SYNTHESIS AND BIOLOGICAL EVALUATION OF 14-ALKOXYMORPHINANS. 14.1 14-ETHOXY-5-METHYL SUBSTITUTED INDOLOMORPHINANS WITH δ OPIOID RECEPTOR SELECTIVITY

Helmut Schmidhammer, *# Dietmar Daurer and Martina Wieser
Institute of Pharmaceutical Chemistry, University of Innsbruck, Innrain 52a, A-6020 Innsbruck, Austria

Krisztina Monory and Anna Borsodi
Institute of Biochemistry, Biological Research Center Szeged, P. O. B. 521, H-6701 Szeged, Hungary

Jackie Elliott and John R. Traynor*
Department of Chemistry, Loughborough University, Loughborough, Leicestershire, LE11 3TU, U. K.

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 substituents 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 cyprodime9 and derivatives10,11).

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

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)\textsuperscript{14} employing \[^{3}H\]DID\textsuperscript{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.\textsuperscript{21-22} EC\textsubscript{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\textsubscript{e} values) were determined by the single-dose method,\textsuperscript{22-24} using dose-ratios determined at the EC\textsubscript{50} points. K\textsubscript{e} values were calculated to allow for direct comparison with K\textsubscript{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.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>14.00 ± 9.51</td>
<td>0.78 ± 0.16</td>
<td>38.70 ± 8.70</td>
<td>59.20 ± 10.00</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>29.90 ± 2.34</td>
<td>10.80 ± 1.55</td>
<td>667.00 ± 203.00</td>
<td>765.00 ± 465.00</td>
<td>62</td>
</tr>
<tr>
<td>4</td>
<td>8.81 ± 2.51</td>
<td>5.75 ± 1.29</td>
<td>715.00 ± 107.00</td>
<td>286.00 ± 101.00</td>
<td>124</td>
</tr>
<tr>
<td>NTI (1)</td>
<td>0.09 ± 0.03</td>
<td>0.33 ± 0.19</td>
<td>30.40 ± 0.69</td>
<td>14.00 ± 3.00$^{b)}$</td>
<td>92</td>
</tr>
</tbody>
</table>

$^{a)}$ The $K_i$ values against $[^3]$HINTI were used for the calculation of the selectivity ratios.

$^{b)}$ $[^3]$H]Cl977 was used as $\kappa$ ligand.

receptor antagonist in the MVD. This compound was about 10-fold weaker than naltrindole, but exhibited considerably higher selectivity ratios ($\mu/\delta$ and $\kappa/\delta$) than naltrindole (Table 2). DPDPE was used as the standard $\delta$ agonist, but since this is a putative $\delta_1$ receptor preferring agonist the putative $\delta_2$ 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 $\delta$ 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 $\mu$M. In contrast, compound 4 was a full agonist in the MVD ($EC_{50}$ 104 ± 33 nM, $n$ = 3), but in the GPI, which contains $\mu$ and $\kappa$ receptors, but not $\delta$ receptors. 4 was a very weak agonist affording 21.0 ± 12% ($n$ = 3) inhibition of twitch height at 10 $\mu$M. This indicates that the agonist action of this compound in the MVD is likely to be mediated purely through an action at $\delta$ receptors.

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_e^{a)}$ (nM) ± SEM</th>
<th>selectivity ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DPDPE ($\delta$)</td>
<td>DAMGO ($\mu$)</td>
</tr>
<tr>
<td>2·HCl</td>
<td>1.3 ± 0.3</td>
<td>133 ± 42</td>
</tr>
<tr>
<td>NTI (1)</td>
<td>0.18 ± 0.02</td>
<td>5.25 ± 0.68</td>
</tr>
</tbody>
</table>

$^{a)}$ $K_e = [\text{antagonist}] / [\text{DR}] - 1$, 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.

Acknowledgement

We wish to thank Prof. Dr. K.-H. Ongania (Institute of Organic Chemistry, University of Innsbruck) for performing the mass spectra and Mag. A. Willeit (Institute of Pharmaceutical Chemistry) for recording the \(^1\)H NMR spectra. The work was in part supported by the Austrian Science Foundation (project P8879-CHE) and the Wellcome Trust (JE and JRT).

References and Notes

15. All new compounds gave satisfactory elemental analyses.
16. 2·HCl: mp > 260 °C (dec.); IR (KBr): 3200 (+NH, NH, OH) cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): 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 (CH₃-C(5))), 1.01 (t, J = 6.8 Hz, 3 H, CH₃CH₂O); CI-MS: m/z 457 (M⁺+1).
17. 3·HCl: mp 168-170 °C; IR (KBr): 3285 (NH, OH) cm⁻¹; ¹H NMR (300 MHz, CD₃OD): 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, CH₃N), 1.94 (s, CH₃-C(5)), 1.02 (t, J = 6.8 Hz, 3 H, CH₃CH₂O); CI-MS: m/z 417 (M⁺+1).
18. 4: mp 165-167 °C; IR (KBr): 3285 (NH, OH) cm⁻¹; ¹H NMR (300 MHz, CDCl₃): 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, CH₃N), 1.94 (s, CH₃-C(5)), 1.02 (t, J = 7 Hz, 3 H, CH₃CH₂O); CI-MS: m/z 417 (M⁺+1).

(Received in Belgium 16 September 1996; accepted 5 December 1996)