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Redefining the structure–activity relationships of 2,6-methano-3-benzazocines. Part 3: 8-Thiocarboxamido and 8-thioformamido derivatives of cyclazocine☆

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Abstract—8-Position variants of cyclazocine have been made where the phenolic 8-OH was replaced by thioamide, amidine, guanidine, urea and thiourea groups. High affinity for opioid receptors was observed for the 8-$CSNH_2$ and 8-NHCHS analogues indicating that these groups are isosteric with not only the 8-OH but with the previously synthesized 8-$CONH_2$ and 8-NHCHO cyclazocine derivatives.

As part of our broad goal to identify long-acting opioid-receptor interactive agents useful in the treatment of cocaine addiction in humans, we recently reported the synthesis and opioid receptor binding properties of 8-carboxamidocyclazocine (8-CAC; **1**)[1] and its reversed amide isoster 8-formamidocyclazocine (**2**) (Fig. 1).[2] Interest in investigating the 8-position was driven by our desire to identify bioisosteric replacements for the 8-OH group of cyclazocine **3**,[3] which like many opioid-receptor interactive agents, is prone to metabolic inactivation via *O*-glucuronidation. We observed that replacement of the 8-OH of cyclazocine with $CONH_2$ and NHCHO resulted in sustained high affinity binding to opioid receptors. These results were unexpected based on previous knowledge that the prototypic phenolic OH group of opiates was an important component of the pharmacophore for binding to opioid receptors.[4] In in vivo studies, 8-CAC showed high antinociception activity and a much longer duration of action than cyclazocine (15 h vs 2 h) when both were dosed at 1 mg/kg ip in mice[5].

To develop additional insight into this new SAR surrounding the 8-position of 2,6-methano-3-benzazocines (a.k.a., benzomorphans), we have prepared a series of analogues of **1** and **2** where the 8-substituent was modified by incorporating thioamide, amidine, guanidine, urea and thiourea groups. We now report the synthesis and opioid receptor binding properties of these new benzomorphans.

Racemic compounds **1**[1] and **2**[2] were converted to their respective thioamide targets, **4** and **6**, via treatment with Lawesson's reagent in toluene (microwaves) in yields of 70% and 86%, respectively (Scheme 1). As shown in

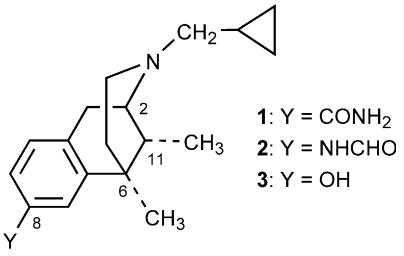

Figure 1. Structures of lead compounds for this study.

Keyword: Opiate SAR.

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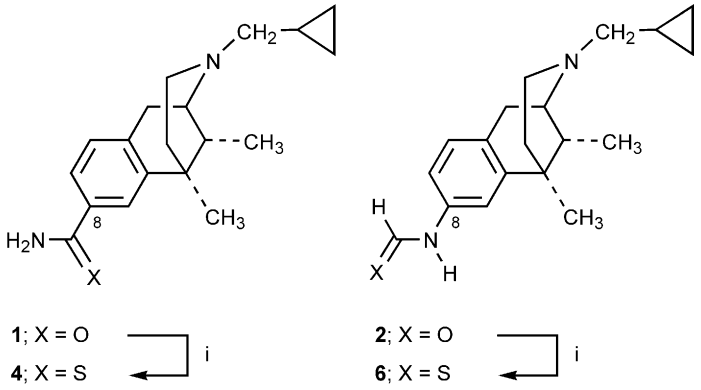
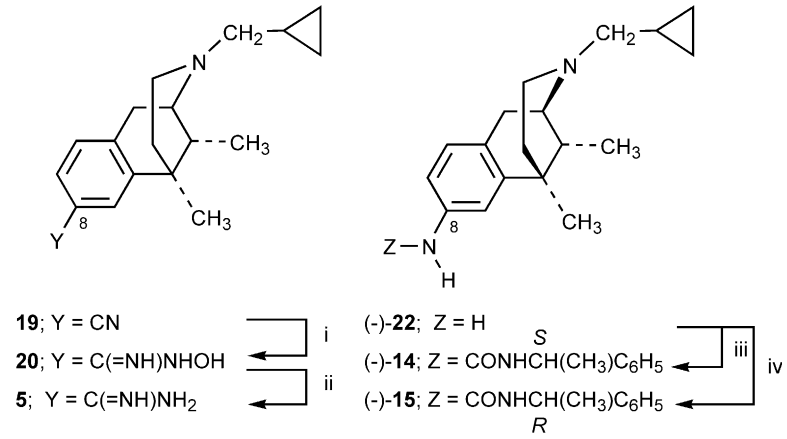


Scheme 1. Syntheses of thioamide targets. Reagents: (i) Lawesson's reagent, tol, microwaves.



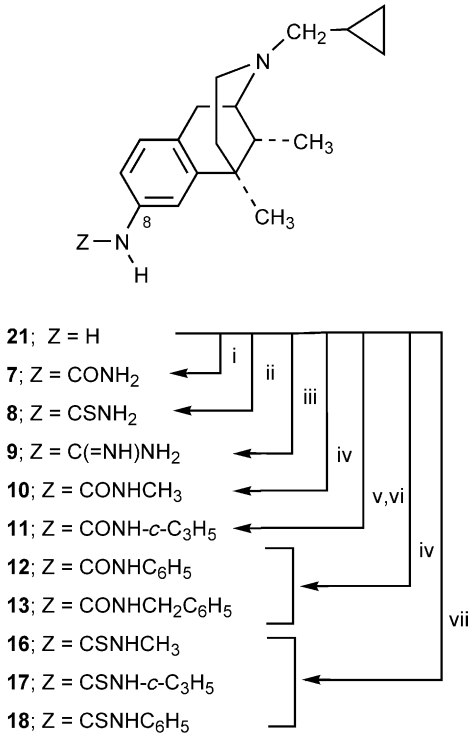
Scheme 2. Syntheses of targets **5**, (−)-**14** and (−)-**15**. Reagents and conditions: (i) $NH_2OH{\cdot}HCl$, Et_3N, EtOH; (ii) Pd/C, H_2, HOAc, H_2O; (iii) (*S*)-(−)-$C_6H_5CH(CH_3)NCO$, CH_2Cl_2; (iv) (*R*)-(+)-$C_6H_5CH(CH_3)NCO$, CH_2Cl_2.

Scheme 2, the amidine analogue (**5**) of carboxamide **1** was made by first treating nitrile **19**[1] with $NH_2OH{\cdot}HCl/Et_3N$ to give a hydoxyamidine intermediate **20**, which without elaborate purification, was reduced (Pd/C, H_2) to give amidine target **5** (75% for two steps). We also attempted to prepare the amidine analogue of **2**; however, its poor aqueous stability (hydrolysis back to **2**) precluded its isolation and biological evaluation.

A number of urea targets were made using 8-aminocyclazocine[6] (**21**) as starting material (Scheme 3). The monosubstituted urea target **7** was made in 77% yield by treating **21** with KNCO in acetic acid.[7] The unsymmetrical disubstituted urea targets **10**, **12** and **13** were made in yields ranging 55–98% by treating **21** with the corresponding commercially available aryl or alkyl isocyanates. *N*-Cyclopropyl urea target **11** was made in 88% overall yield by first treating **21** with 20% phosgene in toluene[8] to give an isocyanate intermediate, which was then treated with cyclopropylamine. Optically active urea derivatives (−)-**14** and (−)-**15** were made by treating optically active amine (−)-(2*R*,6*R*,11*R*)-**22**[6] with commercially available (*S*)-(−)-$C_6H_5CH(CH_3)NCO$ and (*R*)-(+)-$C_6H_5CH(CH_3)NCO$ in yields of 91% and 76%, respectively (Scheme 2).

The thiourea target **8** was prepared in two steps (66% overall yield) by first treating **21** with PhCOCl, and KSCN in acetone followed by hydrolysis of the intermediate with K_2CO_3 in $MeOH/H_2O$.[9] The three thiourea derivatives **16**–**18** were prepared by treating aniline **21** with the appropriate aryl or alkyl isothiocyanates. Guanidine derivative **9** was made from **21** by treatment with MeS–C(=NCbz)NHCbz, $HgCl_2$, Et_3N (93%) to provide an intermediate, which was reduced with H_2, Pd/C, MeOH (58%).[10]

Affinities of target compounds for μ, δ and κ opioid receptors in guinea pig brain membranes or at each human opioid receptor stably expressed in CHO cells were assessed by generating K_i values using well-documented receptor binding assays.[11,12] These data are summarized in Table 1 along with comparative data for the previously reported 8-carboxamido (**1**), formamido (**2**) and OH (**3**) analogues. Opioid receptors from two species were used due to a change in our primary assay midway through this study. Where data are available, absolute and relative affinities, using human or guinea pig receptors were quite similar. For example, affinities (K_i in nM) for guinea pig and human μ receptors for **1** are 0.41 and 0.31, respectively; for **3** they are 0.32 and



Scheme 3. Syntheses of urea and thiourea targets. Reagents: (i) KNCO, HOAc, 35 °C; (ii) (a) PhCOCl, KSCN, acetone, 57 °C; (b) K_2CO_3, MeOH, H_2O; (iii) (a) MeS–C(=NCbz)NHCbz, $HgCl_2$, Et_3N, DMF; (b) H_2, Pd/C, MeOH; (iv) RNCO, solvent; (v) 20% $COCl_2$ in tol, CH_2Cl_2, 25 °C; (vi) *c*-$C_3H_5NH_2$, CH_2Cl_2, 25 °C; (vii) RNCS, DMF.

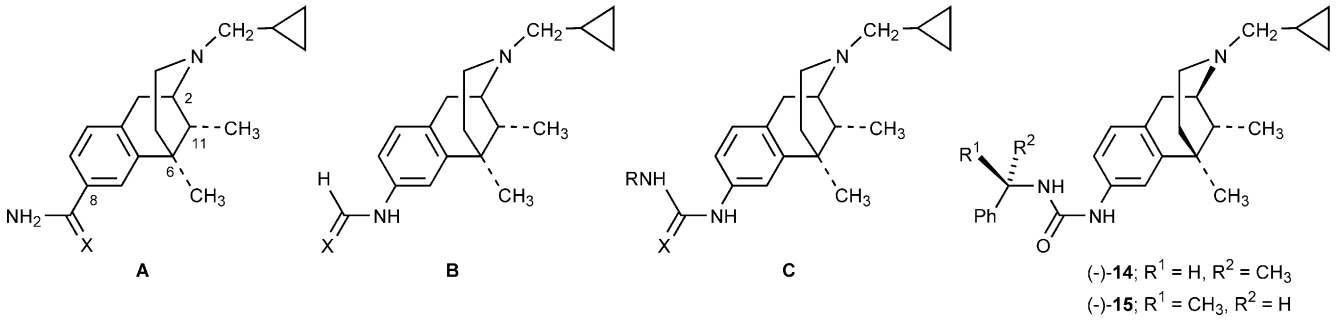
0.16, respectively; and for **8** they are 13 and 10, respectively.

By modifying amides **1** and **2** to their respective thioamides **4** and **6**, comparably high affinity for opioid receptors was observed; these thioamides were very potent with K_i values being <1 nM for μ and κ. The implications of these findings will be detailed in a following paragraph. When the carboxamide group of **1** was modified to give the corresponding amidine **5**, very poor affinity was observed for μ and κ ($K_i \geqslant 180$ nM). As the amidine group of **5** is very basic ($pK_a \sim 12$) and thus protonated to a very high degree at the biological target, this result suggests that the receptors have very low tolerance to positively charged substituents directly attached to the 8-position. This conclusion is supported by another new compound we made, namely the 8-guanidine derivative **9**. K_i values for this highly basic compound were 1000 and 170 nM for μ and κ, respectively.

Previous SAR studies led us to believe that both the H-bond donating and accepting properties of amides **1** and **2** were very important for molecular recognition to opioid receptors.[1,2] To explore the possibility that incorporation of an additional polar group(s) into the 8-substituent would further enhance affinity, we prepared the urea (**7**) and thiourea (**8**) analogues as probes. As was seen with the two amide↔thioamide pairs **1/4** and **2/6**, comparable affinity was also evident for urea derivative **7** and the corresponding thiourea derivative **8**, however the absolute potencies of both (K_i values for μ and κ = 11–20 nM) were substantially lower than **1**, **2**, **4** or **6**. In a related SAR study where a large series of 8-(substituted)amino-cyclazocine (i.e., 8-RNH–) derivatives were evaluated, we found that attaching certain aryl or heteroaryl groups to the 8-NH significantly improved binding affinity relative to the unsubstituted (i.e., 8-NH_2) analogue.[6] For example, the K_i values for 8-PhNH derivative for μ and κ were approximately 1 nM, 7-fold more potent than the parent 8-NH_2 compound. We did not see, however, the same benefit of phenyl substitution when we substituted the carboxamide (i.e., 8-CONHPh) or formamide (i.e., 8-NHCOPh) groups of **1** and **2**, respectively.[1,2] Binding affinity for μ for these compounds was very poor with K_i values of 740 and 570 nM, respectively, leading to the conclusion that the benefit of (hetero)aryl substituents was highly dependent on conformational effects. To that end, we made a small series of unsymmetrical disubstituted urea (**10**–**15**) and thiourea derivatives (**16**–**18**) to probe this putative hydrophobic pocket on the receptor. Whether the target be a urea or thiourea, little advantage was seen by replacing one of the H's of the terminal NH_2 group with phenyl (**12** and **18**, respectively), benzyl (**13**), methyl (**10** and **16**) or cyclopropyl (**11** and **17**) groups. The best affinity for μ and κ was seen with the *N*-benzyl urea target **13** having somewhat higher (4-fold) affinity than the less substituted urea **7**. Since **13** displayed reasonable binding affinity, we then substituted the benzylic CH_2 group of the active enantiomer of **13** with methyl to test our hypothesis that an 8-aryl-containing appendage makes specific molecular contacts with a putative complimentary site on the receptor. This substitution was not a good choice since the addition of this methyl group to give (−)-**14** and (−)-**15** resulted in a significant loss of affinity. Extrapolating from a number of published studies[1,2,6] indicating the active enantiomer of cyclazocine derivatives has the same (2*R*,6*R*,11*R*)-absolute configuration as in (−)-**14** and (−)-**15**, addition of the methyl results in up to a 15-fold decrease in affinity for μ and up to a 27-fold decrease for κ compared to the active enantiomer of **13**. In addition, there is little difference between the binding affinities of the two diasteriomers.

(Thio)amide groups are generally not considered to be classical phenolic-OH replacements[13] although it has been observed that upon replacing the 5-OH group of 5-hydroxytryptamine with $CONH_2$, certain biological properties were retained.[14,15] SAR studies for opiates to date, including this one, suggest the importance of the H-bond donating ability of the five isosteric groups, OH, $CONH_2$, NHCHO, $CSNH_2$ and NHCHS. The induced-fit theory of ligand binding[16] may be used to support a unified binding hypothesis. For the μ opioid receptor, His297 has been proposed as the complementary H-bond acceptor to the phenolic-OH of opiates.[17,18] Subtle movement within the active site of protein upon ligand binding may well position this putative imidazole acceptor to within H-bonding distance to the donor Hs of all five isosteric groups. Other SAR data indicate the importance of the H-bond accepting ability of the (thio)amides.[1,2] However, an irregularity

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



Compd				K_i(nM ± SEM)[a]		
		X	R	[^{3}H]DAMGO (μ)	[^{3}H]Naltrindole (δ)	[^{3}H]U69,593 (κ)
1[b,d]	A	O		0.41 ± 0.07	8.3 ± 0.49	0.53 ± 0.06
2[b,e]	B	O		1.9 ± 0.14	37 ± 3.9	0.85 ± 0.080
3[b,f] (cyclazocine)				0.32 ± 0.02	1.1 ± 0.04	0.18 ± 0.020
4[b,g]	A	S		0.22 ± 0.02	4.0 ± 0.48	0.67 ± 0.01
5[b,g]	A	NH		190 ± 11	> 10 μM	180 ± 9.3
6[b,g]	B	S		0.76 ± 0.09	16 ± 0.30	0.63 ± 0.15
7[b,g]	C	O	H	20 ± 0.66	90 ± 12	15 ± 1.4
8[b,g]	C	S	H	13 ± 3.6	1100 ± 117	11 ± 1.6
9[b,g]	C	NH	H	1000 ± 110	> 10 μM	170 ± 13
10[c,g]	C	O	CH_3	100 ± 7.2	1100 ± 72	29 ± 1.0
11[c,g]	C	O	*c*-C_3H_5	55 ± 4.5	290 ± 47	19 ± 3.7
12[c,g]	C	O	Ph	21 ± 2.2	140 ± 16	18 ± 0.84
13[c,g]	C	O	CH_2Ph	4.8 ± 0.38	51 ± 3.9	5.2 ± 0.045
(−)-**14**[c,g,h]				37 ± 3.7	600 ± 4.7	71 ± 2.6
(−)-**15**[c,g,i]				24 ± 1.5	310 ± 20	47 ± 2.6
16[c,g]	C	S	CH_3	220 ± 6.7	> 10 μM	130 ± 4.4
17[c,g]	C	S	*c*-C_3H_5	190 ± 24	> 10 μM	150 ± 6.5
18[c,g]	C	S	Ph	49 ± 4.7	780 ± 243	28 ± 1.9

[a] Binding assays used to screen compounds are similar to those previously reported (see Refs. 11 and 12). Membrane protein from either guinea pig brain or 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 (κ), 0.25 nM [^{3}H]DAMGO (μ) or 0.2 nM [^{3}H]naltrindole (δ) 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 & Schuell No. 32 glass fibre 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 will be calculated by least squares fit to a logarithm-probit analysis. K_i values of unlabelled compounds were calculated from the equation $K_i = (IC_{50})/1 + S$ where S = (concentration of radioligand)/(K_d of radioligand)—see Ref. 23. Data are the mean ± SEM from at least three experiments performed in triplicate.

[b] Guinea pig membranes.

[c] Human receptors.

[d] See Ref. 1.

[e] See Ref. 2.

[f] See Ref. 3.

[g] 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.

[h] $[\alpha]_D^{25}$ −75.4 (*c* 0.20, $CHCl_3$).

[i] $[\alpha]_D^{25}$ −87.4 (*c* 0.10, $CHCl_3$).

emerges, namely, within each of the two pairs of analogues, $CONH_2/CSNH_2$ and NHCHO/NHCHS, nearly identical binding affinities to the receptors are seen despite the knowledge that sulfur is a weaker H-bond acceptor than oxygen.[19,20] However, our hypothesis that both H-bond donating and accepting properties are important components of the pharmacophore, is supported by studies showing that while a thioamide is a poorer H-bond acceptor relative to the corresponding amide, it is a superior H-bond donor.[21] It is also possible that induced fit places the (thio)amide group of the ligands in close proximity to the complementary bifunctional side chains of Asn150 or Asn328 of the μ receptor. Upon inspection of the coordinates of the μ-norBNI homology model from the rhodopsin X-ray structure, these carboxamide-containing side chains are in close proximity (9–11 Å) to the imidazole of His297.[18] Additionally, it is well known from protein tertiary structural studies that two carboxamides display dual bifunctional H-bond complementary and that Asn

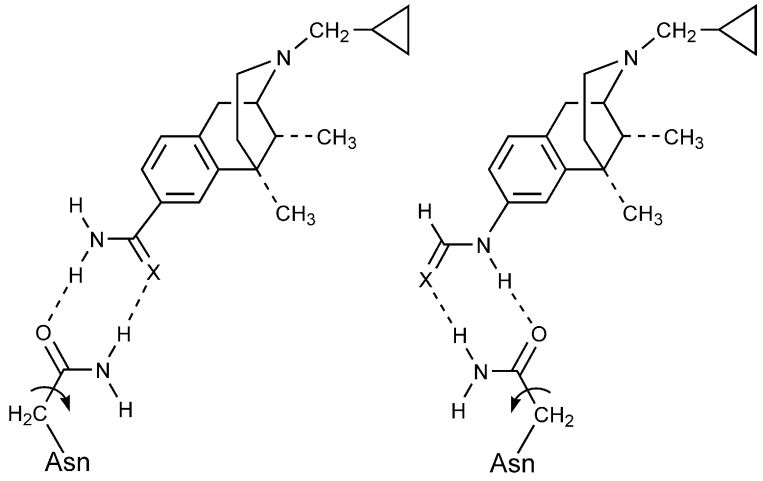


Figure 2. Possible binding mode of (thio)amide targets to receptor protein.

and Gln can readily flip to optimized H-bonding.[22] Figure 2 illustrates such a possibility for the binding in our (thio)amide series.

From these data, we conclude that, when appended to the 8-position of the cyclazocine core, thioamide and thioformamide groups are highly effective bioisosteres not only for each other, but their corresponding amide derivatives as well. Incorporating these findings with our previous results,[1,2] it is apparent that μ and κ opioid receptors have very similar molecular recognition to cyclazocine analogues having the following groups at the 8-position: OH, $CONH_2$, NHCHO, $CSNH_2$ or NHCHS. Incorporation of certain urea or thiourea groups at the 8-position reduces binding affinity somewhat, however, when highly basic amidine and guanidine groups are introduced, very poor activity is seen. Research in our laboratories will continue with the goal being to generate a unified pharmacophore model to better understand the role of these (thio)amide groups in recognition to opioid receptors.

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