3-CARBOMETHOXY FENTANYL: SYNTHESIS, PHARMACOLOGY AND CONFORMATIONAL
ANALYSIS


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Abstract: The synthesis of a novel analogue of fentanyl, 3-carbomethoxy fentanyl or "iso-carfentanyl" has been accomplished in five steps, by simple and efficient route, starting from phenethyl amine and methyl acrylate. Both (α) cis and (α) trans isomers were obtained in pure form and tested pharmacologically for the central analgesic activity. Preliminary results (rat-withdrawal test) revealed significant but substantially reduced potency of both isomers, the trans in particular, compared to carfentanyl. The computational (molecular mechanics) search of the conformational space low energy regions of 5a ((α) cis) and 5b ((α) trans) isomers revealed the difference in their conformational mobility. Besides being more conformationally flexible trans isomer has unfavorable orientation of the 4-N-phenylpropanamide group compared to the other active analogs of fentanyl. This is believed to be the reason of its reduced potency relative to fentanyl.

Introduction

Opioid (narcotic) analgesics present a very important class of drugs, widely used in surgical procedures, in treatment of general postoperative pain, in cancer pain and other conditions. Among various classes of these compounds, 4-anilidopiperidines present the most potent group known to date, including fentanyl (50-100 X morphine), carfentanyl (~7000-8000 X morphine), lofentanil (5000-6000 X morphine), sufentanil (7000-8000 X morphine) and others. A large number of fentanyl analogues have been synthesized so far, both to establish the structure-activity relationship (SAR) and to find novel, clinically useful drugs.
Results and discussion

In this communication, we wish to report: 1) a simple and efficient synthesis of a novel fentanyl analogues, 5a, (±)cis and 5b, (±)trans 3-carbomethoxy fentanyl, or iso-carfentanil, 2) preliminary pharmacological examination of these compounds and 3) conformational studies relevant for the analgesic activity.

Chemistry

The synthesis of 3-carbomethoxy fentanyl ("iso-carfentanil"), was effected in five steps, starting from phenethyl amine and methyl acrylate, as depicted in the Scheme. In the first step, phenethyl amine was reacted with excess methyl acrylate (bis 1:4 addition) to afford amino-diester 1 in quantitative yield. In the next step, Dieckmann condensation of the intermediate 1 (2 eq. NaH, boiling toluene, 5h, then excess of 25% aqueous NaH2PO4 yielded keto-ester 2 in ca. 80% yield. The product was further purified by precipitation as monooxalate salt, then the free base liberated with K2CO3. In the third step, the keto-ester 2 was condensed with aniline in acetic acid (50%, 5h) to yield stable enamine 3 in 70% yield, after recrystallization from i-PrOH. Various unsuccessful attempts to selectively reduce the double bond in the enamine 3, included Zn/AcOH, Mg/MeOH, Mgbuffered MeOH, NaBH4/EtOH and catalytic hydrogenation, while Na/I-PrOH, LiIq, NH2/I-ButOH or LiBH4/Et2O yielded complex mixtures. The reduction was quantitatively carried out with NaBH2CN (MeOH, pH=5, solid NaH2PO4.H2O, rt, 12h) yielding a cis/trans mixture of diastereoisomers in a ratio 1:1. The mixture was separated by chromatography on neutral Al2O3 column, yielding pure 4a, (±) cis and 4b, (±) trans amino-esters respectively. In the final step, both isomers of 3-carbomethoxy fentanyl 5a, (±) cis, and 5b, (±) trans were prepared by acylation of 4a and 4b with propionyl chloride (Et3N, CH2Cl2, rt, 5h) and the products precipitated as monooxalate salts (anh. oxalic acid, MeOH/Et2O, 2/8). The stereochemical assignments for 5a and 5b were made by using 1H NMR spectroscopy. The signals for the methylene hydrogen at position 4 in the piperidine ring of 5a and 5b (as free base) were compared to the corresponding signals in cis 3-methyl fentanyl and trans 3-methyl fentanyl where the absolute stereochemistry was determined by X-ray analysis. 11 Thus, cis 3-methyl fentanyl gave doublet of triplets at 4.40 δ (J= 5 Hz, J= 12.5 Hz) and 5a also gave doublet of triplets (4.50 δ, J= 4.5 Hz, J= 11.6 Hz). On contrary, both trans 3-methyl fentanyl and 5b gave triplet of doublets (4.53 δ, J= 4.5 Hz, J= 12.5 Hz, for the former and 4.98 δ, J= 4.4 Hz, J= 12.4 Hz for the latter).

Pharmacology

Antinociception was determined by the tail-withdrawal test in rats. 12 Percent antinociception was calculated according to the following formula: 100 x (test latency - control latency)/(cut-off - control latency)13 where the control latency was 1.6-2.5s and the cut-off was 6.0s. The ED50 and 95% confidence limits were estimated from log dose-response curve by using a standard computer program of Tallandia. 14

Based on the determined ED50 values for analgesia (I.P. injection), the relative order of potency was found to be: fentanyl (1.00)> 5a (0.52)> 5b (0.12). Apart from analgesia, both compounds tested, 5a and 5b, showed a typical "morphine-like" effects such as Straub tail, catalepsy and respiratory depression. 15 All of the observed effects of 5a and 5b were reversed by opioid antagonist naloxone hydrochloride (1mg/Kg S.C.).

In the view of previous findings 5a (Table1), it is evident that (5a), and (5b), are far less active than (±)cis and (±) trans 3-(methyl) fentanyl, respectively. Therefore, the replacement of methyl by carbomethoxy group in the position 3, caused a considerable decrease in the analgesic potency. Otherwise, it seems that
$5a$ is equipotent to the (+) *cis* 3-(propil) fentanyl and exceeds the potency of (+) *cis* 3-(allyl) fentanyl, while $5b$ is about twice less active than (+) *trans* 3-(propil) fentanyl. The influence of the stereochemistry upon the activity is well documented on the examples of 3-(methyl)$^{16}$ and 3-(propil) fentanyl.$^{17}$ The difference in the activity between $5a$ and $5b$, observed in this study, parallels such examples, since the *cis* isomer $5a$ is about 4 times more active than the *trans*, $5b$. (Table 1). Furthermore, both isomers of 3-(carbomethoxy) fentanyl possesses considerable less analgesic activity in comparison to its regioisomer, carfentanil (Table1).$^{18}$

From the pharmacological standpoint it can be concluded that a relatively small polar group, such as carbomethoxy, at the position 3 of piperidine ring, reduces the analgesic potency compared to fentanyl. The stereochemistry (*cis* or *trans*) is an important factor for the retaining of analgesic activity.
Table 1. Summary of ED₅₀ for analgesia, potency ratio of fentanyl analogues and proposed receptor-recognized conformation of the fentanyl class compounds.

<table>
<thead>
<tr>
<th>No</th>
<th>COMPOUND (FENTANYL ANALOGUE)</th>
<th>ED₅₀ (mg/Kg)</th>
<th>POTENCY RATIO</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fentanyl</td>
<td>0.011²⁺, 17.₁⁶ (0.0095 - 0.0140)</td>
<td>1</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>2</td>
<td>(S)₄₉ 3-Carbomethoxy-fentanyl, 5₉</td>
<td>0.023 (0.009-0.06)</td>
<td>0.52</td>
<td>CO₂Me²⁻</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>3</td>
<td>(S)₄₉ 3-Carbomethoxy-fentanyl, 5₉</td>
<td>0.1 (0.05-0.19)</td>
<td>0.12</td>
<td>H</td>
<td>CO₂Me²⁻</td>
<td>H</td>
</tr>
<tr>
<td>4</td>
<td>(S)₄₉ 3-Allyl-fentanyl</td>
<td>0.08 ⁴⁺, c</td>
<td>0.14</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>(S)₄₉ 3-Propyl-fentanyl</td>
<td>0.02 ⁴⁺, c</td>
<td>0.55</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>(S)₄₉ 3-Propyl-fentanyl</td>
<td>0.04 ⁴⁺, c</td>
<td>0.28</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>(S)₄₉ 3-Methyl-fentanyl</td>
<td>0.0018 ⁴⁺</td>
<td>6.1</td>
<td>Me⁷</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>8</td>
<td>(S)₄₉ 3-Methyl-fentanyl</td>
<td>0.0094 ⁴⁺</td>
<td>1.2</td>
<td>H⁴⁻</td>
<td>Me</td>
<td>H</td>
</tr>
<tr>
<td>9</td>
<td>4-Carbomethoxy-fentanyl (carfentanil)</td>
<td>0.00041 ⁴⁺ (0.00029 - 0.00058)</td>
<td>26.8</td>
<td>H</td>
<td>H</td>
<td>CO₂Me</td>
</tr>
<tr>
<td>10</td>
<td>Morphine</td>
<td>3.15 ²⁻ (2.82 - 3.52)</td>
<td>0.0035</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\[ \phi₁ = 5-4-7-11; \ 0 \ to \ -30^\circ \quad \phi₂ = 1-13-14-15; \ ~ 180^\circ \]

² All ED₅₀'s are expressed as free base weight. ° The ED₅₀ of fentanyl as determined in this study. c Confidence limits are not reported. d (3R, 4S) enantiomer shown; e (3S, 4S) enantiomer shown; f (3R, 4S) enantiomer shown; g (3S, 4S) enantiomer shown;
Conformational analysis

The theoretical studies of the active analogs of fentanyl\textsuperscript{19,22} led to the proposal of some elements of pharmacophore necessary for the optimum interaction with a receptor, Table 1. The active analog approach has been used in all the studies\textsuperscript{19-22} since the structure of a fentanyl receptor is unknown. The postulated\textsuperscript{2} elements of the pharmacophore are: piperidine ring in the chair conformation, N-phenethyl and 4-N-phenylpropanamide substituents trans and both equatorial, trans configuration (C\textsubscript{4} versus E1 group) of the amide group, perpendicular orientation of the aromatic ring (N-Ph) with respect to the amide function. The extended conformation of a N-phenethyl substituent (\( \Phi \approx 180^\circ \)) Table 1, has been proposed,\textsuperscript{19} and it has been calculated\textsuperscript{22} to be the sole low energy conformation common to the seven active analogs of fentanyl bearing different substituents in a N-phenethyl side chain. The postulated receptor recognized conformation of a 4- N-phenylpropanamide side chain, with \( \Phi \) fluctuating between 0° and -30°, Table 1, leans on the low energy conformations\textsuperscript{20} of the most active stereoisomers of cis 3-methyl fentanyl and oxtremifentany. Also the role of the four structure elements, necessary for the optimum receptor recognition, has been postulated.\textsuperscript{19} These are: protonated amine nitrogen capable of electrostatic attraction with negatively charged site on the receptor, polar function (C=O) capable of hydrogen bonding with a receptor, one aromatic ring involved in lipophilic interactions with a receptor, another aromatic ring involved, most likely, in electron transfer interactions with a receptor.

In our earlier studies\textsuperscript{21} we found that activation of the receptor by the fentanyl class of ligands is highly sensitive to the variation in electron density around the C=O function, and to its position in space. In this paper we report the results of the search of conformational space as well as the calculated electronic properties of the cis and trans 3-carbomethoxy fentanyl, \( 5a \) and \( 5b \). The results are compared to the corresponding properties of fentanyl, carfentanil and cis and trans 3-methylfentanyl in an attempt to get further insight to the characteristics of the fentanyl class pharmacophore.

The calculation MC-MM2 method\textsuperscript{25,26} was applied in the search of conformational space. The heats of protonation, as a measure of proton affinities of the polar groups (Table 2), were calculated using PM3 semiempirical method implemented to HyperChem 4.0 program\textsuperscript{27} The receptor-recognized conformations have been used for the calculations of electronic properties, after PM3 optimization of geometry. Only the (4S) enantiomers of 3-methylfentanyl and 3-carbomethoxyfentanyl were used for calculations.

Bioavailability is an important component of drug efficacy which is dependent on the ability of drug molecules to travel from the site of administration to the site of action. Bioavailability is dependent on a transport across biological membranes. It is related to the distribution of the drug molecules between water and organic phase which in turn is related to the octanol/water free energy of transfer. The water accessible surface areas of the molecules and the related octanol/water free energies of transfer (Table 2) were calculated by PCMODEL program.\textsuperscript{28} High portion of the polar area of the \( 5a \) and particularly \( 5b \), and the low values of the related free energies of transfer between organic phase (octanol) and the water suggest the low availability of these molecules at their sites of action. However the ratios between the \( \Delta G \) of compounds in Table 2 and the \( \Delta G \) of fentanyl poorly correlate with their potencies. This indicates that the reduced lipophilicity of \( 5a \) and \( 5b \) relative to fentanyl may be only one of the possible causes of their reduced potencies. The proton affinities of O\textsubscript{12}, which may be related to the strength of a possible hydrogen bond with a receptor, are very similar for all the compounds of Table 2. They differ by less than 3 percent.
The global minimum conformation, and all the other low energy conformations of 5a and 5b have piperidine ring in a chair conformation, with the N-phenethyl and 4-N-phenylpropanamide substituents both equatorial. The global minimum conformations of both, 5a and 5b, have unfavorable, bent conformation of a N-phenethyl side chain, and 5b has unfavorable orientation of a 4-N-phenylpropanamide group, as well. However the conformations corresponding to the fentanyl class receptor-recognized conformations, were found within 11 kJ/mol above the global minimum. The flexibility of the 3-carbomethoxy group is important in both molecules but the rotamer with C=O bond nearly anti to the C2-H is somewhat more stable than the others. The results of the conformational calculations in vacuum should be considered with caution because of the solvent effects which may reverse the stability order of conformations. However it has been suggested that the low dielectric constant (equal 4) should approximate the environment influences of the protein receptor better than the bulk dielectric constant of water (equal 80).

One of the major differences between 5a and 5b, in the low energy conformational region, is flexibility of the 4-N-phenylpropanamide group in 5b. In the 5a isomer, the 4-N-phenylpropanamide group is relatively rigid in a position corresponding to the postulated receptor-recognized conformation of this class of compounds, Table 1. On the other side the trans-3-carbomethoxyfentanyl 5b is considerably more flexible relative to the cis 5a isomer. What is more important, the conformers having inverted 4-N-phenylpropanamide group, with β oriented phenyl, are lower in energy compared to the α phenyl oriented conformations, Fig 2, contrary to the cis-3-carbomethoxyfentanyl 5a where the conformations with inverted 4-N-phenylpropanamide group, β-phenyl, are all at least 12.5 kJ/mol above the global minimum. The other active analogs of fentanyl prefer α phenyl orientation, as well. Combined with the reduced lipophilicity of 5b, Table 2, this unusual orientation of the 4-N-phenylpropanamide group may affect its activity.

<table>
<thead>
<tr>
<th>Compound</th>
<th>HF (kJ/mol)</th>
<th>ΔH°1 (kJ/mol)</th>
<th>ΔΔH1 (kJ/mol)</th>
<th>Total area</th>
<th>Polar area</th>
<th>ΔG (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fentanyl</td>
<td>-30.4</td>
<td>627.5</td>
<td>657.9</td>
<td>5.37</td>
<td>0.18</td>
<td>9.6</td>
</tr>
<tr>
<td>Carfentanil</td>
<td>-326.3</td>
<td>334.6</td>
<td>660.9</td>
<td>5.77</td>
<td>0.23</td>
<td>9.2</td>
</tr>
<tr>
<td>cis-3-Methyfentanyl (3R,4S)</td>
<td>-40.5</td>
<td>667.7</td>
<td>648.1</td>
<td>5.47</td>
<td>0.15</td>
<td>11.7</td>
</tr>
<tr>
<td>trans-3-Methyfentanyl (3S,4S)</td>
<td>-53.7</td>
<td>603.5</td>
<td>657.1</td>
<td>5.80</td>
<td>0.14</td>
<td>12.5</td>
</tr>
<tr>
<td>cis-3-Carbomethoxyfentanyl (3R,4S)</td>
<td>-340.9</td>
<td>329.1</td>
<td>670.0</td>
<td>5.84</td>
<td>0.31</td>
<td>5.9</td>
</tr>
<tr>
<td>trans-3-Carbomethoxyfentanyl (3S,4S)</td>
<td>-352.7</td>
<td>295.1</td>
<td>647.8</td>
<td>5.86</td>
<td>0.42</td>
<td>1.2</td>
</tr>
</tbody>
</table>

* HF is the heat of formation of the neutral form; ΔH°1 is the heat of formation of the protonated form (protonation of O2); ΔΔH1 = ΔH°1-HF represent the proton affinities of neutral forms.
Fig. 1 Postulated receptor recognized conformations of 5a (5a α) and 5b (5b α). Energies (E) are relative to the global minimum.
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