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Syntheses of novel high affinity ligands for opioid receptors

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ABSTRACT

A series of novel high affinity opioid receptor ligands have been made whereby the phenolic-OH group of nalbuphine, naltrexone methiodide, 6-desoxonaltrexone, hydromorphone and naltrindole was replaced by a carboxamido group and the furan ring was opened to the corresponding 4-OH derivatives. These furan ring 'open' derivatives display very high affinity for μ and κ receptors and much less affinity for δ. The observation that these target compounds have much higher receptor affinity than the corresponding ring 'closed' carboxamides significantly strengthens our underlying pharmacophore hypothesis concerning the bioactive conformation of the carboxamide group.

We recently reported the synthesis and exceedingly high μ opioid receptor binding affinity (K_i = 0.052 nM) of a novel derivative **1** of 3-desoxy-3-carboxamidonaltrexone **2**.[1] The design of **1** was based a strategy whereby the 3-carboxamido group was stabilized in the putative bioactive conformation **1a** via an intramolecular H-bond to the adjacent 4-OH donor. The rationale behind this pharmacophore hypothesis arose from the observation that carboxamide derivative **2** had much lower binding affinity than naltrexone (**3**).[2] This result was in conflict with our other SAR studies where the OH→$CONH_2$ switch on non-4,5α-epoxymorphinan core opioid structures (e.g., 2,6-methano-3-benzazocines and morphinans) resulted in sustained or enhanced binding affinity.[1–3] We recently reported studies where the OH→$CONH_2$ switch was performed on fifteen additional diverse opioid core structures; results were entirely consistent with our earlier work.[4] These SAR data coupled with the observation that the proton NMR ($CDCl_3$) spectrum of **2** revealed a strong H-bond between the carboxamide NH (as donor) and the neighboring ether oxygen (i.e., **2a**) led us to reason that the putative carboxamide bioactive conformation was that as shown in **2** rather than **2a** and the compound must pay an energy penalty to adopt the putative bioactive conformation **2** resulting in lower affinity.[1] We also provided strong evidence that (a) the intramolecular H-bond of **1a** was a consequence of the carboxamide acting as acceptor and not as donor (i.e., **1b**) and (b) the benefit of the 4-OH of **1** was to stabilize the putative bioactive conformation **1a** and not via direct contact with the receptor.[1]

X-ray crystal structures were recently obtained for compounds **1** (as the HCl salt; CCDC 710249) and **2** (CCDC 710250).[5] Stick representations are shown in Figure 1. The conformations of the two compounds in the solid state are very similar to those we previously proposed in $CDCl_3$ solution, namely (a) the presence of an intramolecular H-bond in **1** between the carboxamide O and a donor H on the 4-hydroxyl group and (b) the presence of an intramolecular H-bond in **2** between the carboxamide NH and the ether O of the furan ring bridge. These combined NMR and X-ray physical data strongly corroborate our bioactive conformation hypothesis as outlined above.

We now report the syntheses of additional examples of 3-desoxy-3-carboxamido-4-hydroxy opioids related to **1**. The main goal

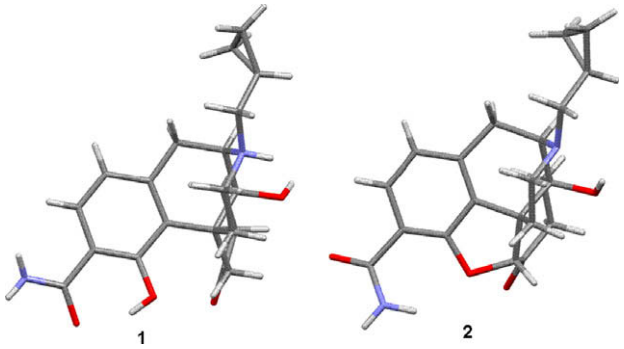

Figure 1. Comparison of the X-ray crystal structures of the furan ring 'open' analogue **1** to the corresponding ring 'closed' derivative 3-desoxy-3-carboxamidonaltrexone **2**.

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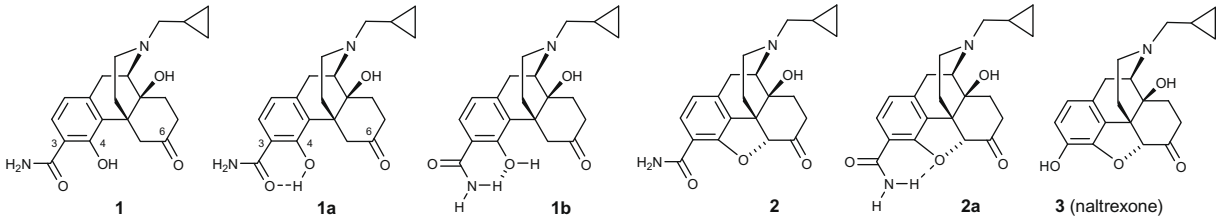
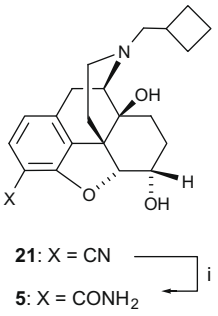


of this study is to confirm the pharmacophore hypothesis that was based on analysis of limited opioid receptor binding previously reported.[1] The phenolic-OH-containing opioids nalbuphine (**6**),[6] naltrexone methiodide (**10**),[7,8] 6-desoxonaltrexone (**13**),[9] hydromorphone (**16**)[10] and naltrindole (**19**)[11] were chosen as substrates for modification to the corresponding 3-desoxy-3-carboxamido-4-hydroxy derivatives related to **1** (i.e., furan ring 'open') and the 3-desoxy-3-carboxamido derivatives analogous to **2** (i.e., furan ring 'closed').

Target compounds related to nalbuphine (**6**)[6] were made using the methodology described in Schemes 1 and 2. Compound **4** having the furan ring 'open' form, was made in 17% yield by the reduction of keto derivative **20**[1] with $NaBH_4$ in MeOH (Scheme 1). This reaction also provided the 6-β-ol analogue **7** (35%); the two isomeric alcohols were easily separated by silica gel flash chromatography. As shown in Scheme 2, carboxamide target compound **5** with the furan ring 'closed' was made in 79% yield via the partial hydrolysis of nitrile intermediate **21**[1] using KOH in refluxing *t*-BuOH.

Target compound **8**, the 'open' carboxamide having the naltrexone methiodide core structure **10** (see Table 1 for structure), was made in 60% yield by heating **1** at 70 °C in a sealed tube for 4 d with 10 equivalents of CH_3I in acetone (Scheme 3). The stereochemistry of the quaternary nitrogen center of **8** was assigned (*R*)- using 2D NOESY NMR (DMSO-d_6, 500 MHz, mixing time = 0.6 s, relax delay = 0.9 s). A cross peak was observed between the proton of 14-OH group and the protons of CH_3 group indicating the CH_3 group occupies the axial conformation with respect the six-membered piperidine ring. This stereochemical assignment is consistent with recent synthetic studies centered on the N-methylation of derivatives of naltrexone.[7] In similar fashion, compounds **2** and **3** (naltrexone) were converted to target compounds **9** (43%) and **10** (41%), respectively; stereochemistry of the newly introduced center of chirality was also assigned as (*R*)- using 2D NOESY NMR. The FDA-approved drug methylnaltrexone,[7,8] is the *N*-methyl quaternary bromide salt of naltrexone. It is unclear from the literature whether the quaternized nitrogen is (*S*)- or (*R*)- or if it is a mixture of the two diastereomers.[7,8] Nevertheless we prepared naltrexone methiodide **10** and used it as a comparator to carboxamide target compounds **8** and **9** (see Table 1 for structures) since all three share the same stereochemistry.

Target compounds **11** and **12** related to 6-desoxonaltrexone (**13**)[9] were made using methodologies shown in Schemes 4 and 5. The known 3-cyano derivative **22**[12] of naltrexone was treated

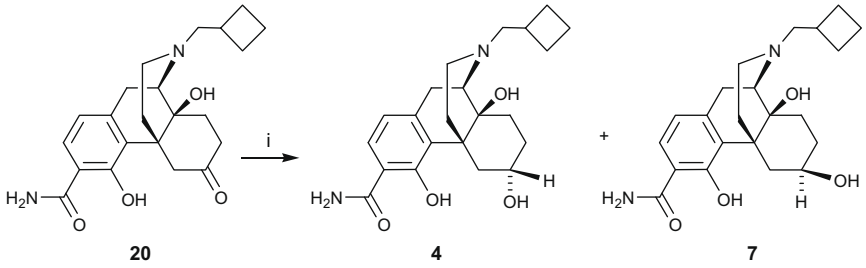


Scheme 2. Reagents and conditions. (i) KOH, *t*-BuOH, 82 °C, 12 h.

with zinc dust, 37% HCl, in refluxing acetic acid to give desired target compound **11** in 22% yield along with **1** (Scheme 4). The corresponding ring 'closed' carboxamide **12** was prepared by a multistep procedure shown in Scheme 5. The 6-keto group of naltrexone (**3**) was reduced to the known 6-desoxonaltrexone **13**[9] in 62% yield using standard Wolff-Kishner conditions of hydrazine hydrate, diethylene glycol and KOH at reflux. Triflate ester **23** was then made in 98% yield by treating **13** with $PhN(SO_2CF_3)_2$ and triethylamine in CH_2Cl_2. Compound **23** was converted to nitrile **24** in 95% yield using $Zn(CN)_2$, $Pd(PPh_3)_4$, in DMF and partial hydrolysis of **24** using KOH in refluxing *t*-BuOH provided target compound **12** in 95% yield.

Carboxamide target compounds **14** and **15** related to hydromorphone **16**[10] were made using the procedure outlined in Scheme 6. The 3-triflate ester **25**[13] of morphine was converted to nitrile **26**[14] in 66% yield using $Zn(CN)_2$, $Pd(PPh_3)_4$, in DMF. Selective reduction of the double bond of **26** to dihydro derivative **27** was accomplished in quantitative yield using H_2, 10% Pd:C in MeOH. Oxidation of **27** using standard Swern conditions provided **28** in 92% yield. Nitrile derivative **28** was then converted to target compound **14** in 63% yield using Zinc dust, NH_4Cl in EtOH and to target compound **15** in 85% yield using KOH in *t*-BuOH at reflux.

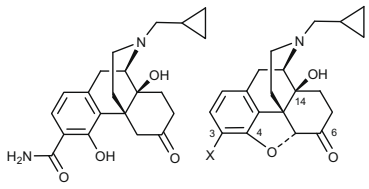
The naltrindole-based carboxamide target compound **17** was made in 31% yield by treating **1** with $PhNHNH_2$, *p*-TsOH in refluxing EtOH (Scheme 7). This procedure is similar to that used to make naltrindole from naltrexone.[11] The carboxamide variant **18** was prepared from naltrindole using procedures similar to those previously described.[1–3] Naltrindole (**19**)[11] was first converted to its tri-



Scheme 1. Reagents and conditions. (i) NaBH4, MeOH, 25 °C, 16 h.

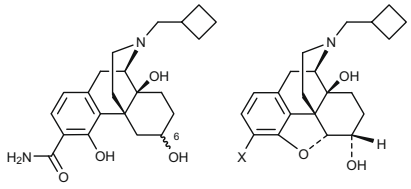
Table 1
Opioid receptor binding data for carboxamido-substituted opioids compared to the OH counterparts

Naltrexone core:



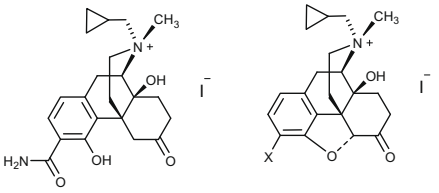
1 **2**: X = $CONH_2$ **3**: X = OH

Nalbuphine core:



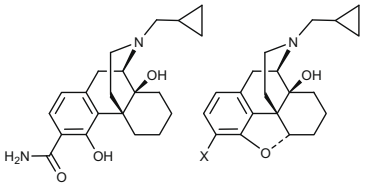
4: 6-α-OH **7**: 6-β-OH **5**: X = $CONH_2$ **6**: X = OH

Naltrexone methiodide core:



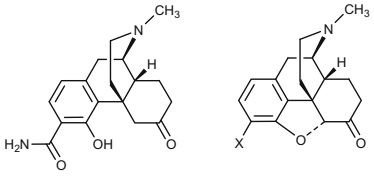
8 **9**: X = $CONH_2$ **10**: X = OH

6-Desoxonaltrexone core:



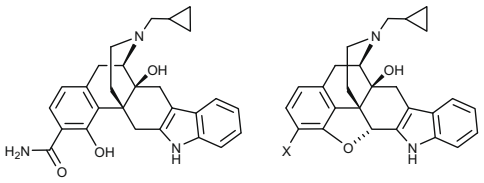
11 **12**: X = $CONH_2$ **13**: X = OH

Hydromorphone core:



14 **15**: X = $CONH_2$ **16**: X = OH

Naltrindole core:



17 **18**: X = $CONH_2$ **19**: X = OH

Compd	K_i (nM)[a]				
	[^{3}H]DAMGO (μ)	[^{3}H]Naltrindole (δ)	[^{3}H]U69,593 (κ)	μ:δ[b]	μ:κ[c]
Naltrexone core					
1[d]	0.052 ± 0.004	2.6 ± 0.26	0.23 ± 0.018	50	4.4
2[d]	0.71 ± 0.058	550 ± 40	11 ± 0.36	780	15
3[e]	0.11 ± 0.006	60 ± 3.2	0.19 ± 0.005	550	1.7
Nalbuphine core					
4[f]	0.52 ± 0.014	78 ± 7.0	9.0 ± 1.9	150	17
5[f]	3.8 ± 0.62	150 ± 82	0.46 ± 0.04	39	0.12
6[e]	1.6 ± 0.37	580 ± 80	3.0 ± 0.63	360	1.9
7[f]	0.072 ± 0.008	3.9 ± 0.42	0.34 ± 0.05	54	4.7
Naltrexone methiodide core					
8[f]	1.3 ± 0.13	280 ± 21	7.7 ± 0.90	220	5.9
9[f]	37 ± 1.6	>10 μM	210 ± 22	>270	5.7
10[e]	2.0 ± 0.27	900 ± 36	6.3 ± 0.46	450	3.2
Naltrexone-6-desoxo core					
11[f]	0.16 ± 0.011	4.2 ± 0.74	0.29 ± 0.015	26	1.8
12[f]	2.5 ± 0.27	630 ± 56	16 ± 1.1	250	6.4
13[e]	0.24 ± 0.0044	79 ± 11	0.24 ± 0.0014	330	1.0
Hydromorphone core					
14[f]	0.30 ± 0.01	8.3 ± 0.60	2.3 ± 0.3	28	7.7
15[f]	1.2 ± 0.053	260 ± 35	19 ± 1.9	220	16
16[e]	0.28 ± 0.02	38 ± 5.2	2.8 ± 0.2	136	10
Naltrindole core				δ:μ[g]	δ:κ[h]
17[f]	0.51 ± 0.010	0.025 ± 0.0013	0.81 ± 0.012	20	32
18[f]	46 ± 9.8	0.30 ± 0.021	47 ± 2.5	150	160
19[e]	5.3 ± 0.23	0.14 ± 0.0043	2.9 ± 0.35	38	21

[a] Binding assays used to screen compounds are similar to those previously reported (see Refs. 15 and 16). 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 [^{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% polyethylenimine for at least 60 min before use. IC_{50} 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. 17. 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 the mean ± SEM from at least three experiments performed in triplicate.

[b] μ:δ = K_i (δ)/K_i (μ) This value shows the selectivity of the compound for the μ receptor over the δ receptor.

[c] μ:κ = K_i (κ)/K_i (μ) This value shows the selectivity of the compound for the μ receptor over the κ receptor.

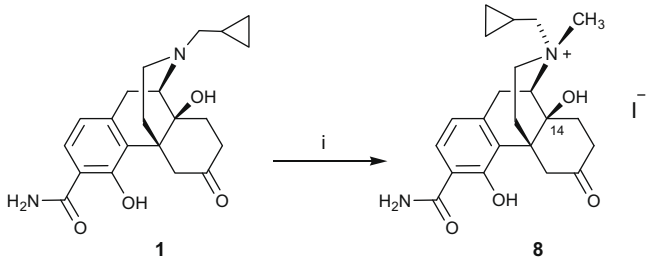
[d] See Ref. 1.

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

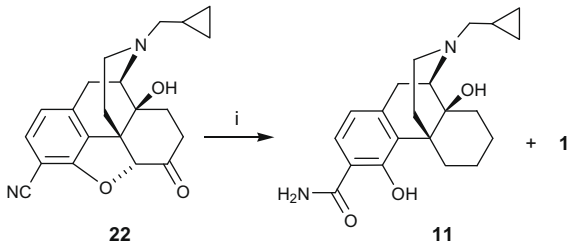
[f] Proton NMR, IR and MS were consistent with the assigned structures of all new compounds. C, H, and N elemental analyses were obtained for all new target compounds and most intermediates and were within ± 0.4% of theoretical values.

[g] δ:μ = K_i (μ)/K_i (δ) This value shows the selectivity of the compound for the δ receptor over the μ receptor.

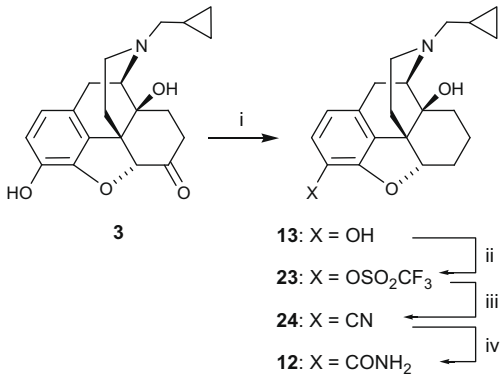
[h] δ:κ = K_i (κ)/K_i (δ) This value shows the selectivity of the compound for the δ receptor over the κ receptor.



Scheme 3. Reagents and conditions. (i) CH_3I, acetone, 70 °C, 4 d (sealed tube).



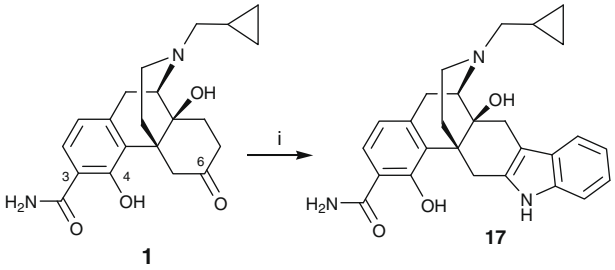
Scheme 4. Reagents and conditions. (i) Zn, 37% HCl, HOAc, 125 °C, 3 h.



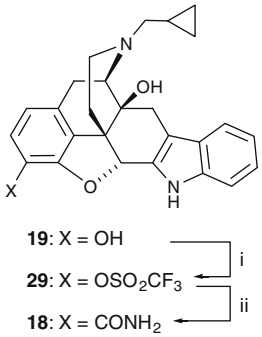
Scheme 5. Reagents and conditions. (i) $NH_2NH_2{\cdot}H_2O$, $(HOCH_2CH_2)_2O$, 210 °C, 1.5 h; (ii) $PhN(Tf)_2$, Et_3N, CH_2Cl_2, 25 °C, 1.5 h; (iii) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 130 °C, 8 h; and (iv) KOH, *t*-BuOH, 82 °C, 5 h.

flate ester **29** in 56% yield using $PhN(SO_2CF_3)_2$, Et_3N in CH_2Cl_2. Compound **29** was then subjected to CO, NH_3, $Pd(OAc)_2$ and DPPF in DMSO to provide target compound **18** in 27% yield (Scheme 8).

Both furan ring 'open' and 'closed' carboxamide target compounds as well as their phenolic-OH counterparts were evaluated for their affinity for μ, δ and κ opioid receptors. Binding data are detailed in Table 1. Membrane protein from CHO cells that stably



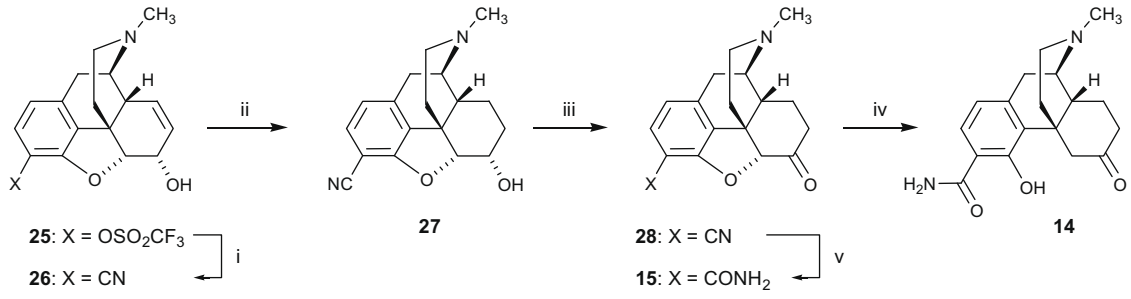
Scheme 7. Reagents and conditions. (i) $PhNHNH_2$, *p*-TsOH, EtOH, 78 °C, 2 h.



Scheme 8. Reagents and conditions. (i) $PhN(Tf)_2$, Et_3N, CH_2Cl_2, 25 °C, 11 h; (ii) CO, $Pd(OAc)_2$, NH_3, DPPF, DMSO, 70 °C, 15 h.

expressed one type of the human opioid receptor was used.[15,16] The objectives of this study are to compare the binding affinities of (a) the 'open' to the 'closed' form in each carboxamide pair, and secondarily (b) the 'closed' carboxamide target compounds to the corresponding parent phenolic-OH opioids.

Consistent with our earlier observations for naltrexone derivatives **1** and **2**,[1] there is a convincing trend in the SAR that the ring 'open' carboxamide partner has much higher affinity for μ, δ and κ opioid receptors than the corresponding ring 'closed' carboxamide. As shown in Table 1, against μ, the 'open' derivatives **4** (nalbuphine core), **8** (naltrexone methiodide core), **11** (6-desoxonaltrexone core), **14** (hydromorphone core) and **17** (naltrindole core) have 7-, 28-, 16-, 4- and 90-fold higher affinity, respectively, than the corresponding furan ring 'closed' carboxamides, **5**, **9**, **12**, **15**, and **18**. Doing the identical comparison for δ, the increase in potency is 2-, >35-, 150-, 31- and 12-fold. For the κ receptor, the increase in binding affinity was 27-, 55- 8- and 58-fold for 'open' derivatives **8**, **11**, **14** and **17**; however, for the pair with a nalbuphine core, the 'open' analogue **4** had 20-fold *lower* affinity than the 'closed' form **5**. For 14 of 15 comparisons of ring 'open' to 'closed' carboxamides (5 novel pairs against 3 receptor subtypes), the observation that the 'open'



Scheme 6. Reagents and conditions. (i) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 120 °C, 20 h; (ii) 40 psi H_2, 10% Pd/C, MeOH, 25 °C, 4 h; (iii) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C, 20 min then Et_3N at 25 °C; (iv) Zn, NH_4Cl, EtOH, 78 °C, 4 h; (v) KOH, *t*-BuOH, 82 °C, 2 h.

analogues have much higher affinity strongly corroborates our pharmacophore hypothesis regarding carboxamide bioactive conformation of naltrexone as outlined earlier in this Letter. For the single exception, we do not have an explanation as to why the nalbuphine-based pair **4** and **5** against κ does not follow this SAR trend. Absolute affinity of ring 'open' target compounds **4**, **8**, **11**, **14**, and **17** for μ is very high; K_i values were all subnanomolar (0.16–0.52 nM) except for compound **8**, a derivative of naltrexone methiodide which was slightly lower (K_i = 1.3 nM). This latter result is not surprising since the affinity of parent OH compound **10** is also in the single digit range (K_i = 2.0 nM). Compound **7**, obtained as a byproduct, is a diastereomer of nalbuphine-derived target compound **4** and displays outstanding affinity for μ (K_i = 0.072 nM).

With the exception of naltrindole-derived target compound **17,** ring 'open' carboxamides **4**, **7**, **8**, **11**, and **14** have much lower affinity for δ than μ where K_i values ranged between 3.9 and 280 nM. However for target compound **17**, not only is affinity for δ extremely high (K_i = 0.025 nM), it has 6-fold higher affinity than the well known δ-selective parent, naltrindole (**19**).[11] Three ring 'open' carboxamides, **7**, **11** and **17**, have very high affinity for the κ receptor (K_i = 0.34, 0.29 nM and 0.81 nM, respectively); the other carboxamides in this class (**4**, **8** and **14**) have somewhat lower affinity with K_i values in the 2.3–9.0 nM range.

In addressing the secondary objective of this study, we find that novel ring 'closed' carboxamides **9**, **12**, **15** and **18** display lower affinities for the three receptors than their corresponding phenolic-OH counterparts **10**, **13**, **16** and **19**. These reductions in binding affinity are between 4- and 150-fold except for the naltrindole-based carboxamide **18** which has nearly comparable (twofold lower) affinity for δ as naltrindole itself. These findings are consistent with our previous SAR studies[2,4] and add strong support for our underlying pharmacophore hypothesis. There is an important exception, however. In the nalbuphine case, the 'closed' carboxamide **5** has much *higher* affinity for δ and κ receptors than nalbuphine (**6**) by factors of fourfold and sixfold, respectively. Against μ, the two have comparable affinity. We have no rationale why the 3-desoxy-3-carboxamido analogue **5** of nalbuphine (**6**) does not follow this trend in SARs.

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 agonis *t*-stimulated [^{35}S]GTPγS binding to the human μ, δ 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	120 ± 4	NI[b]	NI
1	Antagonist	NA[c]	3.8 ± 0.67	0.88 ± 0.14	92 ± 2.9
2	Weak agonist/antagonist	4.9 ± 1.2	10 ± 1.7	270 ± 37	91 ± 3.6
3 (NTRX)	Weak agonist/antagonist	16 ± 8.6	14 ± 0.82	4.8 ± 0.42	93 ± 0.87
4	Weak agonist/antagonist	3.1 ± 1.9	17 ± 3.0	26 ± 1.6	84 ± 3.2
5	Weak antagonist	NA	3.2 ± 4.5	NA	56% @ 10 μM
6	Agonist/antagonist	46 ± 9.6	26 ± 1.3	96 ± 19	76 ± 1.9
7	Weak agonist/antagonist	0.86 ± 0.29	13 ± 3.7	6.4 ± 0.35	81 ± 1.7
8	Antagonist	NA	−0.28 ± 0.39	52 ± 20	96 ± 1.2
10	Antagonist	NA	5.6 ± 1.4	170 ± 4.0	98 ± 0.62
11	Weak agonist/antagonist	10 ± 8.3	25 ± 3.9	3.8 ± 0.70	92 ± 4.7
12	Antagonist	NA	−0.36 ± 1.3	54 ± 5.9	93 ± 0.46
13	Antagonist	NA	<20%	4.4 ± 1.1	89 ± 5.1
14	Agonist	1.6 ± 0.04	62 ± 3.4	NI	NI
15	Agonist	110 ± 26	140 ± 4.2	NI	NI
16	Agonist	2.6 ± 0.14	70 ± 6.2	NI	NI
17	Antagonist	NA	1.9 ± 1.3	3.5 ± 0.47	97 ± 2.0
Delta opioid receptor					
SNC80	Agonist	4.8 ± 0.60	120 ± 4.7	NI	NI
1	Agonist/antagonist	1.8 ± 0.5	35 ± 4.2	6.9 ± 2.1	56 ± 3.0
3 (NTRX)	weak Agonist/antagonist	21 ± 6.7	14 ± 4.3	130 ± 30	88 ± 1.5
7	Weak agonist/antagonist	3.7 ± 0.18	28 ± 2.4	130 ± 62	58 ± 6.5
11	Antagonist	NA	2.3 ± 0.91	5.8 ± 1.7	79 ± 1.8
17	Antagonist	NA	3.1 ± 2.3	0.080 ± 0.031	88 ± 2.6
18	Antagonist	NA	−3.4 ± 1.4	0.77 ± 0.11	89 ± 1.2
19	Antagonist	NA	−0.47 ± 1.5	0.39 ± 0.070	84 ± 0.88
Kappa opioid receptor					
U50,488	Agonist	36 ± 5.0	77 ± 11	NI	NI
1	Agonist/antagonist	3.3 ± 1.2	36 ± 0.98	38 ± 8.8	57 ± 0.71
3 (NTRX)	Agonist/antagonist	3.3 ± 0.52	39 ± 2.4	130 ± 15	54 ± 0.90
4	Weak agonist/antagonist	3.9 ± 0.27	35 ± 5.1	NA	36 ± 5.2 @ 10 μM
5	Agonist	220 ± 57	39 ± 8.8	NI	NI
6	Agonist	56 ± 9.5	74 ± 7.2	NI	NI
7	Weak agonist/antagonist	2.7 ± 2.2	32 ± 0.22	NA	25 ± 2.0 @ 10 μM
8	Antagonist	NA	3.0 ± 2.6	7800 ± 530	88 ± 1.4
10	Weak agonist/antagonist	9.0 ± 5.0	16 ± 1.9	NA	60 ± 4.9 @ 10 μM
11	Agonist/antagonist	2.3 ± 0.47	35 ± 4.9	57 ± 21	55 ± 3.1
13	Antagonist	NA	<20%	13 ± 2.6	86 ± 2.4
14	Agonist	3.9 ± 0.52	71 ± 3.1	NI	NI
16	Agonist	11 ± 2.9	78 ± 5.6	NI	NI
17	Antagonist	NA	2.1 ± 1.7	28 ± 12	97 ± 1.6

[a] See Ref. 18 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 [^{35}S]GTPγS binding was set at 0%. For inhibition studies, a concentration of 200 nM DAMGO was used to measure inhibition of DAMGO-stimulated [^{35}S]GTPγS binding. A concentration of 10 nM SNC 80 was used to measure inhibition of [^{35}S]GTPγS binding, mediated by the δ opioid receptor and 100 nM U50,488 was used to measure inhibition of U50,488-stimulated [^{35}S]GTPγS binding. %.

[b] NI→no inhibition.

[c] NA→not applicable.

Intrinsic opioid-receptor mediated activity for high affinity (K_i values <5 nM) carboxamide-containing ligands was determined using [^{35}S]GTPγS binding assays at μ, δ and/or κ opioid receptors; results are shown in Table 2. In cases where the parent phenol had high affinity for a particular receptor, they were also assayed. Procedures similar to those previously reported were used.[18] Like naltrexone (**3**), a μ antagonist with a small amount of μ agonist effects in the [^{35}S]GTPγS binding assay,[19] its two carboxamide derivatives **1** and **2** were also found to be potent antagonists at μ; antagonist potency at μ correlated reasonably well with binding affinities. Naltrexone and its 'closed' carboxamide derivative **2** produced a weak stimulation of [^{35}S]GTPγS binding mediated by the μ receptor. The higher affinity of the 'open'; ring carboxamide derivative **1** of naltrexone for the μ receptor may account for its lack of agonist effect in the [^{35}S]GTPγS binding assay. At δ and κ receptors, both **1** and naltrexone were found to be mixed agonists/antagonists. For compounds **4–7** having the nalbuphine core, **4**, **6** and **7** were mixed agonists/antagonists at μ of varying potency while **5** was a weak antagonist. Compound **7** had very high affinity in the receptor binding assay, and it was primarily an antagonist at the μ receptor. At the κ receptor, **4** and **7** were weak agonists/antagonists while **5** and **6** were agonists; potencies in this case correlated reasonable well with binding affinities. Naltrexone methiodide (**10**) and its 'open' carboxamide analogue **8** were found to be antagonists at both μ and κ; potencies for the two were similar at μ and somewhat divergent at κ. 6-Desoxonaltrexone (**13**) and both of its carboxamido analogues **11** and **12**, were all antagonists at μ of varying potencies. Compound **11** was an antagonist at δ and a mixed agonist/antagonist at the κ receptor. Hydromorphone (**16**), a prototypic μ agonist, shares this agonist profile with its two carboxamide analogues, **14** and **15** and potencies in the GTPγS binding assay correlated well with binding affinities at μ. At the κ receptor, both **14** and **16** were also agonists having similar potency that correlated well with binding affinity. Comparing the known δ-selective antagonist naltrindole (**19**)[11] to carboxamide analogues **17** and **18**, the data show that all three were antagonists at δ in our assays. Good correlation was observed between GTPγS and binding potencies at δ. Due to its relatively high affinity to μ and κ compared to **18** and **19**, 'open' carboxamide derivative **17** was evaluated at these receptors where it displayed an antagonist profile.

A series of novel carboxamido-substituted, furan ring 'open' derivatives of nalbuphine, naltrexone methiodide, 6-desoxonaltrexone, hydromorphone and naltrindole generally display very high affinity for opioid receptors and are much more potent than the corresponding ring 'closed' carboxamides. These data significantly strengthen our underlying pharmacophore hypothesis that the bioactive conformation of the carboxamide group of, for example, **1** and **2** is that represented by structures **1a** and **2**. Further support of the hypothesis was gained by our observation that binding affinities of the furan ring 'closed' carboxamides **9**, **12**, **15**, and **18** are lower than their phenolic-OH counterparts **10**, **13**, **16** and **19**. It is interesting to note that the only significant exceptions seen in both SAR trends ('open' carboxamide is preferred over 'closed' and phenolic-OH is preferred over 'closed' carboxamide) are for the nalbuphine core structures **4–6**. Compound **4** against κ, but not at μ or δ, is the only 'open' carboxamide that displays lower affinity than the corresponding 'closed' form **5**. Additionally, compound **5** against δ and κ is the only 'closed' form to have higher binding affinity than the corresponding phenolic-OH parent **6**. At this point, we can not offer any explanation for this divergence in SAR. For those analogues studied in [^{35}S]GTPγS binding assays, a trend was observed where the 'open' and 'closed' carboxamide analogues of a particular phenolic-OH opioid displayed nearly the same functional activity as the OH counterpart. This trend was the strongest when studying the receptor type for which the parent OH opioid had the highest binding affinity (e.g., hydromorphone core compounds at μ and naltrindole core derivatives at δ). For certain cores at certain receptors (e.g., hydromorphone core compounds at μ and naltrindole core derivatives at δ), potency in the GTPγS binding assay correlated nicely with binding affinity. However, for other cores (e.g., nalbuphine) a poor correlation was observed. The value of the SAR data generated in this study is not only the strengthening of our underlying pharmacophore hypothesis, but also in the identification of a number of novel high affinity opioid receptor ligands. Additional research in this area is ongoing in our laboratories and will be the subject of future communications.

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