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-carfentanil" has been accomplished in five steps, by simple and efficient route, starting from phenethyl amine and methyl acrylate. Both (±) *cis* and (±) *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 carfentanil. The computational (molecular mechanics) search of the conformational space low energy regions of ***5a*** ((±) ***cis***) and ***5b*** ((±) ***trans***) isomers revealed the difference in their conformational mobility. Besides being more conformationaly 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.[1-4] Among various classess of these compounds, 4-anilidopiperidines present the most potent group known to date,[1,2] including fentanyl[5] (50-100 X morphine), carfentanil[6] (~7000-8000 X morphine), lofentanil[5] (5000-6000 X morphine), sufentanil[5] (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*-cafentanil"), 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 NaH_2PO_4) yielded keto-ester ***2*** in ca. 80% yiled. The product was further purified by precipitation as monooxalate salt, then the free base liberated with K_2CO_3. 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 recrystalization from i-PrOH. Various unsuccessful attempts to selectively reduce the double bond in the enamine ***3***, included Zn/AcOH,[7] Mg/MeOH,[8] Mg/buffered MeOH,[9] $NaBH_4$/EtOH and catalytic hydrogenation, while Na/i-PrOH,[10] Li/lq. NH_3/t-BuOH or $LiBH_4/Et_2O$ yielded complex mixtures. The reduction was quantitatively carried out with $NaBH_3CN$ (MeOH, pH~5, solid $NaH_2PO_4 H_2O$, rt, 12h) yielding a ***cis/trans*** mixture of diastereoisomers in a ratio 1: 1. The mixture was separated by chromatography on neutral Al_2O_3 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 (Et_3N, CH_2Cl_2, rt, 5h) and the products precipitated as monooxalate salts (anh. oxalic acid, MeOH/Et_2O, 2/8). The stereochemical assignments for ***5a*** and ***5b*** were made by using ^1H NMR spectroscopy. The signals for the methyne hydrogen at possition 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_t= 5 Hz, J_d= 12.5 Hz) and ***5a*** also gave doublet of triplets (4.50 δ, J_t= 4.5 Hz, J_d= 11.6 Hz). On contrary, both ***trans*** 3-methyl fentanyl and ***5b*** gave triplet of doublets (4.53 δ, J_d= 4.5 Hz, J_t= 12.5 Hz, for the former and 4.98 δ, J_d= 4.4 Hz, J_t= 12.4 Hz for the later).

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 ED_{50} and 95% confidence limits were estimated from log dose-response curve by using a standard computer program of Tallarida.[14]

Based on the determined ED_{50} 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 reveresed by opioid antagonist naloxone hydrochloride (1mg/Kg S.C.).

In the view of previous findings[16] (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.[17] 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, paralels 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.

SCHEME

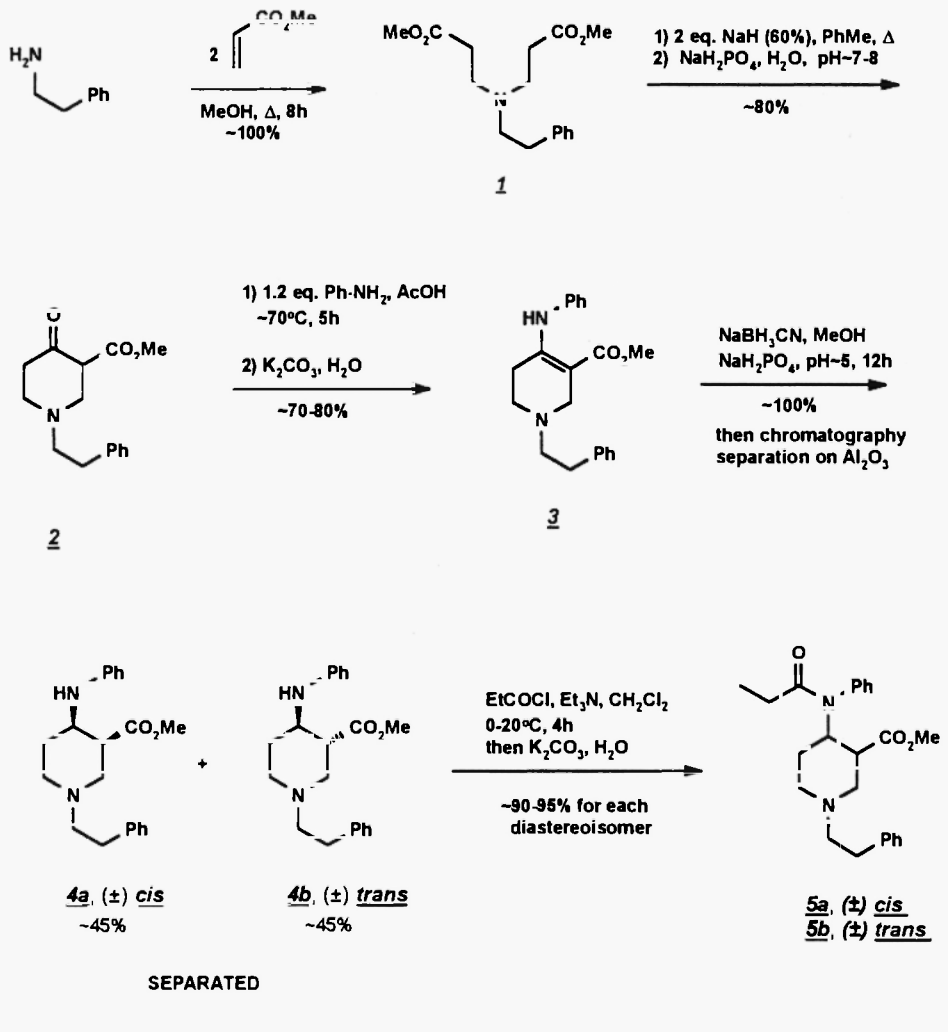
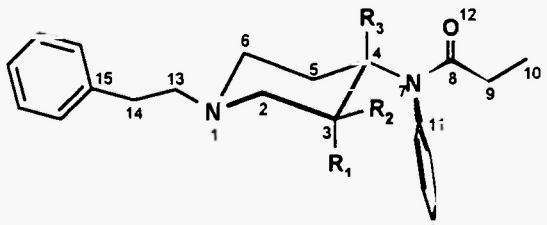


Table 1. Summary of ED_{50} for analgesia, potency ratio of fentanyl analogues and proposed receptor-recognized conformation of the fentanyl class compounds.

No	COMPOUND (FENTANYL ANALOGUE)	ED_{50} (mg/Kg) [A] (95% confidence limits)	POTENCY RATIO	R_1	R_2	R_3
1	Fentanyl	0.011[16, 17, 18] (0.0095 - 0.0140) 0.012[B] (0.006-0.02)	1	H	H	H
2	(±)*cis* 3-Carbomethoxy-fentanyl, *5a*	0.023 (0.009-0.06)	0.52	CO_2Me[D]	H	H
3	(±)*trans* 3-Carbomethoxy-fentanyl, *5b*	0.1 (0.05-0.19)	0.12	H	CO_2Me[E]	H
4	(±)*cis* 3-Allyl-fentanyl	0.08 [17, C]	0.14	-	-	-
5	(±)*cis* 3-Propil-fentanyl	0.02 [17, C]	0.55	-	-	-
6	(±)*trans* 3-Propil-fentanyl	0.04 [16, C]	0.28	-	-	-
7	(+)*cis* 3-Methyl-fentanyl	0.0018 [16] (0.0013-0.0024)	6.1	Me[F]	H	H
8	(±)*trans* 3-Methyl-fentanyl	0.0094 [16] (0.0070-0.0127)	1.2	H[G]	Me	H
9	4-Carbomethoxy-fentanyl (carfentanil)	0.00041 [18] (0.00029 - 0.00058)	26.8	H	H	CO_2Me
10	Morphine	3.15 [18] (2.82 - 3.52)	0.0035	-	-	-



Φ_1 = 5-4-7-11; 0 to −30° Φ_2 = 1-13-14-15; ~ 180°

[A] All ED_{50}'s are expressed as free base weight. [B] The ED_{50} 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[2,19-23] 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[2,19-23] since the structure of a fentanyl receptor is unknown. The postulated[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_4 versus Et 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 (ϕ_2 around 180°) Table 1, has been proposed,[19] and it has been calculated[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 ϕ_1 fluctuating between 0° and -30°, Table 1, leans on the low energy conformations[20] of the most active stereoisomers of *cis* 3-methyl fentanyl and ohmefentanyl. Also the role of the four structure elements, necessary for the optimum receptor recognition, has been postulated.[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[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 fentanyls, ***5a*** and ***5b***. The results are compared to the corresponding properties of fentanyl, carfentanil and *cis* and *trans* 3-methylfentanyls in an attempt to get further insight to the characteristics of the fentanyl class pharmacophore.

The calculational MC-MM2 method[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[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-methylfentanyls and 3-carbomethoxyfentanyls 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.[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 ΔG of compounds in Table 2 and the ΔG 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_{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,[2 19-23] Fig.1 , 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 C_3-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[29,30] 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*-phenlypropanmide 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,[1] as well. Combined with the reduced lipophilicity of ***5b***, Table 2, this unusual orientation of the 4-*N*- phenlypropanmide group may affect its activity.

Table 2. Proton Affinities[a] (kJ/mol) and Water Accessible Surface Areas (nm^2)

Compound	HF (kJ/mol)	ΔH_{O1} (kJ/mol)	$\Delta\Delta H_1$ (kJ/mol)	Total area	Polar area	ΔG (kJ/mol)
Fentanyl	-30.4	627.5	657.9	5.37	0.18	9.6
Carfentanil	-326.3	334.6	660.9	5.77	0.23	9.2
cis-3-Methylfentanyl (3R,4S)	-40.5	607.7	648.1	5.47	0.15	11.7
trans-3-Methylfentanyl (3S,4S)	-53.7	603.5	657.1	5.60	0.14	12.5
cis-3-Carbomethoxy fentanyl (3R,4S)	-340.9	329.1	670.0	5.84	0.31	5.9
trans-3-Carbomethoxyfentanyl (3S,4S)	-352.7	295.1	647.8	5.86	0.42	1.2

[a] HF is the heat of formation of the neutral form; ΔH_{O1} is the heat of formation of the protonated form (protonation of O_{12}); $\Delta\Delta H_1 = \Delta H_{O1}$-HF represent the proton affinities of neutral forms.

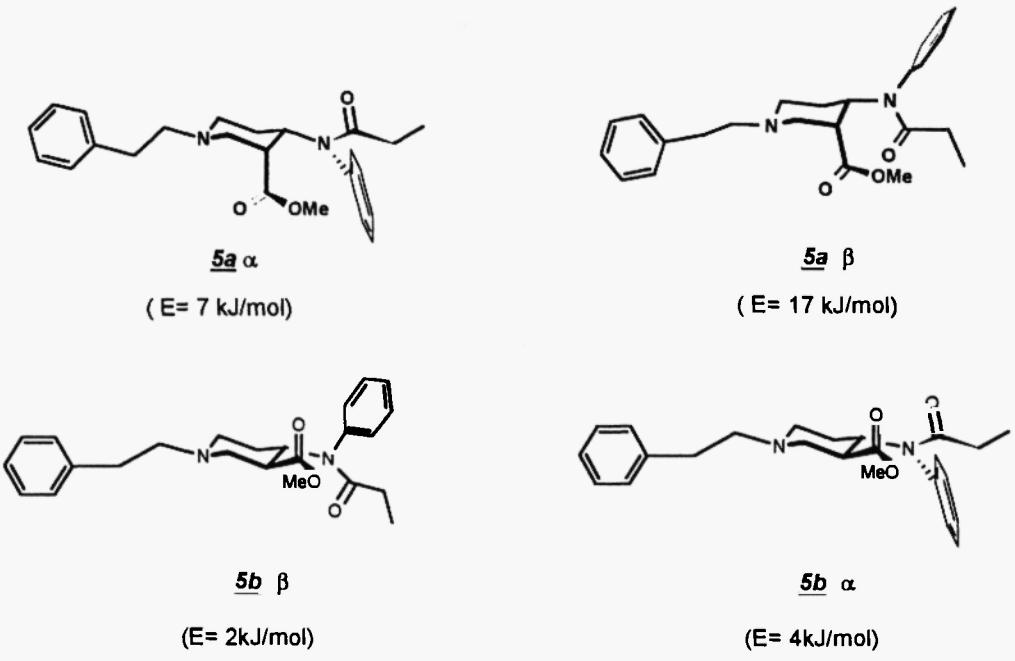


Fig. 1 Postulated receptor recognized conformations of ***5a*** ***(5a α)*** and ***5b*** ***(5b α)***. Energies (E) are relative to the global minimum.

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