Graphical Abstract

Fentanyl Derivatives Bearing Aliphatic Alkane--guanidinium Moieties: a New Series of Hybrid Molecules with Significant Binding Affinity for μ-Opioid Receptors and I_2-Imidazoline Binding Sites

Bioorg. Med. Chem. Lett. **2004**, *14*, 491-493

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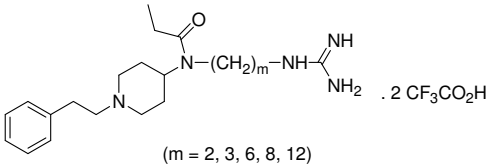

(m = 2, 3, 6, 8, 12)

Bioorganic & Medicinal Chemistry Letters

Fentanyl Derivatives Bearing Aliphatic Alkaneguanidinium Moieties: a New Series of Hybrid Molecules with Significant Binding Affinity for μ-Opioid Receptors and I_2-Imidazoline Binding Sites

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Abstract− A new series of fentanyl derivatives (i.e. *N*-[1-(2-phenethyl)-4-piperidyl]-*N*-(guanidinoalkyl)propanamide) bearing aliphatic alkaneguanidinium moieties were prepared. Their affinities for the μ opioid receptors and for the I_2-imidazoline binding sites (I_2-IBS) were determined on human post-mortem prefrontal cortex membranes. All of these hybrid compounds had significant and/or very high affinity for both receptors in the nanomolar range, meaning an improvement compared to the prototype *N*-[1-(2-phenethyl)-4-piperidyl]-*N*-(guanidinopropyl)propanamide previously reported.

Key words: I_2-Imidazoline binding site affinity; μ-opioid affinity; alkane guanidine; dual acting drug; opioid tolerance; opioid withdrawal; human brain.

Several pharmacological studies revealed the interactions between I_2-imidazoline binding site (IBS) and opioid systems.[1-4] Very recently, it has been shown that 2-BFI, an I_2-IBS ligand, when chronically administrated, led to an attenuation of both the hyperactivity of rat locus coeruleus neurons during opiate withdrawal and the development of tolerance to morphine.[5] Other studies demonstrated the potential of the putative endogenous IBS ligand agmatine[6] (1-amino-4-guanidinobutane) to attenuate the escalation of intravenous cocaine and fentanyl self-administration in rats[7] and to regulate morphine tolerance/dependence and withdrawal symptoms.[8] Thus, I_2-IBS ligands might be useful agents for the management of opioid dependence and tolerance.

One approach that seems interesting and promising for pharmaceutical regulation of opioid dependence and tolerance is the use of hybrid drugs in which opioid (e.g. fentanyl) and I_2-imidazoline agents (e.g. guanidine group) are combined in one molecule. Among structure-activity relationships (SAR) that have been published earlier, studies on the replacement of the phenyl ring of fentanyl by a butyl or propyl group afforded μ-opioid molecules that retained some analgesic activity of the parent compound.[9]

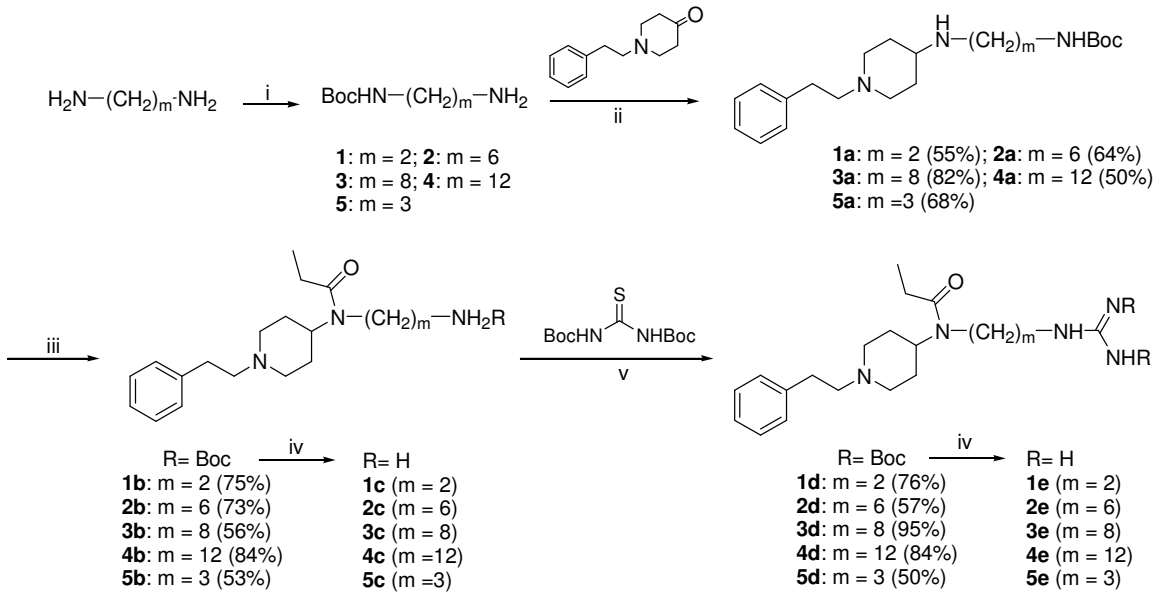
A first attempt to obtain hybrid molecules with opioid activity and I_2-IBS affinity led us to identify the prototype **5e** (Scheme 1).[10] This compound displayed high affinity for the μ opioid receptor whereas it only showed low affinity for the I_2-IBS.
In a previous SAR studies of a series of alkane bisguanidinium aliphatic derivatives,[11] we found out that increasing the number of methylene units between two guanidine moieties led to an increase of the I_2-IBS affinity. We rationalised that modulating the chain length of the methylenic spacer between the fentanyl pharmacophore and the guanidine moiety should increase the affinity of the prototype **5** for I_2-IBS. Hence, a new series of fentanyl hybrids compounds bearing a guanidine at the end of a methylene chain of 2, 6, 8 and 12 units was prepared (Scheme 1) and their binding affinity to both kinds of receptors was evaluated.

Chemistry

Compounds **1e**-**4e** were synthesised in 6 steps using a shorter procedure than the one previously described by Montero *et al.*[10] The mono Boc-protected diaminoalkanes (m = 2, 6, 8 and 12) were directly reacted with 1-phenethyl-4-piperidone in the presence of $NaBH_3CN$ maintaining the pH at *ca* 7-8 with AcOH. The resulting amines (**1a**-**4a**) were acylated with propionic anhydride/$DMAP_{cat.}$/pyridine/CH_2Cl_2 to afford the corresponding propanamides **1b**-

4b which were easily isolated by flash chromatography.[12] Following the removal of the Boc-protecting group, the guanidines were introduced with the classical Boc-thiourea reagent.[13] Compounds **1e**-**4e** were finally isolated as their trifluoroacetate salt and purified by crystallisation.[14] The NMR spectra of these series of propanamides (**1b-e** to **4b-e**) show characteristic duplicated signals corresponding to the observation of the *cis-trans* isomerism around the N-CO bond as previously reported for **5e**.[10]

Scheme 1



Reagents and conditions. (i) Amine (5 eq.), Boc_2O (1 eq.), $CHCl_3$, rt; (ii) $NaBH_3CN$, MS (3Å), MeOH, rt; (iii) $(EtCO)_2O$, DMAP, Pyridine, CH_2Cl_2, rt; (iv) CF_3CO_2H, CH_2Cl_2, 30 min; (v) $HgCl_2$, Et_3N, CH_2Cl_2, rt

Pharmacology

The binding affinity of the new synthesised compounds (**1e**-**4e**) and **5e** was evaluated through competition binding studies against the selective I_2-IBS radioligand [^{3}H]-2-BFI and the selective μ-opioid ligand [^{3}H]-DAMGO following the procedure previously described.[10,11,15,16] The assays were performed in membranes from post-mortem human frontal cortex, a brain area that shows an important density of I_2-IBS and μ-opioid receptors. Drug competition studies were performed with either [^{3}H]-2-BFI (1 nM) or [^{3}H]-DAMGO (2 nM) in the absence or presence of various concentrations of competing drugs (10^{-12}, 10^{-10} and 10^{-3} M concentrations). Non-specific binding was estimated in the presence of 10^{-3} M

idazoxan in experiments with [^{3}H]-2-BFI, or with 10^{-4} M naloxone in [^{3}H]-DAMGO assays. The opioid agonist fentanyl and the α_2-adrenergic/I_2-IBS ligand idazoxan were used as references (Table 1). Compounds **1e**-**5e** were studied as their trifluoroacetate salts.

All the new compounds synthesised (**1e**-**4e**) showed submicromolar to nanomolar affinity for I_2-IBS which represented a raise from 4- (**1e**) to almost 300-fold (**4e**) compared to the prototype **5e** (Table 1). Remarkably, compound **4e**, with a 12 methylene units spacer between the propanamide and guanidine groups, was a more potent ligand than idazoxan (K_i = 28 nM) for I_2-IBS with K_i = 6.5 nM. In this series, we observed a good correlation between the number of methylene units in the spacer and the affinity for I_2-IBS.

Table 1. Affinity data [K_i (nM)] for I_2-IBS and μ-opioid receptors.

Compound	-(CH_2)m-m[a]	[^{3}H]-2BFI **I_2**	[^{3}H]-DAMGO **μ**	n[b]
Idazoxan	--	28±11	nd[c]	5
Fentanyl	--	5462±1343[d]	2.9±1.5	4
1e	2	437±228	433±83	3
5e	3	1920±996	23±4.5	3
2e	6	409±238	1.04±0.28	3
3e	8	126±72	37±9.7	3
4e	12	6.5±3.0	477±75	3

[a] Number of methylene units in the spacer;[b] Values expressed as mean ± standard error mean of n experiments;[c] Not determined; [d] K_i value for mouse brain membrane(from reference 10)

The clear improvement in I_2-IBS binding affinity observed when increasing the number of methylene units from m = 3 (**5e**) to m = 12 (**4e**) was in agreement with our previous observations on alkane bis-guanidinium compounds in which the affinity for I_2-IBS increased with the chain length. However, in that study, the presence of both guanidinium at the end of the aliphatic chain appeared essential for I_2-IBS activity since monoguanidinium counterparts (e.g. octyl, nonyl and dodecylguanidinium) showed extremely poor I_2-IBS affinity.[11] Thus, in the case of the hybrid molecules **1e**-**5e**, it is interesting to note that the presence of the piperidyl propanamide pharmacophore proceeding from the fentanyl structure confers significant I_2-IBS affinity to the alkyl guanidinium moiety. Since fentanyl alone is a very poor I_2-IBS ligand (see Table 1), there is some evidence for a possible synergic effect of both pharmacophoric groups for I_2-receptor affinity in these hybrid compounds.

Regarding μ-opioid receptor, all the molecules bound with affinities in the nanomolar range (Table 1). One compound (**3e**, K_i = 37 nM) was roughly equipotent with **5e** (K_i = 23 nM), two molecules (**1e**, **4e**) showed lower affinity whereas **2e** (K_i = 1.04 nM) was an excellent μ-opioid ligand with a binding affinity comparable to that of fentanyl (2.9 nM).

With respect to the spacer chain length, these results showed that the best μ-opioid ligand was obtained with six methylene units (**2e**, m = 6) whereas either increasing (m = 8, 12) or decreasing (m = 2, 3) the length of the spacer affords less potent molecules. This observation would need confirmation by the assay of more spacers of different lengths (e.g. m = 4, 5, 7, 9).

To conclude, we have reported a new series of *N*-[1-(2-phenethyl)-4-piperidyl]-*N*-(guanidinoalkyl)propanamide hybrid molecules with improved affinity for the I_2-IBS and μ-opioid receptor compared to the prototype **5e**.

This study showed that the incorporation in a same molecule of μ-opioid and I_2-IBS pharmacophoric moieties (i.e. fentanyl and alkylguanidine respectively), linked by a methylene spacer, led to a synergistic effect for the binding to both receptors. The modulation of the affinity for both receptors by modification of the length of the methylene spacer between the two pharmacophoric moieties afforded two hybrid compounds (**1e** and **3e**) with balanced I_2/μ affinity, a 400-fold μ-opioid selective compound (**2e**) and an 80-fold I_2-IBS selective compound (**4e**). The *in vitro* and *in vivo* functional activity of these new dual acting drugs is currently being evaluated and will be reported elsewhere.

Finally, these results are relevant in the field of IBS since they further demonstrate that aliphatic alkaneguanidines can afford high affinity ligands for the I_2-IBS.

Acknowledgments. This work was supported by the Spanish Grant SAF00-0114-C02 and the Basque Government (INTEK-ER02ME04). C. D. is recipient of a postdoctoral fellowship from the Spanish Ministerio de Educación Cultura y Deporte (SB2001-0174). We thank the staff members of the Instituto Vasco de Medicina Legal for their cooperation in the study.

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(14) Spectroscopic data: **1e.** (2TFA): NMR (400MHz, D_2O/CD_3OD) ^{1}H δ ppm 7.3-7.1 (m, 5H); 4.0-3.8 (m, 1H); 3.55 (m, 2H); 3.35 (m, 1H); 3.2 (br m, 4H); 3.12 (br m, 1H); 3.05-2.85 (m, 4H); 2.33 (m, 2H); 2.25-2.0 (m, 1H); 1.95-1.75 (m, 3H); 0.88 (m, 3H); ^{13}C δ ppm 178.3; 157.2; 136.2; 129.2; 128.9; 127.5; 57.8; 52.5; 52.0; 40.4; 39.7; 30.0; 27.4; 26.7; 9.0; ES^+MS *m/z* 346 [M+H]; 173.6 [(M+2H), 100%]. **2e.** (2TFA): NMR (400MHz, D_2O) ^{1}H δ ppm 7.63-7.49 (m, 5H); 4.45 (m, 0.6H); 4.30 (m, 0.4H); 3.91 (br m, 2H); 3.56 (m, 2H); 3.45 (t, 2H); 3.35 (m, 3H); 3.27 (m, 3H); 2.67 (q, 1H, J = 7.5Hz); 2.60 (q, 1H, J = 7.5 Hz); 2.46-2.1 (m, 4H); 1.77 (br m, 3H); 1.68 (m, 1H); 1.54 (br m, 4H); 1.27 (m, 3H); ^{13}C δ ppm 178.3 (s); 177.7 (s); 157.7 (s); 137.17 (s); 137.1 (s); 130.0 (d); 129.7 (d); 128.3 (d); 58.6 (d); 53.4 (d); 53.1 (d); 53.0 (d); 52.0 (t); 46.3 (d); 42.8 (d); 42.0 (d); 30.9 (t); 30.8 (t); 29.5 (t); 28.9 (t); 28.8 (t); 28.4 (t); 27.7 (t); 27.6 (t); 27.4 (t); 26.9 (t); 26.6 (t); 10.1 (q); 10.0 (q); ES^+MS *m/z* 402.5 [M+H]; 201.9 [(M+2H), 100%]. **3e.** (2TFA): NMR (300MHz, CD_3OD) ^{1}H δ ppm 7.3-7.1 (m, 5H); 4.2 (br m, 0.5H); 4.0 (br m, 0.5H); 3.61 (br d, 2H); 3.3-2.85 (m, 10H); 2.43-2.0 (m, 4H); 1.85 (br t, 2H); 1.5 (br m, 4H); 1.27 (br m, 8H); 1.01 (br t, 3H, J= 7.3 Hz); ^{13}C δ ppm 177.0 (s); 176.3 (s); 163.8 (s); 163.3 (s); 138.0 (s); 130.4 (d); 130.2 (d); 128.7 (d); 59.4 (d); 54.0 (d); 53.6 (t); 52.7 (t); 46.9 (t); 43.5 (t); 42.8 (t); 32.4 (t); 31.9 (t); 30.9 (t); 30.65 (t); 30.60 (t); 30.5 (t); 30.2 (t); 29.3 (t); 28.5 (t); 28.3 (t); 28.27 (t); 28.05 (t); 27.97 (t); 10.4 (q); ES^+MS *m/z* 430.5 [M+H]; 215.9 [(M+2H), 100%]. **4e.** (2TFA): NMR (500MHz, CD_3OD) ^{1}H δ ppm 7.55-7.43 (m, 5H); 4.49 (br t, 0.6H); 4.32 (m, 0.4H); 3.91 (br m, 2H); 3.6-3.2 (m, 10H); 2.67 (q, 0.8H, J = 7.3 Hz); 2.58 (q, 1.2H, J = 7.3 Hz); 2.47 (br q, 1.2H); 2.37 (br q, 0.8H); 2.19 (br d, 0.8H); 2.13 (br d, 1.2 H); 1.81-1.65 (m, 4H); 1.6- 1.4 (br m, 16H); 1.30 (td, 3H, J = 7.3 Hz); ^{13}C δ ppm 177.2; 176.5; 158.8; 137.8; 130.2; 130.1; 128.5; 59.3; 53.8; 53.4; 52.5; 46.7; 43.4; 42.7; 32.2; 31.7; 30.9; 30.8; 30.6; 30.53; 30.48; 30.0; 29.1; 28.4; 28.13; 27.97; 27.87; 10.43; 10.38; ES^+MS *m/z* 486.5 [M+H]; 243.9 [(M+2H), 100%].
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