was accomplished using K_2CO_3, H_2O_2 in DMSO in 78% yield. Lastly, the carboxamide analogue **18b** of nor-BNI (**18a**)[24] was made (Scheme 3) in 51% yield by treating **4b** with hydrazine hydrochloride using conditions very similar to those used to make **18a**.[24]

Target compounds were evaluated for their affinity and selectivity for μ, δ and κ opioid receptors. Membrane protein from CHO cells that stably expressed one type of the human opioid receptor was used.[26] In three instances where indicated, membrane protein from guinea pig brain was used. Opioid receptors from two species were used due to a change in our primary assay midway through this study, however, where data are available, absolute and relative affinities, using human or guinea pig receptors were quite similar. Binding data for all new carboxamide targets compared to their phenolic-OH counterparts are detailed in Table 1. Also included in Table 1 are columns that summarize the K_i Binding Ratio ('*K*BR') for each core structure against the three receptors [*K*BR = K_i (OH)/K_i ($CONH_2$)]. A *K*BR that is ⩾0.5 and ⩽2 means the K_i values for the pair of compounds are within 2-fold and have comparable binding affinity for that receptor. A *K*BR of >2 indicates the carboxamide has higher affinity for the receptor than does its OH counterpart; if it is <0.5, the OH partner has higher affinity.

For 2,6-methano-3-benzazocine core structures (**5**–**10**), high affinity for μ and κ receptors is seen in all $CONH_2$ targets; lower affinity for δ is observed. The *K*BRs for μ range from 1.3 (for **5**) to 13 (for **7**) indicating the carboxamide partners have, in several instances, much higher potency than the OH counterpart. For δ and κ receptors, within each pair, both partners have comparable affinity (*K*BRs ⩾ 0.5 and ⩽2) except for the phenazocine core (**7**) where the carboxamide has considerably higher affinity (*K*BR = 4.2 and 3.6, respectively). For the butorphanol pair **11**, an example of the morphinan class, a very similar trend in *K*BRs (i.e., near unity) and



Scheme 3. Reagents and conditions: (i) $H_2NNH_2 \cdot HCl$, DMF, 150 °C, 1 h.

receptor selectivity (i.e., higher affinity for μ and κ receptors than for δ) was seen compared to published data for the morphinans levorphanol, cyclorphan and MCL-101.[5] Comparing receptor selectivities of carboxamides **5b**–**11b** to their phenolic-OH counterparts **5a**–**11a**, the κ:μ and κ:δ selectivity ratios are similar within each pair with two exceptions. The exceptions are the metazocine (**6**) and phenazocine (**7**) examples where a significant divergence in the κ:μ selectivity is seen; the κ:μ ratios of 0.08 and 0.03, respectively, are much lower (i.e., the carboxamide has much lower affinity for κ than μ) than seen in the other core structures.

As predicted from our previous results with morphine and naltrexone,[3,4] there is an overwhelming trend in the seven 4,5α-epoxymorphinan pairs (**12a/b**–**18a/b**) studied that the carboxamide partner has lower receptor affinity than the corresponding OH-containing opioid. *K*BRs versus all three receptors were <0.5. For μ, *K*BRs ranged from 0.3 for the buprenorphine core **17a/b** to 0.01 for the nalorphine and nor-BNI cores, **16a/b** and **18a/b**, respectively. Against the δ receptor, binding affinity for the carboxamide partner was also very low compared to the traditional OH partner and *K*BRs ranged from 0.1 (**12a/b**, **15a/b** and **17a/b**) to <0.01 for **13a/b** and **14a/b**. Carboxamide targets **12b**–**18b** displayed relatively low affinity for κ and *K*BRs of all pairs (**12a/b**–**18a/b**) for κ were low and ranged from 0.3 (buprenorphine core **17a/b**) to as low as 0.001 (nor-BNI core **18a/b**); more than half of these values were 0.03 or lower. For the seven known phenolic-OH 4,5α-epoxymorphinans **12a**–**18a** studied, six have comparable affinity for μ and κ and much less affinity for δ; these mixed μ/κ compounds are **12a**–**17a**. Comparing this receptor selectivity pattern to that of corresponding carboxamides **12b**–**17b**, little correlation is observed since κ affinity is very low for **12b**–**17b** compared to affinity for μ; the κ:μ [K_i (μ)/K_i (κ)] ratios ranged from 1 for nalmefene analogue **15b** to 0.07 for the naltrexone-6-β-ol analogue **14b**. Receptor selectivity for **18b** showed a similar trend to **18a**, the well-known κ selective ligand nor-BNI,[24] in that both were κ-selective. However, the carboxamide partner displayed very low affinity for all receptors, including κ. The carboxamide partner **19b** of the active metabolite **19a**[19] of tramadol **19c** had comparable binding affinities (*K*BRs = 0.9–1.3) and a similar receptor selectivity pattern to **19a** for all three receptors. Following characterization of these compounds in our laboratories, an independent study describing their syntheses and biological properties appeared.[25]

Intrinsic opioid-receptor mediated activity for a number of high affinity carboxamide-containing ligands compared to the corresponding phenolic-OH prototype was determined using [^{35}S]GTPγS binding assays at μ and κ receptors; results are shown in Table 2. Due to the relatively poor binding affinity to δ receptors, these compounds were not evaluated for functional activity at δ. Procedures similar to those previously reported were used.[29] For the

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	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
1a	Agonist/antagonist	4.0 ± 1.3	24 ± 2.7	13 ± 2.2	67 ± 3.1
1b	Weak agonist/antagonist	1.0 ± 0.70	20 ± 2.4	15 ± 4.0	76 ± 3.6
6a	Agonist	40 ± 4.6	78 ± 4.6	NI	NI
6b	Agonist/antagonist	24 ± 7.3	23 ± 1.5	25 ± 15	70 ± 2.6
7a	Agonist	27 ± 3.1	79 ± 0.82	NI	NI
7b	Agonist	5.3 ± 0.87	110 ± 2.9	NI	NI
8a	Agonist/antagonist	2.7 ± 0.72	31 ± 4.9	17 ± 3.3	61 ± 3.9
8b	Weak agonist/antagonist	NA	~20%	58 ± 14	89 ± 3.9
9a	Weak agonist	NA	~20%	NI	NI
9b	Agonist/antagonist	101 ± 50	42 ± 2.0	250 ± 36	68 ± 2.9
10a	Agonist/antagonist	21 ± 3.2	39 ± 0.21	130 ± 24	63 ± 5.2
10b	Agonist/antagonist	4.2 ± 1.4	23 ± 1.5	150 ± 57	73 ± 3.1
11a	Weak agonist/antagonist	NA	22 ± 5.8	14 ± 3.3	54 ± 2.7
11b	Weak agonist/antagonist	NA	11 ± 2.6	30 ± 5.6	86 ± 6.9
17a	Agonist/antagonist	0.11 ± 0.021	32 ± 6.2	0.59 ± 0.21	48 ± 0.87
17b	Antagonist	NA	9.0 ± 7.0	4.0 ± 0.80	57 ± 8.4
Kappa opioid receptor					
U50,488	Agonist	36 ± 5.0	77 ± 11	NI	NI
1a	Agonist	1.3 ± 0.20	57 ± 3.8	NI	NI
1b	Agonist	2.4 ± 0.65	59 ± 2.4	NI	NI
6a	Agonist	120 ± 26	50 ± 2.3	NI	NI
6b	NT	NT	NT	NT	NT
7a	Agonist	8.1 ± 0.93	95 ± 4.5	NI	NI
7b	Agonist/antagonist	4.4 ± 1.2	60 ± 2.6	640 ± 137	49 ± 4.4
8a	Agonist	1.0 ± 0.13	60 ± 2.0	NI	NI
8b	Agonist	30 ± 9.9	75 ± 3.6	NT	NT
9a	Agonist	41 ± 11	83 ± 10	NT	NT
9b	Agonist	170 ± 29	65 ± 3.3	NT	NT
10a	Agonist	3.3 ± 0.17	84 ± 1.2	NI	NI
10b	Agonist	9.6 ± 4.1	60 ± 0.97	NI	NI
11a	Agonist	2.9 ± 1.0	73 ± 8.8	NI	NI
11b	Agonist	3.8 ± 0.42	55 ± 4.1	NI	NI
17a	Agonist/antagonist	0.18 ± 0.014	35 ± 2.5	15 ± 6.8	24 ± 1.7
17b	Antagonist	NA	−1.3 ± 2.5	140 ± 18	59 ± 5.1

NA, not applicable.
NT, not tested.
[a] See Ref. 29 for experimental details. 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, U50,488 at final concentration of 100 nM was used for the κ receptor.
[b] NI, no inhibition.

benzomorphan pair cyclazocine (**1a**) and 8-CAC (**1b**), both are mixed agonists/antagonists at the μ receptor and agonists at κ. Both displayed similar potencies that correlated well with binding affinities. In the case of the **6a**/**6b** pair (metazocine core) against μ, the carboxamide partner **6a** is an agonist/antagonist while the OH partner is an agonist. Because the affinity of **6b** for κ receptors was relatively weak, it was not studied in the [^{35}S]GTPγS binding assay. For **7a**/**7b** having a phenazocine core, both were agonists at μ having qualitatively similar potencies. Against κ, **7b** was a mixed agonist/antagonist and **7a** was an agonist. The agonist potencies of the two were similar. A divergence in functional activity at the μ receptor was noted for **8a** (agonist/antagonist) and **8b** (weak agonist/antagonist). At the κ receptor, both were agonists, although **8a** had much higher potency. Divergence was also seen at the μ receptor for the **9a** (weak agonist) and **9b** (agonist/antagonist) pair. Against κ, both were agonists, however, **9a** had much higher potency. For the **10a**/**10b**, another benzomorphan pair, both were mixed agonists/antagonists at μ and agonists at κ; agonist potencies at κ were similar. For the butorphanol core, an example of a tetracyclic morphinan, the OH partner **11a** exhibited a weak agonist/antagonist profile at μ, whereas the carboxamide **11b** was an antagonist. At κ, both were agonists having similar potencies. At μ and κ receptors, the carboxamide derivative **17b** of buprenorphine displayed a somewhat different profile (antagonist) than buprenorphine (**17a**) (agonist/antagonist). As μ and κ antagonists, **17a** was more potent than **17b**. In the [^{35}S]GTPγS binding assay mediated by the κ opioid receptor, some compounds showed a less than maximal activation of [^{35}S]GTPγS binding, but did not have antagonist properties. While most partial agonists have antagonist properties, not all compounds that produce less than a maximal effect in an assay have antagonist effects. Some compounds are less efficacious than an agonist that produces a maximal effect. While many of these compounds have antagonist properties, too, making them partial agonists, there are some compounds that are less efficacious than full agonists but do not have antagonist properties. Compounds **1a**, **1b**, **6a**, **8a** and **10b** are examples of compounds that have a lower efficacy in the [^{35}S]GTPγS binding assay mediated by the κ receptor, but these compounds do not antagonize [^{35}S]GTPγS binding that was induced by the κ agonist U50,488.

A series of novel opioids **5b**–**19b** have been prepared where the phenolic-OH group of traditional and well-studied opioids **5a**–**19a** was replaced by a carboxamide ($CONH_2$) group. Characterization of target and known compounds in opioid receptor binding assays revealed that carboxamide targets **5b**–**11b** and **19b** derived from 2,6-methano-3-benzazocine (a.k.a. benzomorphans), morphinan or tramadol-based core structures have high affinity to μ and κ receptors and relatively low affinity for δ receptors. Compared to their phenolic-OH counterparts **5a**–**11a** and **19a**, comparable or enhanced affinity was seen for all three receptors. Receptor selectivity for these carboxamides was, in general, similar to the OH partners. It is interesting to note that carboxamide **7b** having the phenazocine core, has *K*BRs for all three receptors considerably higher than the other 2,6-methano-3-benzazocine cores and has the least degree of selectivity between μ, δ and κ. Another note of interest is the observation that when a divergence in selectivity ratios is seen, there is a trend toward higher selectivity for μ than κ.

A divergent SAR was seen for carboxamides **12b**–**18b** having the 4,5α-epoxymorphinan core. Without exception, these carboxamides had lower and in many instances, much lower affinity for μ, δ and κ opioid receptors than their phenolic-OH counterparts **12a**–**18a**. These observations were, in fact, predicted from our earlier studies and are consistent with our pharmacophore hypothesis concerning the bioactive conformation of the carboxamide group.[4] Receptor selectivity of carboxamides **12b**–**18b** for μ, δ and κ receptors was, in general, similar to that seen for corresponding OH partners **12a**–**18a**. SAR findings that we now report are consistent with our previous OH → $CONH_2$ switch studies[1,3–5] and as well as those from other laboratories.[5–9] For those OH/$CONH_2$ pairs studied in [^{35}S]GTPγS binding assays, within each pair, similarities in their function and potency profiles were frequently observed especially at the κ receptor. At the μ receptor, more divergence was observed with a trend towards the carboxamide partner displaying less agonist activity.

Acknowledgments

We gratefully acknowledge the contributions of Rensselaer's mass spectroscopist Dr. Dmitri Zagorevski and the technical assistance provided by Brian I. Knapp of the University of Rochester. Funding of this research was from NIDA (DA12180 and KO5-DA00360) and the NSF (Agilent 1100 series LC/MSD system).

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. Wentland, M. P.; Lou, R.; Dehnhardt, C. M.; Duan, W.; Cohen, D. J.; Bidlack, J. M. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1717.
4. Wentland, M. P.; Lu, Q.; Lou, R.; Knapp, B. I.; Bidlack, J. M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2107.
5. 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.
6. Le Bourdonnec, B.; Belanger, S.; Cassel, J. A.; Stabley, G. J.; DeHaven, R. N.; Dolle, R. E. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4459.
7. Diaz, N.; Benvenga, M.; Emmerson, P.; Favors, R.; Mangold, M.; McKinzie, J.; Patel, N.; Peters, S.; Quimby, S.; Shannon, H.; Siegel, M.; Statnick, M.; Thomas, E.; Woodland, J.; Surface, P.; Mitch, C. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3844.
8. Dolle, R. E.; Machaut, M.; Martinez-Teipel, B.; Belanger, S.; Cassel, J. A.; Stabley, G. J.; Graczyk, T. M.; DeHaven, R. N. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3545.
9. Weltrowska, G.; Lemieux, C.; Chung, N. N.; Schiller, P. W. *J. Pept. Res.* **2005**, *65*, 36.
10. Archer, S.; Albertson, N. F.; Harris, L. S.; Pierson, A. K.; Bird, J. G. *J. Med. Chem.* **1964**, *7*, 123.
11. Ager, J. H.; Fullerton, S. E.; May, E. L. *J. Med. Chem.* **1963**, *6*, 322.
12. Sargent, L. J.; Ager, J. H. *J. Med. Chem.* **1963**, *6*, 569.
13. Merz, H.; Stockhaus, K. *J. Med. Chem.* **1979**, *22*, 1475.
14. Neumeyer, J. L.; Bidlack, J. M.; Zong, R.; Bakthavachalam, V.; Gao, P.; Cohen, D. J.; Negus, S. S.; Mello, N. K. *J. Med. Chem.* **2000**, *43*, 114.
15. Monkovic, I.; Conway, T. T.; Wong, H.; Perron, Y. G.; Pachter, I. J.; Belleau, B. *J. Am. Chem. Soc.* **1973**, *95*, 7910.
16. Cone, E. J. *Tetrahedron Lett.* **1973**, 2607.
17. Chatterjie, N.; Inturrisi, C. E.; Dayton, H. B.; Blumberg, H. *J. Med. Chem.* **1975**, *18*, 490.
18. Lewis, J. W. *Drug Alcohol Depend.* **1985**, *14*, 363.
19. Subrahmanyam, V.; Renwick, A. B.; Walters, D. G.; Young, P. J.; Price, R. J.; Tonelli, A. P.; Lake, B. G. *Drug Metab. Dispos.* **2001**, *29*, 1146.
20. Linder, C.; Fishman, J. *J. Med. Chem.* **1973**, *16*, 553.
21. Schmidhammer, H.; Spetea, M.; Schuetz, J.; Greiner, E.; Schuellner, F.; Sailer, B.; Stuebegger, K. WO 2004005294, 2004.
22. Hahn, E. F.; Fishman, J.; Heilman, R. D. *J. Med. Chem.* **1975**, *18*, 259.
23. Kubota, H.; Rice, K. C. *Tetrahedron Lett.* **1998**, *39*, 2907.
24. Portoghese, P. S.; Lipkowski, A. W.; Takemori, A. E. *J. Med. Chem.* **1987**, *30*, 238.
25. Carson, J. R.; Pitis, J. M. US Patent Appl. US2005/0256203.
26. 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.
27. Archer, S.; Seyed-Mozaffari, A.; Jiang, Q.; Bidlack, J. M. *J. Med. Chem.* **1994**, *37*, 1578.
28. Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
29. Wentland, M. P.; VanAlstine, M.; Kucejko, R.; Lou, R.; Cohen, D. J.; Parkhill, A. L.; Bidlack, J. M. *J. Med. Chem.* **2006**, *49*, 5635.