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SYNTHESIS AND BIOLOGICAL EVALUATION OF 14-ALKOXYMORPHINANS. 14.[1] 14-ETHOXY-5-METHYL SUBSTITUTED INDOLOMORPHINANS WITH δ OPIOID RECEPTOR SELECTIVITY

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Abstract: The 5-methyl and 14-ethoxy substituted analogues (compounds **2** - **4**) of the δ opioid receptor antagonist naltrindole showed similar selectivity when compared with the reference drug. Compound **2** was a δ receptor antagonist in the mouse vas deferens preparation (MVD) exhibiting considerably higher selectivity ratios than naltrindole, while compound **4** was found to be a full and potent δ receptor agonist in the MVD.

Naltrindole (NTI; **1**) is a non-peptidic δ opioid receptor antagonist which is widely employed.[2-4] Interestingly, this compound exhibits potent immunosuppressive effects.[5-7] The conformationally constrained indolic benzene moiety is suggested as a key "address" component affording selectivity by increasing δ-affinity and reducing affinity for μ and κ opioid receptor sites.[3] In an attempt to improve on the selectivity of naltrindole, to develop potent δ agonists and to uncover structure-activity relationships in this series of compounds we decided to prepare indolomorphinans with a 14-alkoxy substituent and a 5-methyl substituent, from the corresponding morphinan-6-ones by Fischer indole synthesis. 14-Alkoxy substitutents on morphinans are reported to improve receptor affinity providing potent agonists or antagonists depending on the substituent at the nitrogen.[8] Many of these compounds interact preferentially with μ opioid receptors (e.g. the μ-selective opioid receptor antagonist cyprodime[9] and derivatives[10,11]).

Indolomorphinans **2** and **3** were prepared from the μ opioid antagonists 14-O-ethyl-5-methylnaltrexone (**5**) and 14-O-ethyl-5-methylnaloxone (**6**)[12], respectively, while the potential δ agonist **4** was prepared from the

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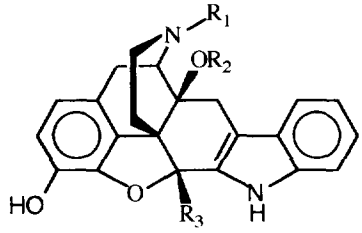


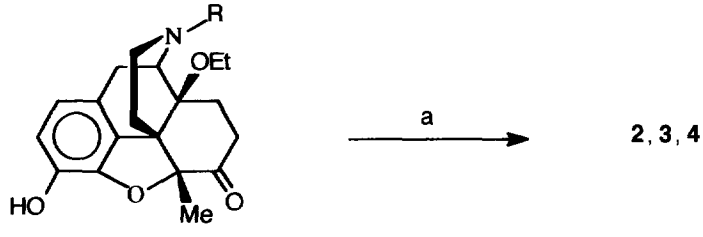
1 R_1 = cyclopropylmethyl, $R_2 = R_3 = H$

2 R_1 = cyclopropylmethyl, R_2 = Et, R_3 = Me

3 R_1 = allyl, R_2 = Et, R_3 = Me

4 $R_1 = R_3$ = Me, R_2 = Et

highly potent μ agonist 14-ethoxymetopon (**7**).[13,14] The indolomorphinans[15] **2**[16], **3**[17] and **4**[18] were obtained from the corresponding morphinan-6-ones (**5**, **6** and **7**) by reaction with phenylhydrazine hydrochloride (Scheme).



5 R = cyclopropylmethyl

6 R = allyl

7 R = Me

Scheme: a) 1.5 equ. phenylhydrazine hydrochloride, AcOH, 7 h reflux.

The biological properties of synthesized compounds were performed using radioligand binding assays (rat brain homogenates) and bioassays (guinea-pig ileum myenteric plexus preparation (GPI) and mouse vas deferens preparation (MVD)). The binding affinities of **2**·HCl, **3**·HCl and **4** were assessed in homogenates of rat brain in Tris·HCl buffer (50 mM, pH 7.4)[14] employing [^{3}H]DIDI[19,20] (δ agonist), [^{3}H]naltrindole (NTI; δ antagonist), [^{3}H]DAMGO (μ agonist) and [^{3}H]U69593 (κ agonist) as radioligands (Table 1). The ligand binding results confirm the selectivity of naltrindole for δ opioid receptors and show that the inclusion of 5-methyl and 14-ethoxy groups do not greatly alter δ-selectivity, though a slightly different selectivity is seen for each compound.

Compounds **2**·HCl and **4** were tested in the bioassay preparations which were performed as described previously.[21-22] EC_{50} values were determined from concentration-effect curves. Compounds were tested for antagonism by the ability to shift the dose-effect curve for standard opioid agonists to the right. Where shifts were seen apparent equilibrium dissociation constants for the antagonists (K_e values) were determined by the single-dose method,[22-24] using dose-ratios determined at the EC_{50} points. K_e values were calculated to allow for direct comparison with K_i values determined from ligand-binding assays. Compound **2**.HCl was a potent δ opioid

Table 1: Opioid Receptor Binding of Compounds **2**, **3** and **4**.

	K_i (nM) ± SEM				selectivity ratio[a]	
Cpd.	[^{3}H]DIDI (δ)	[^{3}H]NTI (δ)	[^{3}H]DAMGO (μ)	[^{3}H]U69593 (κ)	μ/δ	κ/δ
2	14.00 ± 9.51	0.78 ± 0.16	38.70 ± 8.70	59.20 ± 10.00	50	76
3	29.90 ± 2.34	10.80 ± 1.55	667.00 ± 203.00	765.00 ± 465.00	62	71
4	8.81 ± 2.51	5.75 ± 1.29	715.00 ± 107.00	286.00 ± 101.00	124	50
NTI (**1**)	0.09 ± 0.03	0.33 ± 0.19	30.40 ± 0.69	14.00 ± 3.00[b]	92	42

a) The K_i values against [^{3}H]NTI were used for the calculation of the selectivity ratios.
b) [^{3}H]CI977 was used as κ ligand.

receptor antagonist in the MVD. This compound was about 10-fold weaker than naltrindole, but exhibited considerably higher selectivity ratios (μ/δ and κ/δ) than naltrindole (Table 2). DPDPE was used as the standard δ agonist, but since this is a putative δ_1 receptor preferring agonist the putative δ_2 preferring agonist deltorphin II was also used. However a similar antagonist equilibrium constant (K_e) for **2** (1.6 ± 0.2 nM) was obtained. These K_e values of **2** at the δ receptor are in line with the affinity of **2** determined in binding assays against the antagonist [^{3}H]naltrindole rather than determined against the agonist [^{3}H]DIDI. Compound **2**.HCl showed only very weak agonism in either the GPI or the MVD affording just 32% and 23% inhibition of the electrically evoked twitch respectively at 10 μM. In contrast, compound **4** was a full agonist in the MVD (EC_{50} 104 ± 33 nM, n = 3), but in the GPI, which contains μ and κ receptors, but not δ receptors, **4** was a very weak agonist affording 21.0 ± 12% (n = 3) inhibition of twitch height at 10 μM. This indicates that the agonist action of this compound in the MVD is likely to be mediated purely through an action at δ receptors.

Table 2: Antagonist K_e Values of Compound **2**·HCl and Naltrindole Determined in the Mouse Vas Deferens Preparation (MVD)

	K_e[a] (nM) ± SEM			selectivity ratio	
Compound	DPDPE (δ)	DAMGO (μ)	CI977 (κ)	μ/δ	κ/δ
2·HCl	1.3 ± 0.3	133 ± 42	529 ± 92	102	455
NTI (**1**)	0.18 ± 0.02	5.25 ± 0.68	32.4 ± 1.1	29	178

a) K_e = [antagonist]/DR -1, where DR is dose ratio (i. e. ratio of equiactive concentrations of the test agonist in the presence and absence of the antagonist).

The results show that introduction of a 5-methyl and a 14-ethoxy group onto the selective δ antagonist naltrindole does not greatly alter the ligand binding profile of the compound for δ, μ and κ receptors, except where [^{3}H]DIDI, rather than [^{3}H]naltrindole, is used as the labelled ligand. The reason for the markedly higher affinity of **2**.HCl when measured against the antagonist [^{3}H]naltrindole is unknown. However, since [^{3}H]DIDI being an agonist would be expected to label agonist affinity states of the receptor then the results suggest that compound **2** has a preference for δ-antagonist binding. This would confirm the antagonist nature of the compound, though it is usual for antagonists to have similar affinity when determined against both agonist and antagonists.[25,26] Alternatively the difference may be caused by some additional selectivity by virtue of the additional groups on **2**, although the N-cyclopropylmethyl group seems essential to see this difference. Indeed, replacement of the N-cyclopropylmethyl group with N-allyl (compound **3**) or with N-Me (compound **4**) does lead to a considerable reduction (approximately 20-fold) in affinity at all three receptor sites, indicating an important role for the cyclopropylmethyl group in binding. [^{3}H]DIDI is a deltorphin analogue reported to have preference for the δ_2 site[20] and thus the results may indicate that **2** does have some preference for δ_1 over δ_2 sites. On the other hand the K_e value obtained for compound **2** in bioassay in the MVD was similar using both DPDPE (δ_1 preferring) and deltorphin II (δ_2 preferring) as agonists. The lack of differentiation in this tissue would be expected, however, since previous studies do suggest the MVD contains a single δ opioid receptor type.[24,27,28]

In marked contrast to the cyclopropylmethyl (**1** and **2**) and N-allyl (**3**) analogues the N-Me analogue (**4**) is a potent δ agonist showing full agonism in the MVD preparation. Previously synthesized compounds of the naltrindole type (e. g. oxymorphindole) only show partial agonism in the MVD[3] and although the novel structure BW373U86 is a full agonist in the MVD it also acts as an agonist in the GPI, although 700-times higher concentrations are needed.[29]

In conclusion, replacement of the 5-H and 14-OH functions in naltrindole with Me and ethoxy groups, respectively, improves the δ-selectivity of the antagonist in bioassay preparations. Furthermore, replacement of the N-cyclopropylmethyl group with N-Me affords a change in efficacy resulting in a compound with good potent δ agonist properties in the MVD, but without appreciable μ agonist properties.

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15. All new compounds gave satifactory elemental analyses.
16. **2**·HCl: mp > 260 °C (dec.); IR (KBr): 3200 ($^+$NH, NH, OH) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 11.34, 9.21 and 8.55 (3 s, $^+$NH, NH, OH), 7.32 (m, 2 arom. H), 7.08 (dd, J = 8.1, 8.1 Hz, 1 arom. H), 6.94 (dd, J = 8.1, 8.1 Hz, 1 arom. H), 6.62 (d, J = 8.2 Hz, 1 arom. H), 6.55 (d, J = 8.2 Hz, 1 arom. H), 1.86 (s (CH_3-C(5)), 1.01 (t, J = 6.8 Hz, 3 H, CH_3CH_2O); CI-MS: m/z 457 (M^++1).
17. **3**·HCl: mp 168-170 °C; IR (KBr): 3200 ($^+$NH, OH) cm^{-1}; ^1H-NMR (300 MHz, CD_3OD): δ 7.39 (dd, J = 7.8, 7.8 Hz, 2 arom. H), 7.14 (dd, J = 7.8, 7.8 Hz, 1 arom. H), 7.01 (dd, J = 7.8, 7.8 Hz, 1 arom. H), 6.67 (s, 2 arom. H), 6.02 (m, 1 olef. H), 5.75 (m, 2 olef. H), 1.99 (s, CH_3-C(5)), 1.09 (t, J = 6.8 Hz, 3 H, CH_3CH_2O); CI-MS: m/z 443 (M^++1).
18. **4**: mp 165-167 °C; IR (KBr): 3285 (NH, OH) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.15 (br s, NH, OH), 7.35 (d, J = 8 Hz, 1 arom. H), 7.26 (d, J = 8 Hz, 1 arom. H), 7.13 (dd, J = 8, 8 Hz, 1 arom. H), 7.01 (dd, J = 8, 8 Hz, 1 arom. H), 6.64 (d, J = 8.2 Hz, 1 arom. H), 6.55 (d, J = 8.2 Hz, 1 arom. H), 2.40 (s, CH_3N), 1.94 (s, CH_3-C(5)), 1.02 (t, J = 7 Hz, 3 H, CH_3CH_2O); CI-MS: m/z 417 (M^++1).
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