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6N-CINNAMOYL-β-NALTREXAMINE AND ITS *p*-NITRO DERIVATIVE. HIGH EFFICACY κ-OPIOID AGONISTS WITH WEAK ANTAGONIST ACTIONS.

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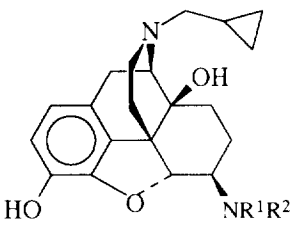
Abstract : 6N-cinnamoyl-β-naltrexamine and its *p*-nitro derivative (**7** and **8**) are κ-opioid agonists of high potency and exceptional efficacy and are only weakly effective μ opioid antagonists. This contrasts with the *p*-methyl analogue which has only low efficacy κ-agonism but is a selective irreversible μ-antagonist like β-FNA.

Introduction

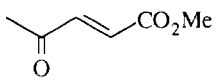
The study of opioid receptors and their subtypes to a substantial extent has depended on the availability of irreversible antagonists for those receptors. β-funaltrexamine (β-FNA; **2**), a fumaroyl derivative of β-naltrexamine (β-NTA; **1**), has proved of particular value since it is an irreversible antagonist specific for μ-receptors.[1] The common feature of the available ligands is that they posses a reactive electrophilic group appropriately located so as to be able to form a covalent bond to a thiol group near the active site of the receptor. The fumaroylamido group of β-FNA is a Michael acceptor to which a receptor group can add to form a covalently bound ligand-receptor complex.

In some *in-vivo* assays β-FNA shows relatively short lasting κ-agonist activity which must be allowed to decay before the selective μ-antagonist activity can be utilised.[1] This means that β-FNA is administered 24 h before testing in order to utilise its ability to antagonise an opioid agonist effect. Clocinnamox (C-CAM; **9a**) shows no agonist activity after systemic administration and as an irreversible antagonist is selective for μ-receptors.[2]

Since the cinnamoylamido group in C-CAM and its close analogues was not associated with any agonist activity it was of interest to determine whether the κ-agonist activity of β-FNA could be avoided by replacing the fumaroylamido group with a cinnamoylamido group. However, we showed that the *p*-methyl substituted cinnamoylamido derivative (**5**), though an irreversible μ-selective antagonist, like β-FNA had κ-agonist activity in the guinea-pig *ileum* preparation and showed low-efficacy κ-agonist activity in the mouse acetic acid-induced writhing test.[3] In further investigation of cinnamoyl derivatives of β-NTA, we here report that the unsubstituted cinnamoyl derivative (**7**) and its *p*-nitro analogue (**8**) are κ-agonists of very high efficacy with only modest antagonist activity.



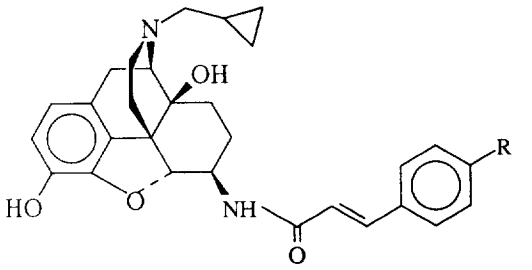
1 $R^1=R^2=H$ (β-NTA)

2 $R^1=$ 

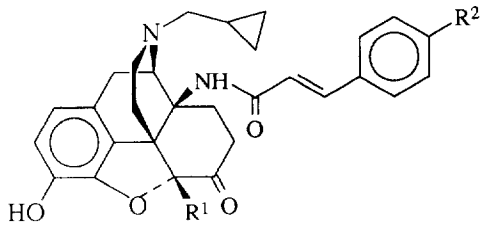
$R^2=H$ (β-FNA)

3 $R^1=CH_2C_6H_5$, $R^2=H$

4 $R^1=R^2=CH_2C_6H_5$



5 R=Me
6 R=Cl
7 R=H
8 $R=NO_2$



9 (a) $R^1=H$, $R^2=Cl$, (C-CAM)
(b) $R^1=Me$, $R^2=NO_2$

Chemistry

The literature method for the preparation of β-NTA,[4] involving catalytic hydrogenation of the 6N,6N-dibenzyl derivative (**4**), in our hands gave extremely poor yields with evidence of substantial amounts of the 6N-benzyl derivative (**3**) in the product. We found that by use of hydrogen-transfer catalysis,[5] β-NTA could be obtained smoothly from **4** in 83% yield when cyclohexene was used as the hydrogen source.[6] The cinnamoylamido derivatives were prepared from β-NTA by acylation with the appropriate acid chloride in the presence of triethylamine.

Biological Results

The new cinnamoyl derivatives (**7**, **8**) were tested in receptor binding assays in guinea-pig brain homogenates and in the electrically stimulated guinea-pig *ileal* longitudinal muscle (GPI) and mouse *vas-deferens* (MVD) preparations. They were also tested *in-vivo* in mouse tail withdrawal (TW) and acetic acid-induced writhing (AW) assays. These assays are described in reference 3.

In the receptor competition binding assay the radioligands (~1 nM) used to label opioid receptors were [^{3}H][D-Ala^2,(Me)Phe^4,Gly(ol)^5]-enkephalin (DAMGO) for μ-sites, [^{3}H][D-Pen^2,*p*-Cl-Phe^4,D-Pen^5]-enkephalin (Cl-DPDPE) for δ-sites, [^{3}H]-(5α,7α,8β)-(-)N-methyl-N-1-pyrrolidinyl-1-oxaspiro-[4,5]-dec-8-yl

benzeneacetamide (U-69593) for κ_1-sites and bremazocine in the presence of 100nM each of DAMGO, U-69593 and [D-Ser^2,Leu^5]-enkephalin-Thr^6 (DSLET) for κ_2-sites. The results are shown in Table 1.

Table 1. Opioid Receptor Binding of β-naltrexamine Derivatives in Guinea-Pig Brain Homogenate.

	K_i (nM)			
Compound	[^{3}H] DAMGO μ	[^{3}H] Cl-DPDPE δ	[^{3}H] U-69593 κ_1	[^{3}H] bremazocine[a] κ_2
7	0.07	3.1	0.2	12.8
8	0.2	5.1	0.8	30.5
5	0.23	9.6	0.6	171.4
6	0.1	5.0	0.2	48.3
β-FNA	0.4	22.4	0.9	66.5

[a] IC_{50} (nM), obtained in the presence of 100nM DAMGO, DSLET and U-69593.

7 and **8** showed high affinity for μ and κ_1-sites though in each case μ affinity was 5-fold higher than κ_1 affinity. Affinity for δ-sites was 130- and 70-fold lower than for μ for **7** and **8** respectively, and for κ_2-sites more than 20-fold lower than for κ_1-sites. These binding profiles were broadly similar to those of the methyl- and chloro- derivatives (**5**) and (**6**).

Table 2. Activity of **7** and **8** in Isolated Tissue Preparations.

	Guinea-Pig *Ileum*		Mouse *Vas Deferens*	
Compound	IC_{50} (nM)	K_e (nM) Nor-BNI	IC_{50} (nM)	K_e (nM) NTI[f]
7	0.4±0.1	0.5±0.04	5.6±1.5	5.1±0.2
8	13.4±1.3	[b]	[c,d]	
5[a]	12.6±4.0	0.2±0.05	17100±3000	N.D.
6[a]	2.2±0.6	0.3±0.04	[c,e]	

[a] Data from reference 3. [b] The inhibition could not be reversed by nor-BNI.
[c] Not an agonist. [d] μ, κ antagonist. [e] μ, δ antagonist. [f] Naltrindole. N.D.=Not determined.

In GPI, **7** and **8** were both full agonists though the potency of **7** was substantially reduced when the *p*-nitro group was introduced (Table 2). The previously reported methyl- and chloro- derivatives (**5**) and (**6**)

were partial agonists with peak responses about 60-70% of maximum. The agonist effect of **7** in GPI was κ-receptor mediated as shown by the K_e values for its antagonism by the selective κ-antagonist nor-binaltorphimine (nor-BNI). The agonist effect of **8** was not reversed by either D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-NH_2 (CTAP); (μ-antagonist) or nor-BNI. The agonist effects of all the other cinnamoyl derivative tested (**5**, **6** and **7**) were reversed by nor-BNI, though not by CTAP.

In MVD **7** had significant agonist activity that was wash resistant. With a 60 min wash the tissue did not show full recovery. This agonist response had a significant δ-receptor component as shown by the K_e value for its antagonism by naltrindole (Table 2). In contrast **8** like the methyl- and chloro- analogues (**5**) and (**6**)[3] showed very little agonist effect in MVD so that its antagonism of selective agonists for the individual types of opioid receptor could be determined. The K_e values for the antagonism of DAMGO (μ) and U-69593 (κ) by **8** showed that it is a very potent antagonist with about 5-fold selectivity for κ over μ.

7 and **8** were very high efficacy agonists in the TW assay in mice and were about 10 times more potent than morphine (see Figure 1 for sample data on **8**). They produced nearly maximum possible effect when the water temperature was 50 °C which was maintained when the water temperature was raised to 55 °C. They were also potent agonists in the AW assay, producing total inhibition of writhing at 1 mg/kg. This contrasted with the methyl- and chloro- analogues (**5**) and (**6**) which showed very modest effects in TW though they totally inhibited writhing at 32 mg/kg by a κ agonist mechanism.[3] The selectivity of the agonist action of **8** in TW assay was determined using selective antagonists β-FNA (μ), nor-BNI (κ) and NTI (δ). Only nor-BNI produced a significant rightward shift in the dose-response curve of **8**, (Figure 1) showing that the TW response of **8** was κ-receptor mediated. The agonist effects of **7** and **8** in the AW assay were also investigated using selective antagonists (Figure 2). This confirmed that the agonist effects are largely due to κ-opioid receptor activation.

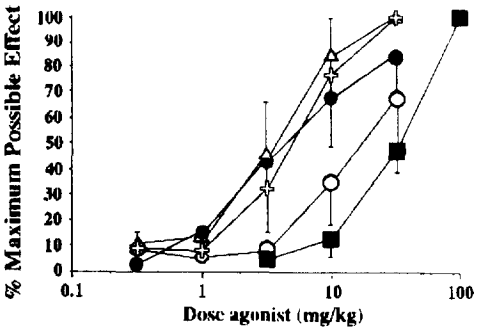


Figure 1 - Antagonism of the effects of **8** (closed circles) by β-FNA, 32 mg/kg, 3 h pretreatment (triangles); nor-BNI, 32 mg/kg, 24 h pretreatment (open circles) and NTI 10 mg/kg, 15 min (crosses) in the TW assay (water at 55 °C).
Effect of morphine (closed squares).
All doses administered *intraperitonealy* (ip).

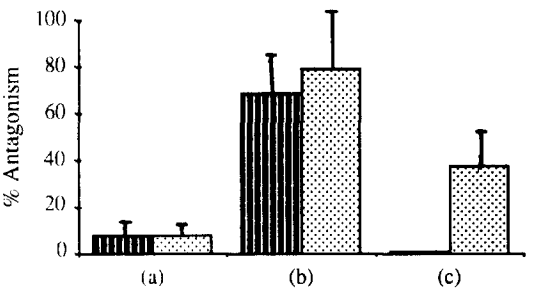


Figure 2 - Antagonism of the effects of **7** (vertical stripes) and **8** (dots) at 1mg/kg (ip) by (a) β-FNA (32 mg/kg (sc), 24 h pretreatment), (b) nor-BNI (32 mg/kg (sc), 24 hr pretreatment) and (c) NTI (10mg/kg (sc), 15 min pretreatment) in the AW assay. (sc = *subcutaneously*).

The antagonist actions of **7** and **8** could only be determined when the agonist actions had waned. Their long duration antagonist actions were assessed by administering them 24 h before challenge with the appropriate agonist. In the TW assay, **7** displaced the morphine dose-response curve slightly to the right but **8** showed no antagonism of morphine (data not shown). Since κ- and δ- agonists give unreliable responses in TW, the AW assay was used to determine whether **8** was a κ- or δ- antagonist. Figure 3 shows that it produced modest antagonist activity with 24 h pretreatment and that this activity was predominantly κ- with some δ- but no μ- involvement. These data were consistent with **8**'s lack of morphine antagonist activity in the TW assay. A similar study with **7** revealed similar modest antagonist activity; this was κ- and δ- mediated, again with very little μ- involvement (Figure 3).

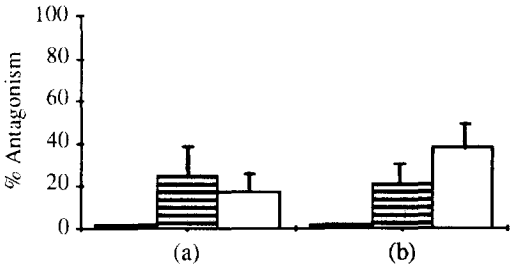


Figure 3 - Antagonism of the effects of morphine, 3.2 mg/kg (solid bar); BW373U86, 10 mg/kg (horizontal stripes) and bremazocine, 0.1 mg/kg (clear bar) by (a) **7** and (b) **8**, both administered ip at 32 mg/kg, 24 h pretreatment, in the AW assay.

Discussion

This work confirms our previous report that replacement of the fumaroylamido group of β-FNA with cinnamoylamido groups does not eliminate κ-agonist activity.[3] In fact the unsubstituted cinnamoyl analogue (**7**) and its *p*-nitro derivative (**8**) had considerably greater κ-agonist potency and efficacy *in-vivo* than either β-FNA or the methyl- and chloro- substituted cinnamoylamido analogues (**5**) and (**6**). The full agonist response shown by **7** and **8** in the TW assay with water at 55 °C is exceptional in our experience for a κ-opioid receptor mediated response. This suggests that they may have higher efficacy than previously reported κ-agonists. The agonist effect of the unsubstituted cinnamoyl derivative (**7**) in the GPI preparation was antagonised by nor-BNI (indicating κ agonism) as was the effect of the methyl- and chloro- derivatives (**5**) and (**6**). These effects returned after washing the tissue repeatedly. In this respect they differed from β-FNA which had a κ agonist effect in GPI which could be removed by washing the tissue. The nitro derivative (**8**) differed from the other cinnamoyl derivatives in that its GPI response was not antagonised by nor-BNI. This suggests that the κ-agonist effect of **8** in GPI may be irreversible.

Thus the profiles of the cinnamoyl derivatives (**5-8**) differ with the substituent in the cinnamoyl aromatic ring. They are all κ agonists but whereas the methyl- and chloro- derivatives have low potency and efficacy,[3] the unsubstituted and nitro- derivatives (**7**) and (**8**) are potent and highly efficacious. Only the methyl-substituted (**5**) was an irreversible μ antagonist comparable to β-FNA.[3] These differences are difficult to explain in terms of standard substituent effects. Molecular modelling of the cinnamoyl derivatives shows very little difference between them. They show no evidence of conjugation involving the double bond with the amide group,[7] which precludes covalent bond formation to a receptor thiol group by a Michael addition mechanism. A similar conclusion was reached for the 5-methyl-*p*-nitrocinnamoyl analogue (**9b**) of C-CAM which failed to demonstrate Michael addition *in vitro* with acetylcysteine.[8] The most likely explanation for the irreversible actions of the cinnamoyl derivatives is very powerful lipophilic receptor interactions involving

the aryl group. In this case the methyl- substituted derivative (**5**) would be expected to be superior to the nitro- (**8**) and unsubstituted (**7**) derivatives. However the chloro- derivative (**6**) should be comparable to **5** whereas it had only modest antagonist activity.[3]

Conclusion

The pharmacological profiles of cinnamoyl derivatives of β-NTA are sensitive to substitution in the aromatic ring. The profiles do not correlate with their expected reactivity as Michael acceptors and covalent bond formation to the receptor protein through a Michael addition reaction does not appear to operate in this series. It seems likely that the observed *pseudo*-irreversible actions in tissue preparations and *in vivo* are due to powerful lipophilic interactions involving the cinnamoyl aromatic group.

Acknowledgement

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5. Johnstone, R. A. W.; Wilby, A. H. and Entwiste, I. D. *Chem. Rev.* **1985**, *85*, 129.
6. A solution of (**4**) (14.0 g, 26.99 mmol) and cyclohexene (700 mL) in methanol (700 mL) was added rapidly to 10% palladium on carbon catalyst (14.0 g). The resulting suspension was heated to reflux for 6 h, after which the catalyst was removed by filtration through a celite bed. The residue was washed with methanol (60 mL) and the filtrate was evaporated under reduced pressure to give (**1**) as a brown solid (7.7 g, 83%) which could be used without further purification, mp 292 °C; EIMS, m/z 55 (base peak), 342 (M^+, 75%); IR 3684, 3366 and 3302 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.66, 6.52 (2H, 2d, J = 8 Hz, H-1 and H-2), 6.05 (4H, brs, 2 O*H* and N*H* 2), 4.50 (1H, d, J = 7 Hz, H-5), 3.10 (1H, d, J = 5Hz, H-9), 2.99 (1H, d, J = 18 Hz, H-10β), 2.62 to 1.40 (12H, m, H-6, 7α, 7β, 8α, 8β, 10α, 15α, 15β, 16α, 16β and cyclopropylmethyl C*H* 2), 0.83 (1H, m, cyclopropyl C*H*), 0.53 (2H, m, 2 cyclopropyl C*H* cis),), 0.13 (2H, m, 2 cyclopropyl C*H* trans).
7. For the modelling methods used and their application to similar systems see Piggot, N. G.; Derrick, I.; Moynihan, H. A.; Husbands, S. M.; Lewis, J. W and Woods, J. H. *Analgesia*, 1995, 1, 647.
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